Universitat Autònoma de Barcelona

Clinical Impact of Colonization and Infection by Multidrug-Resistant Pseudomonas aeruginosa in the Critically Ill Patient

Bárbara Borgatta Barzé

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Universitat Autònoma de Barcelona

Department of Medicine

Doctoral Programme in Medicine RD99

Clinical Impact of Colonization and Infection by Multidrug-Resistant *Pseudomonas aeruginosa* in the Critically Ill Patient

Thesis presented by Bárbara Borgatta Barzé to opt to the grade of Doctor.

Supervisors:

Prof. Jordi Rello Condomines

Prof. Manel Luján Torné

Barcelona, November 2017

Universitat Autònoma de Barcelona

Departament de Medicina

Programa de Doctorat en Medicina RD99

Impacte Clínic de la Colonització i Infecció per *Pseudomonas aeruginosa* Multiresistent en el Malalt Crític

Tesi presentada per Bárbara Borgatta Barzé per optar al grau de Doctor.

Directors:

Prof. Jordi Rello Condomines

Prof. Manel Luján Torné

Barcelona, Novembre 2017

The supervisors, Jordi Rello Condomines (Full Professor at UAB), and Manel Luján Torné (Associate Professor at UAB), certify that Bárbara Borgatta Barzé who presents ¨Clinical Impact of Colonization and Infection by Multidrug-Resistant *Pseudomonas aeruginosa* in the Critically Ill Patient¨ has been completed under their direction, meets the compulsory methodological and scientific requirements and authorize its presentation for defense in front of the corresponding panel, according to the MDI modality.

Els directors, Jordi Rello Condomines (Professor Titular UAB) i Manel Luján Torné (Professor Associat UAB) certifiquem, que Bárbara Borgatta Barzé qui presenta ¨Impacte Clínic de la Colonització i Infecció per *Pseudomonas aeruginosa* Multiresistent en el Malalt Crític¨ ha estat realitzada sota la seva direcció, reuneix les exigències metodològiques i científiques necessàries i autoritzen la seva presentació per ser defensada davant del tribunal corresponent, sota la modalitat MDI.

“ Yesterday I was clever, so I wanted to change the world. Today I am wise, so I am changing myself ”

— Jalaluddin Rumi

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PREFACE

This dissertation by compendium of publications has been organized structurally in accordance with the recommendations contained in the Regulatory Framework for Studies of Doctorate **RD 99/2011** of the Universitat Autònoma de Barcelona. According to point II.18.2 of Framework, it is recommended that the thesis should contain an introduction that present the works and justify the thematic unit of the thesis; an overall summary of the results and its discussion; final conclusions; and a copy of the published works already admitted by the Comissió d'Estudis de Postgrau (Commission of Postgraduate Studies).

We have also added three review articles and two editorials in relation to the topic of the thesis written by the doctoral student and published during the period of preparation of the same. These articles complete and expand the content of the two original articles that constitute this thesis, which have been considered relevant to incorporate as appendixes to it.

ABREVIATIONS

aHR= adjusted hazard ratio

AKI= acute kidney injury

APACHE-II= acute physiology and chronic health evaluation

ARDS= acute respiratory distress syndrome

CI= confidence interval

COPD= chronic obstructive pulmonary disease

ESBL= extended-spectrum beta-lactamase

HAP= hospital acquired pneumonia

IIAT= inadequate initial antibiotic treatment

ICU= intensive care unit

IQR= interquartile range

KPC= *Klebsiella pneumoniae* carbapenemase

LOS= length of stay

MDR= multidrug-resistant

MIC= minimum inhibitory concentration

MV= mechanical ventilation

OR= odds ratio

PA= *Pseudomonas aeruginosa*

SD= standard deviation

SDD= selective digestive decontamination

SOFA= sequential organ failure assessment

VAP= ventilator-associated pneumonia

VAT= ventilator-associated tracheobronchitis

XDR = extensively drug-resistant

I. INTRODUCTION

1. Definitions and Epidemiology

Pseudomonas aeruginosa (PA) is a gram-negative non-fermenting bacillus opportunistic pathogen. Is one of the most important causative microorganisms of nosocomial infections, especially in the intensive care unit (ICU) **[1-4].** The spread of multidrug resistant (MDR) strains over the last decade is a matter of profound concern in the critically ill setting **[2-5]** and poses a serious threat. It is of great concern as well, since it has a preference for immunocompromised patients, it causes highly virulent respiratory infections; and has many reservoirs in the ICU **[6]**. Furthermore, even when patients with PA infections receive with appropriate empiric antibiotic, the attributable mortality is 13.5 % [**4**]. In the early twenty-first century, the increase and dissemination of clones with progressive resistance are a cause of concern. In an international study of over 1,200 ICUs in 75 countries, the risk of infections, including those due to Pseudomonas species, was found to increase with duration of ICU stay and increased mortality [**2**].

PA is the also most common multidrug-resistant gram-negative pathogen causing pneumonia in hospitalized patients. It has been reported to be one of the top three organisms causing respiratory infections resistant to carbapenems **[7]**. It is both intrinsically resistant to a number of antibiotics and can acquire resistance during therapy, which further assists its bacterial virulence. Known mechanisms of antibiotic resistance are many: AmpC beta-lactamase, extended-spectrum beta-lactamase, down regulation of the outer membrane protein OprD, multi-drug efflux pumps, the ability to form a biofilm; and others **[8]**. Acquired resistance, specifically, can result from mutation or acquisition of exogenous resistance determinants. Approximately 20% of strains are resistant to piperacillin/tazobactam, imipenem and ceftazidime; and 30% or more to quinolones and aminoglycosides **[9]**.

2. Risk Factors for *P. aeruginosa* Isolation in the ICU

Risk factors for PA acquisition in the ICU are advanced age, length of mechanical ventilation, previous antibiotic exposure, transfer from a medical unit or ICU, and admission to a ward with a high incidence of patients with Pseudomonas infection **[10]**. Multiple studies have confirmed the association of risk factors for exogenous cross-contamination (5 days and prior antibiotic exposure) and endogenous colonization such as chronic obstructive pulmonary disease (COPD) **[11]**. It is particularly associated with underlying fatal medical condition, previous fluoroquinolone exposure, initial disease severity and developing ventilator-associated pneumonia (VAP) **[12]**. Whilst for MDR-PA strains, risk factors include isolation are ICU stay, being bedridden, having high invasive devices scores, diabetes mellitus, undergoing surgery and prior exposure to broad-spectrum cephalosporins, aminoglycosides, carbapenems and flouroquinolones **[13,14]**.

3. Outcomes associated with *P. aeruginosa*

In survivors, PA infections increase ICU length of stay (LOS) and use of healthcare resources, which translates to extra-costs as high as US$40,000 per VAP episode **[15]**. In patients with VAP, MDR has been identified as an independent predictor of hospital death and is the single strongest predictor of initial inadequate antibiotic therapy **[3,16]**.

We already know that ventilator-associated pneumonia (VAP) is associated with increased days on mechanical ventilation (MV) **[17]**, and that PA pneumonia is associated with poorer outcomes **[13,18-20]**. When compared to susceptible strains, resistant PA infections have increased mortality **[16]**. Furthermore, it has been shown that MDR-PA isolation and infection in the critically ill is associated with poor prognosis **[5,13,16]**, though with conflicting results **[21]**. Despite extensive research, the spectrum of outcomes in patients with MDR-PA isolated in different respiratory infections in the ICU has not been studied to date. Isolates in respiratory specimens correspond to pneumonia, tracheobronchitis or colonisation depending on the inflammatory response.

II. JUSTIFICATION OF THE THEMATIC UNITY OF THE THESIS

Both the investigation studies that constitute this doctoral thesis analyse the clinical significance of MDR-PA isolation in the critically ill patient. First, we performed a study comparing colonisation versus infection in patients without previous MDR-PA isolation, in order to identify predictors of infection in a population in high-risk of MDR-PA acquisition. We identified non-modifiable predictors related to infection and its severity, but also a modifiable predictor that may be of use for the clinical management and for the control of the spread of this organism. Subsequently, we studied patients with MDR-PA respiratory infections to assess its outcomes in terms of attributable morbidity and mortality in adult ICU patients. We found that MDR-PA pneumonia significantly increases mortality and it bears tremendous morbidity; whereas ventilator-associated tracheobronchitis (VAT) behaves as respiratory colonization. Also, our results do not support guidelines' recommendations of management of MDR-PA pneumonia and shows that adequate antibiotic treatment does not influence the outcome; guiding efforts towards development of new antibiotics and prophylactic/adjunctive therapies.

Finally, the manuscripts enclosed as appendixes summarize the management of respiratory infections in critically ill patients and also specifically due to *P. aeruginosa* (both susceptible and MDR):

1. The first review provides a succinct update on the risk factors for *P.aeruginosa* pneumonia in the ICU.
2. The second review addresses the management of severe community-acquired pneumonia and hospital-acquired pneumonia (where *P. aeruginosa* is one of the main pathogens). Focusing on the rationale, benefits and risks of combination antibiotic therapy.
3. The third review addresses the current management of ventilator-associated pneumonia caused by *P. aeruginosa*, providing an update of the most recent antipseudomonal agents and new adjunctive therapies.
4. The first editorial discusses the complexity of the management of *P. aeruginosa* pneumonia in the ICU and the importance of tailoring therapy.
5. The second editorial summarise the relevant evidence in the management of VAP in ICU patients, providing as well an expert opinion in the field by Dr. Rello.

III. HYPOTHESIS AND OBJECTIVES

Hypothesis.

Our hypothesis were:

1. ICU patients who develop MDR-PA infection differ from those who are only colonised by it; and so, there are specific predictors of MDR-PA infection.
2. MDR-PA respiratory infections are associated with elevated morbidity and mortality, with specific predictors of mortality.

Objectives.

In order to test our hypothesis, we selected the following objectives:

1. To characterise ICU patients in whom MDR-PA was isolated from clinical samples.
2. To characterise MDR-PA infection in ICU patients.
3. To identify predictors of MDR-PA infection in ICU patients that might help the control the burden of disease of this organism.
4. To characterise MDR-PA pneumonia cases in ICU patients.
5. To identify predictors of MDR-PA pneumonia 30-day ICU mortality in order to improve these patient's outcomes'.

IV. METHODS

1. Population

We conducted a retrospective case-control study in 4 medical-surgical ICUs at Vall d'Hebron University Hospital. The study population comprised all consecutive adult patients admitted to the ICU between January 2010 and April 2015. We included all the positive isolates from clinical samples (that were taken when infection was suspected, requested by attending physician). Patients with MDR-PA isolation previous to ICU admission or prior to the study period and samples from surveillance studies (rectal, skin and nasopharyngeal swabs) were excluded.

2. Design

In the first part of this doctoral thesis, we analysed all samples included in the study. Given that standard ICU care includes performance of multiple and simultaneous cultures when infection is suspected, all clinical samples retrieved simultaneously from the same patient were included and recognized as one case. Epidemiological, clinical and microbiological data were recorded and each case was then classified as either infection or colonization. Further isolations were recorded and included only if they were infections.

In the second part, we analysed the respiratory samples and we classified the cohort into a case group formed by patients with pneumonia (hospital-acquired pneumonia [HAP] and ventilator-associated pneumonia) and a control group composed by patients with respiratory samples without evidence of pneumonia (colonisation and ventilator-associated tracheobronchitis) and without subsequent MDR-PA infection. In patients with multiple infections, only the last episode was retained. Statistical analysis was performed in the pneumonia and control cohorts. To identify predictors of mortality among ICU adults harbouring MDR-PA in respiratory samples, a univariate analysis of survivors versus non-survivors was performed in patients with pneumonia (HAP plus VAP).

Patients' records were reviewed up to death or ICU discharge. The study was

approved by the local institutional review board, and the need for written consent was waived due to the observational nature of the study. Variables included were demographic data, comorbidities, and risk factors for MDR-PA isolation (diabetes mellitus, chronic kidney disease, chronic obstructive pulmonary disease, cystic fibrosis, immunosuppressive state, malignancy, neurological sequel and pressure-ulcers) **[13]**. Baseline comorbidity was assessed with Charlson's comorbidity score **[22]**. Severity scores were recorded at admission (APACHE-II **[23]** and SOFA **[24]**) and at the time of sample collection (SOFA and PIRO). PIRO (Predisposition, Infection, Response and Organ dysfunction) is a severity score that allows the prediction of the mortality risk in patients with ventilator-associated pneumonia (VAP) **[25]**. ΔSOFA was defined as SOFA score at culture minus SOFA score at admission, in order to assess the patient's progress at the time of the culture. Clinical, microbiological and laboratory data related to infection status, severity of illness and the appropriateness of initial antibiotic therapy were also recorded.

3. Definitions

a. First Part

- *Previous P. aeruginosa colonization:*
    - At least two positive samples for *P.aeruginosa* within the six months previous to current admission, OR
    - One positive sample for *P.aeruginosa* during current admission (minimum 14 days earlier).
- *Index isolate:* first isolation of MDR-PA in ICU (either infection or colonization). It could include isolates from more than one sample site taken simultaneously.
- *Infection:* specimens associated with clinical signs of inflammation (fever, alteration in WBC, presence of purulence), and categorized as:
    - Respiratory: Ventilator-associated pneumonia (VAP) and hospital-acquired pneumonia (HAP) were diagnosed according to ATS/IDSA 2005 guidelines **[26]**. Ventilator-associated tracheobronchitis (VAT) was defined according to Craven *et al.* **[27]**.

- Non-respiratory: Defined following international sepsis forum consensus definitions **[28]**.
- Bloodstream: Blood culture isolates.

- *Superinfections:* MDR-PA positive sample occurring 14 days after a case, in addition to meeting the infection criteria described above.
- *Colonization:* MDR-PA positive specimens that did not fulfill infection criteria.
- *Shock:* New presence of sustained hypotension and/or vasopressor initiation (noradrenaline) or increase in ≥20% in less than 24 hours.

b. Second Part

- *Pneumonia cases:* patients who met VAP or HAP diagnostic criteria according to 2005 ATS/IDSA guidelines **[26]**.
- *Controls:* patients who met diagnostic criteria for ventilator-associated tracheobronchitis (VAT) and colonisation. VAT was diagnosed according to the definition proposed by Craven *et al.* **[27]**. Colonisation was diagnosed in positive specimens with MDR-PA that did not meet criteria for infection.
- *Shock:* new presence of sustained hypotension and/or initiation of vasopressors (noradrenaline) or increase of ≥20% in less than 24 hours.
- *Moderate-Severe hypoxemia:* PaO_2/FiO_2 or $SpO_2/FiO_2 \leq 200$ only if FiO_2 was increased by ≥10% in less than 24 hours.
- *Acute respiratory distress syndrome (ARDS)*: according to the Berlin definition **[29]**.
- *Acute Kidney Injury (AKI)*: according to the KDIGO criteria **[30]**.
- *ΔSOFA:* SOFA at culture minus SOFA at admission, in order to assess patient's evolution from admission to culture.
- *Crude mortality*: all deaths occurring during ICU stay.
- *Attributable* 30-day ICU *mortality:* the difference between observed 30-day ICU mortalities in the pneumonia and the control groups. **[31]**

4. Microbiology

The microbiology laboratory identified MDR-PA using the VITEK-MS automated system (bioMérieux, Marcy l'Etoile, France). Antimicrobial susceptibility of isolates was tested using disk diffusion, and resistant strains were checked using the gradient diffusion method. Minimum inhibitory concentrations (MICs) were classified using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints **[32]**. MDR was defined as non-susceptibility to at least one agent in ≥3 antimicrobial categories, and extensive drug resistance (XDR) as non-susceptibility to at least one agent in all but ≤2 antimicrobial categories **[33]**. Inadequate initial antibiotic treatment was defined as the absence of any antibiotic with *in vitro* susceptibility administered at the adequate dose **[34]**.

5. Statistical Analysis

Continuous variables were tested with the Kolmogorov-Smirnov test to assess deviations from normality. Discrete variables were summarized as frequency (%) and continuous variables as mean-standard deviation (SD) or median-interquartile range (IQR). Univariate analysis was performed using Pearson's Chi ^{2}, two-tailed Fisher exact test or Mann-Whitney U test, as appropriate. Statistical analysis was performed using Stata for Mac version 13 (StataCorp LP, Texas, USA) and SPSS for Mac, version 18 (SPSS, Chicago, IL, USA). Statistical significance was set at $p < 0.05$.

a. <u>First part</u>

Multivariate logistic regression analysis was used to determine predictors for MDR-PA infection/colonization, and variables were included if they were statistically significant in the univariate analysis and/or if they were considered clinically relevant according to current knowledge with p≤0.10 level of significance. A logistic regression analysis with the stepwise forward method was applied to identify association with infections among the MDR-PA isolates. Odds ratios (OR) are presented with 95% confidence intervals (CI).

b. Second part

ANOVA and Kruskal-Wallis test were performed in variables with more than two categories. Kaplan-Meier curves were performed for the survival analysis in the different cohorts. A Cox proportional hazards model using the enter method was applied to analyse predictors of ICU mortality. Variables investigated as predictors of mortality were included if they reached statistical significance in the univariate analysis and if they were considered clinically relevant according to current knowledge with a p value <0.10. Adjusted hazard ratio (aHR) were expressed with 95% CI. Part of the statistical analysis was carried out in the Statistical and Bioinformatics Unit of the Vall Hebron Hospital Research Institute .

V. SUMMARY OF RESULTS

Study No.1. Infections in Intensive Care Unit adult patients harbouring MDR Pseudomonas aeruginosa: Implications for prevention and therapy.

- 5,667 patients were admitted to the ICU, and *P. aeruginosa* was isolated in 504 (8.8%). MDR-PA was identified in 142 clinical samples from 104 patients (20.6%); sixty-two (43.6%) of these samples appeared to be true infections.
- Twenty MDR-PA were isolated from blood and 108 (76.1%) from respiratory tract: 30 VAP and 26 ventilator-associated tracheobronchitis (VAT).
- One hundred and eighteen (83.1%) isolates were susceptible only to amikacin and colistin, and 13 (9.2%) were susceptible only to colistin.
- One hundred and thirty-one (92.3%) had MIC to meropenem> 8ug/mL.
- Independent predictors for MDR-PA infection were fever/hypothermia (OR 6.65), PIRO score>2 (OR 5.81), vasopressors at infection onset (OR 5.89) and recent antipseudomonal cephalosporin therapy (OR 5.71).

Study No.2. The clinical significance of pneumonia in patients with respiratory specimens harbouring multidrug-resistant Pseudomonas aeruginosa: a 5-year retrospective study following 5667 patients in four general ICUs

- Of 5,667 adults admitted to the ICU, 69 had MDR-PA in respiratory samples: 31 were identified as having pneumonia (pneumonia): 21VAP and 10 HAP.
- Twenty-one (67.7%) adults with MDR-PA pneumonia died after a median of 4 days (18 of the 21 deaths within 8 days), compared with one (2.6%) without pneumonia at day 8.
- In a Cox proportional regression model, MDR-PA pneumonia was an independent variable (aHR 5.92) associated with 30-day ICU mortality.
- Most strains (85.1%) were susceptible to amikacin and colistin. Resistance to betalactams (3rd generation cephalosporins and piperacillin-tazobactam) ranged from 44.1% to 45.3%.
- Meropenem showed poor overall activity ($MIC_{[50/90]}$ 16/32 mg/dL), with 47.0% having MIC breakpoint >8 mg/L.

- Twenty-four (77.4%) pneumonia episodes received inappropriate empirical therapy. Although empirical combination therapy was associated with less inappropriate therapy than monotherapy (16.7% vs 88.3%, $p<0.01$), there was no difference in survival (30% vs. 33.3%, $p=0.8$).

VI. MANUSCRIPT No.1

Infections in Intensive Care Unit adult patients harbouring MDR Pseudomonas aeruginosa: Implications for prevention and therapy.

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ORIGINAL ARTICLE

Infections in intensive care unit adult patients harboring multidrug-resistant *Pseudomonas aeruginosa*: implications for prevention and therapy

B. Borgatta[1,2,3] · **L. Lagunes**[2] · **A. T. Imbiscuso**[4] · **M. N. Larrosa**[5] · **M. Lujàn**[6] · **J. Rello**[2,3,7]

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Abstract The purpose of this paper was to report the burden and characteristics of infection by multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA) in clinical samples from intensive care unit (ICU) adults, and to identify predictors. This was a retrospective observational study at four medical-surgical ICUs. The case cohort comprised adults with documented isolation of an MDR-PA strain from a clinical specimen during ICU stay. Multivariate analysis was performed to identify predictors for MDR-PA infection. During the study period, 5667 patients were admitted to the ICU and *P. aeruginosa* was isolated in 504 (8.8%). MDR-PA was identified in 142 clinical samples from 104 patients (20.6%); 62 (43.6%) of these samples appeared to be true infections. One hundred and eighteen (83.1%) isolates were susceptible only to amikacin and colistin, and 13 (9.2%) were susceptible only to colistin. Overall, the MIC_{50} to meropenem was 16 μg/mL and the MIC_{90} was >32 μg/mL, with 60.4% of respiratory samples being MIC >32 μg/mL to meropenem. Independent predictors for MDR-PA infection were fever/hypothermia [odds ratio (OR) 9.09], recent antipseudomonal cephalosporin therapy (OR 6.31), vasopressors at infection onset (OR 4.40), and PIRO (predisposition, infection, response, and organ dysfunction) score >2 (OR 2.06). This study provides novel information that may be of use for the clinical management of patients harboring MDR-PA and for the control of the spread of this organism.

Electronic supplementary material The online version of this article (doi:10.1007/s10096-016-2894-3) contains supplementary material, which is available to authorized users.

✉ B. Borgatta
bborgattab@gmail.com

1 Critical Care Department, Vall d'Hebron University Hospital, Pg Vall d'Hebron, 119–129, 08035 Barcelona, Spain

2 CRIPS, Vall d'Hebron Institute of Research (VHIR), Barcelona, Spain

3 Universitat Autònoma de Barcelona, Barcelona, Spain

4 Anesthesiology Department, Institut Hypnos, Hospital General de Catalunya, Barcelona, Spain

5 Microbiology Department, Vall d'Hebron University Hospital, Barcelona, Spain

6 Respiratory Medicine Department, Fundació Sanitària Parc Taulí, Sabadell, Spain

7 CIBERES, Madrid, Spain

Background

Multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA) has become a significant problem in the intensive care unit (ICU). A report by the Centers for Disease Control and Prevention (CDC) published in 2013 classified it as a "serious antibiotic resistant threat" [1], estimating that 6700 patients were affected by MDR-PA (resistant >3 antibiotics) in the USA in 2013 and that around 440 had died as a result. *Pseudomonas aeruginosa* possesses an impressive ability to develop antibiotic resistance; it represents a serious challenge in the management of infections in the ICU, especially since MDR-PA pneumonia is associated with higher morbidity than infection by susceptible strains [2]. In the last decade, there has been a rise in MDR-PA strains, especially in the ICU setting [3–5]. ICU patients usually have concomitant problems and elevated inflammatory markers that may be due to either infectious or non-infectious causes. Consequently, differentiating between colonization and infection in clinical samples in this setting can be challenging, and failure to do so effectively may result in unnecessary antibiotic treatment, with the detrimental consequences that this entails. Factors associated with the isolation of MDR-PA have been studied in hospitalized patients,

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but the current guidelines are not reliable predictors of infection in the ICU [2, 6, 7].

Thus, the main objective of this study was to identify predictors of MDR-PA infection in ICU patients with positive clinical samples. A secondary objective was to take a snapshot of the characteristics of clinical samples with MDR-PA isolates. Outcome considerations are far from the objectives of this study. Our hypothesis was that risk factors for MDR-PA differ in patients who develop infection and in patients who are only colonized.

Methods

Patients and study design

A retrospective cohort study was carried out at four general ICUs (36 beds) of a major tertiary teaching hospital in Barcelona, Spain (Vall d'Hebron University Hospital). These ICUs were not used as recovery rooms (except for transplant) and burns, cardiac surgery, cardiology, and head trauma patients were admitted to other ICUs. We included all clinical samples in which MDR-PA was isolated for the first time in the ICU between January 2010 and April 2015, taken from all adult patients consecutively admitted to these ICUs with suspected infection (clinical samples) and without previous isolation of MDR-PA during the current admission. Surveillance samples (rectal, skin, and nasopharyngeal swabs) and patients with prior MDR-PA isolation during the current admission were excluded. Given that standard ICU care includes the performance of multiple and simultaneous cultures when infection is suspected, all clinical samples retrieved simultaneously from the same patient were included and recognized as one case. Epidemiological, clinical, and microbiological data were recorded and each case was then classified as either infection or colonization. Patients' records were reviewed up to death or ICU discharge. Further isolations were recorded and included only if they were infections. The study was approved by the local institutional review board, and the need for written consent was waived due to the observational nature of the study.

Variables included were demographic data, comorbidities, and risk factors for MDR-PA isolation (diabetes mellitus, chronic kidney disease, chronic obstructive pulmonary disease, cystic fibrosis, immunosuppressive state, malignancy, neurological sequel, and pressure ulcers) [5]. Baseline comorbidity was assessed with Charlson's comorbidity score [8]. Severity scores were recorded at admission (APACHE II [9] and SOFA [10]) and at the time of sample collection (SOFA and PIRO). The PIRO (predisposition, infection, response, and organ dysfunction) is a severity score that allows the prediction of the mortality risk in patients with ventilator-associated pneumonia (VAP) [11]. ΔSOFA was defined as SOFA score at culture minus SOFA score at admission, in order to assess the patient's progress at the time of the culture. Clinical, microbiological, and laboratory data related to infection status, severity of illness, and the appropriateness of initial antibiotic therapy were also recorded.

ICU characteristics and infection control

Patients were admitted to four independent units on two different floors. Two beds in five closed rooms were available in each ICU, which each admitted eight patients. Overall, the nurse to patient ratio was 1:2. Infection control measures were standardized, and included the standard precautions applied in all ICU admissions and contact precautions in patients known to be colonized by methicillin-resistant *Staphylococcus aureus* (MRSA) or MDR Gram-negative agents. Surveillance cultures were limited to transfers from other centers, patients colonized in prior admissions, or in the presence of potential outbreaks. Patients with extended-spectrum beta-lactamase or carbapanemase-producing Enterobacteriaceae, MDR-PA, *Acinetobacter baumannii*, resistant *Stenotrophomonas maltophilia*, MRSA, and *Clostridium difficile* were maintained alone in the ICU rooms. Hand washing with 2% chlorhexidine was performed with dispensers at each room gate. Overall compliance with hand washing (assessed by blinded audits) was estimated to be 70.5% over the study period. All patients used disposable bedpans. Selective digestive decontamination (SDD) was performed in all patients with mechanical ventilation (MV) and comprised administration every 6 h of an antibiotic solution containing tobramycin 0.8% and colistin 1% through a nasogastric tube and topical oral paste containing tobramycin 2%, colistin 2%, amphotericin B 2%, and vancomycin 4%.

Microbiology

Pseudomonas aeruginosa was isolated from respiratory samples (sputum, endotracheal aspirate, and bronchoalveolar lavage), blood, urine, cerebrospinal fluid (CSF), peritoneal fluid, catheter tips, surgical wounds, and ear exudate. The microbiology laboratory identified MDR-PA using the VITEK MS automated system (bioMérieux, Marcy l'Etoile, France). Antimicrobial susceptibility of isolates was tested using disk diffusion, and resistant strains were checked using the gradient diffusion method. Minimum inhibitory concentrations (MICs) were classified using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [12]. MDR was defined as non-susceptibility to at least one agent in ≥3 antimicrobial categories, and extensive drug resistance (XDR) as non-susceptibility to at least one agent in all but ≤2 antimicrobial categories [13]. Inadequate initial antibiotic treatment was defined as the absence of any antibiotic with in vitro susceptibility administered at the adequate dose [14].

Definitions

- *Previous P. aeruginosa colonization*:

– At least two positive samples for *P. aeruginosa* within the 6 months prior to current admission, OR
– One positive sample for *P. aeruginosa* during current admission (minimum of 14 days earlier).

- *Index isolate*: first isolation of MDR-PA in ICU (either infection or colonization). It could include isolates from more than one sample site taken simultaneously.
- *Infection*: specimens associated with clinical signs of inflammation (fever, alteration in WBC, presence of purulence) and categorized as:

– Respiratory: Ventilator-associated pneumonia (VAP) and hospital-acquired pneumonia (HAP) were diagnosed according to American Thoracic Society (ATS)/Infectious Diseases Society of America (IDSA) 2005 guidelines [15]. Ventilator-associated tracheobronchitis (VAT) was defined according to Craven et al. [16].
– Non-respiratory: Defined following International Sepsis Forum consensus definitions [17].
– Bloodstream: Blood culture isolates.

- *Superinfections*: MDR-PA positive sample occurring 14 days after a case, in addition to meeting the infection criteria described above.
- *Colonization*: MDR-PA positive specimens that did not fulfil the infection criteria.
- Shock: New presence of sustained hypotension and/or vasopressor initiation (noradrenaline) or increase of ≥20% in less than 24 h.

Statistical analysis

Continuous variables were tested with the Kolmogorov–Smirnov test to assess deviations from normality. Discrete variables were summarized as frequency (%) and continuous variables as mean and standard deviation (SD) or median and interquartile range (IQR). Univariate analysis was performed using Pearson's Chi^2, two-tailed Fisher's exact test, or the Mann–Whitney *U*-test, as appropriate. Multivariate logistic regression analysis was used to determine predictors for MDR-PA infection/colonization, and variables were included if they were statistically significant in the univariate analysis and/or if they were considered clinically relevant according to current knowledge with a $p \leq 0.10$ level of significance. A logistic regression analysis with the stepwise forward method was applied to identify association with infections among the MDR-PA isolates. Odds ratios (ORs) are presented with 95% confidence intervals (CIs). Statistical analysis was performed using Stata for Mac version 13 (StataCorp LP, College Station, TX, USA) and SPSS for Mac version 18 (SPSS, Chicago, IL, USA). Statistical significance was set at $p < 0.05$.

Results

Clinical isolates: incidence and distribution

During the study period, 5667 patients were admitted to the ICU and *P. aeruginosa* was isolated in 504 (8.8%). A total of 142 (21%) MDR-PA clinical samples (from 104 patients) were identified, 20 (14.1%) in blood, 108 (76.1%) respiratory, and 54 (38%) non-respiratory. Bacteremia was secondary to respiratory, urinary, and catheter samples in 9, 4, and 3 isolates, respectively. Distribution between ICUs was balanced (data not shown). MDR-PA was isolated in more than one site (per case) in 32 cases (22.5%). Respiratory MDR-PA were mainly associated with MV [30 VAP: median onset 25.5 days (IQR: 19–32) and 26 VAT: median onset 20.5 days (IQR: 9–31)], 83.9% being associated with tracheotomy. Twenty-nine (43.3%) required vasopressors. Non-respiratory sites were the urinary tract in 34 cases (23.9%), abdomen in 10 (7%), catheter tip in 7 (4.9%), and other in 10 (7%). In 47.7% of these samples, another microorganism was also isolated; 11 (25%) were MDR. Enterobacteriaceae were the most common concomitant microorganisms isolated (38.6%), followed by *Enterococcus* spp. (27.3%), other Gram-positive cocci (18.2%), and yeasts (13.6%). Eleven (7.8%) isolates were MDR, of which seven were isolated from the same MDR-PA sample.

Index isolates appeared to be true infections in 62 samples (43.6%). Twenty-four (23.1%) of these patients developed subsequent infections. Indeed, a total of 100 infections were identified in 72 patients (69.2%). A group flow diagram of patients and cases is shown in Fig. 1.

Demographics

The patient population was predominantly male (72/104, 69.2%), with a median age of 59 years (IQR: 48–69) and low baseline comorbidities (median Charlson's index: 2, IQR: 1–3.5), but high severity at ICU admission (median APACHE II: 22.5, IQR: 17–28). At the time the samples were obtained, the median SOFA score was 6 (IQR: 3–8), similar to the score at admission (median ΔSOFA 0, IQR: −2–2).

The median pre-ICU ward stay was 1 day (IQR: 0–16) and the median ICU stay was 15.5 days (IQR: 3–34) before the index isolate. Of the 104 patients with MDR-PA, 22 were admitted from the emergency department (21%), 19 from

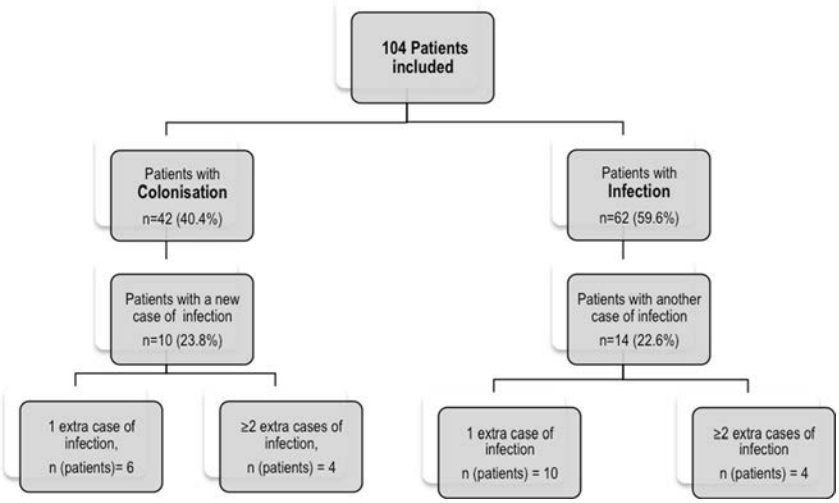

Fig. 1 Group flow diagram of patients and cases of multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA). One hundred and six patients were included: 62 were classified as infected and 44 as colonized. Twenty-five patients had infection after the first isolation: 12 were previously colonized and 13 were previously infected. Patients had more than one infection following the first isolation, resulting in a total of 100 infections in 74 patients

the surgical theater (18.3%), eight from the recovery room (7.7%), and 36 from hospitalization wards (34.6%). Nineteen were referred from another hospital (18.3%). Sixty-seven were medical admissions, 15 had undergone thoracic surgery, three cardiac surgery, 14 abdominal surgery, and two neurosurgery; none had trauma and three were miscellaneous. Regarding comorbidities, immunosuppression was present in almost half of the cohort (51, 49%), of whom 25 had undergone solid organ transplant (17 lung/4 liver/3 kidney/1 hepatorenal) and two bone marrow transplant. Moreover, 32 (30.8%) had chronic lung disease; 22 (21.2%) had either chronic obstructive pulmonary disease (COPD) or cystic fibrosis (CF) and 23 (22.1%) had chronic heart disease. Patients' baseline characteristics and risk factors are detailed in Table 1. When clinical samples were retrieved, 121 patients (85.2%) underwent MV, 102 (71.8%) with a tracheostomy, and 54 (38.3%) with parenteral nutrition.

Prior antibiotic exposure

Most MDR-PA isolates (118, 83.1%) were susceptible only to amikacin and colistin, and 13 (9.2%) were extensively multidrug-resistant (susceptible only to colistin). Overall, the MIC_{50} to meropenem was 16 μg/mL and MIC_{90} was >32 μg/mL, with 60.4% of respiratory samples being MIC >32 μg/mL to meropenem. Figures for prior antibiotic exposure were as follows: 36.5% for carbapenems, 18.3% for quinolones, 58.7% for penicillins, and 38.5% for cephalosporins (cefepime 9.7% and ceftazidime 13.6%). Only 13.5% had prior aminoglycoside exposure. Overall, prior antipseudomonal exposure was 77.9%. Additionally, 80.8% of patients had prior exposure to colistin due to SDD. Previous antibiotic exposure to antipseudomonal cephalosporins was significantly associated with infection [OR 3.88 (95% CI: 1.21–12.49)], which, in turn, was associated with cefepime exposure (10 vs. 0 patients, $p < 0.01$) but not with ceftazidime.

MDR-PA infection

In total, 100 infections were recorded, of which 67 (67%) were respiratory and 33 (33%) were non-respiratory. In the order of frequency, we observed: 30 VAP, 26 VAT, 18 urinary infections, 11 HAP, seven mixed infection (including abdominal), and three catheter infections (see figure in the electronic supplementary material). Initial antibiotic therapy was inadequate in 78 of 100 infections, and was more frequent (80.7% in the first infection vs. 73.7% in secondary infections, $p = 0.4$). The mean time to adequate antibiotic therapy was 1.3 days (±1.7 SD) and was significantly longer in the first infection (1.9 ± 1.9 vs. 0.7 ± 1.3, days ± SD, $p < 0.01$). The majority of demographic data did not differ between the infection and colonization subgroups. Only the Charlson's index was significantly higher in the infection group than in the colonization group among clinical isolates: median 3 (IQR: 1–4) vs. 1 (IQR: 0.3); $p < 0.05$. Variables at the time of sampling that were associated with infection are detailed in Table 2. In the univariate analyses, multiple sites of MDR-PA isolation were also significantly associated with infection (OR 8.57, 95% CI [1.95–37.78], $p < 0.01$).

Respiratory infections had lower rates of associated bloodstream infections than non-respiratory infections (13.4% vs. 33.3%, $p < 0.05$). However, HAP was significantly associated with bloodstream infection: 5 of 11 cases (45.5%) vs. 15 of 89 (16.9%) in the rest of the infections, $p < 0.01$. VAT was not associated with any positive blood cultures ($p < 0.05$). Among non-respiratory infections, the urinary tract was the most common site. Out of the 62 clinical isolates that appeared to be infections, 14 (22.6%) developed at least another infection during the ICU stay. The median time between infections was 23.5 days (IQR: 18–32). The median time between index colonization and subsequent infection was 15.5 days

Table 1 Univariate analysis of demographics and risk factors associated with multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA) infection and colonization

	All, 104	Infection, $n = 62$	Colonization, $n = 42$	Infection OR (95% CI), p
Age, years, median (IQR)	59 (48.5–68)	58.5 (49–66)	59.5 (47–70)	
Sex				
Male	72 (69.2%)	45 (72.6%)	27 (64.3%)	
Female	32 (30.8%)	17 (27.4%)	15 (35.7%)	
Charlson comorbidity index, median (IQR)	2 (1–3.5)	2.5 (1–4)**	2 (1–3)**	
Origin				
Ward	36 (34.6%)	24 (38.7%)	12 (28.6%)	
Emergency room	22 (21.2%)	16 (26.3%)	6 (13.9%)	
Operating theater	19 (18.3%)	11 (17.7%)	8 (19.1%)	
Other center	19 (18.3%)	7 (11.3%)*	12 (28.6%)*	0.32 (0.11–0.89), $p = 0.03$
Recovery room	8 (7.7%)	4 (6.5%)	4 (9.5%)	
Admission diagnosis				
Infectious	33 (31.7%)	20 (32.3%)	13 (30.9%)	
Respiratory	26 (25%)	18 (29%)	8 (19.1%)	
Neurological	16 (15.4%)	8 (12.9%)	8 (19.1%)	
Cardiovascular	11 (10.6%)	5 (8.1%)	6 (14.3%)	
Gastrointestinal	9 (8.7%)	6 (9.7%)	3 (7.1%)	
Oncohematologic	3 (2.9%)	2 (3.2%)	1 (2.4%)	
Other	5 (4.8%)	2 (3.2%)	3 (7.1%)	
Severity at admission				
APACHE II, median (IQR)	22.5 (17–28)	22.5 (17–28)	22.5 (17–29)	
SOFA at admission, median (IQR)	6 (4–8)	6 (4–9)	5.5 (3–7)	
Risk factors for MDR-PA isolation				
Comorbidities				
Diabetes mellitus	27 (25.9%)	16 (25.8%)	11 (26.2%)	
Neurological sequelae	7 (6.7%)	4 (6.5%)	3 (7.1%)	
Skin ulcers (chronic or previous pressure ulcers)	4 (3.8%)	3 (4.8%)	1 (2.4%)	
Chronic lung disease (not COPD/CF)	32 (30.8%)	20 (32.3%)	12 (28.6%)	
COPD or CF	22 (21.2%)	17 (27.4%)	5 (11.9%)	
Chronic renal disease	19 (18.3%)	9 (14.5%)	10 (23.8%)	
Chronic heart disease	23 (22.1%)	15 (24.2%)	8 (19.1%)	
Chronic liver disease	14 (13.5%)	9 (14.5%)	5 (11.9%)	
Immunocompromised	51 (49%)	35 (56.5%)	16 (38.1%)	
Malignancy (active)	19 (18.3%)	14 (22.6%)	5 (11.9%)	
Neutropenia	5 (4.8%)	4 (6.5%)	1 (2.4%)	
HIV/AIDS	5 (4.8%)	5 (8.1%)	0	
Solid organ transplant (previous and current)	25 (24%)	14 (22.6%)	11 (26.2%)	
Pharmacological (non-SOT)	8 (7.7%)	7 (11.3%)	1 (2.4%)	
Admission to hospital in the previous 90 days***	27 (27.6%)	15 (26.3%)	12 (29.3%)	
Admission to ICU facility in the previous 90 days	34 (32.7%)	20 (32.3%)	14 (33.3%)	
ICU admission >5 days	84 (80.8%)	53 (85.5%)	31 (73.8%)	
Pre-culture total LOS, median (IQR)	29.5 (11–45)	23 (11–43)	33 (15–59)	
Hospital LOS pre-ICU, days, median (IQR)	1 (0–16)	2 (0–13)	1 (0–17)	
ICU LOS pre-culture, days, median (IQR)	15.5 (3–34)	14.5 (5–33)	15.5 (1–35)	
Previous MV	77 (74%)	44 (70.9%)	33 (78.6%)	
MV pre-culture, days, median (IQR)	11.5 (0–35)	11 (0–35)	15 (5–38)	
Previous antibiotic (30 days)	101 (97.1%)	60 (98.4%)	41 (95.4%)	
With antipseudomonal activity	81 (77.9%)	47 (77.1%)	34 (79.1%)	
Antipseudomonal cephalosporin	22 (21.2%)	18 (29%)**	4 (9.5%)**	3.88 (1.21–12.49), $p = 0.02$
Previous CRRT	21 (20.2%)	13 (20.9%)	8 (19.1%)	
Previous colonization with *Pseudomonas* (non-MDR)	16 (15.4%)	10 (16.4%)	6 (13.9%)	

CF = cystic fibrosis;' *COPD* = chronic obstructive pulmonary disease; *CRRT* = continuous renal replacement therapy; *LOS* = length of stay; *MV* = mechanical ventilation; *OR* = odds ratio; *SOT* = solid organ transplant

*$p < 0.01$

**$p < 0.05$

(IQR: 10–25.5). Variables associated with developing multiple infections were: transfer from post-surgical reanimation unit (21.3% vs. 2.1%, $p < 0.05$) and Rh-negative type of blood (35.7% vs. 10.4%, $p < 0.05$).

Multivariate analysis

Variables introduced in the logistic regression analysis using the stepwise forward method were: Charlson index,

Table 2 Univariate analysis of clinical findings associated with MDR-PA infection and colonization

	All, 42	Infection, $n = 100$	Colonization, $n = 42$	Infection OR (95% CI)
Severity at the time of culture				
SOFA, median (IQR)	6 (3–8)	6 (3–9)	5 (3–7)	
ΔSOFA, median (IQR)	0 (−2–2)	0 (−2–3)	0 (−2–2)	
PIRO, median (IQR)	1.9 ± 1.4	2.4 ± 1.5*	1.2 ± 1*	
Risk factors at the time of culture				
Previous PA colonization	44 (30.9%)	38 (38%)*	6 (14.3%)*	3.84 (1.48–9.96)**
Proton pump inhibitors	142 (100%)	100 (100%)	42 (100%)	
Selective digestive decontamination	114 (80.3%)	76 (76%)**	38 (90.5%)**	0.32 (0.10–0.98)**
Parenteral nutrition	54 (38.3%)	43 (43%)	11 (26.8%)	
Mechanical ventilation	121 (85.2%)	83 (83%)	39 (90.7%)	
Tracheostomy	102 (71.8%)	73 (73%)	29 (69.1%)	
High-flow nasal cannulae	8 (5.6%)	5 (5.1%)	3 (6.9%)	
Arterial/venous catheter	141 (99.3%)	99 (99%)	42 (100%)	
Nasogastric tube	115 (80.9%)	78 (78%)	37 (88.1%)	
Vesical catheter	140 (98.6%)	98 (98%)	42 (100%)	
Other tube or drain	51 (35.9%)	35 (35%)	16 (38.1%)	
Pressure skin ulcers***	10 (7.3%)	7 (7.1%)	3 (7.7%)	
Clinical findings at the time of culture				
Temperature <36 or ≥38 °C	66 (46.8%)	59 (59%)*	7 (16.7%)*	6.19 (2.60–14.71)*
Leukocyte count <4 or ≥9.9 × 10E9/L	92 (66.7%)	74 (74%)*	18 (42.9%)*	3.52 (1.63–7.59)*
CRP (mg/dL), median (IQR)***	13.45 (6–24.6)	14.7 (6–27.5)	9.2 (4.9–23.7)	
Shock	51 (35.9%)	47 (47%)*	4 (9.5%)*	6.59 (2.39–18.16)*
Other source of SIRS/active infection***	26 (27.4%)	16 (21.9%)**	10 (45.5%)**	0.34 (0.12–0.92)**
Antibiotic susceptibility				
Susceptible to colistin and amikacin	118 (83.1%)	82 (82%)	36 (85.7%)	
Susceptible only to colistin	13 (9.2%)	9 (9%)	4 (9.5%)	
R ≥ 3 antipseudomonal families, other	11 (7.8%)	9 (9%)	2 (4.8%)	

CRP = C-reactive protein; *SIRS* = systemic inflammatory response syndrome

*$p < 0.01$

**$p < 0.05$

*** Missing data >5%

immunosuppression, COPD/CF, previous PA colonization, previous exposure to antipseudomonal cephalosporins, SDD, fever/hypothermia, vasopressors at infection onset, PIRO score >2 when sampling, and isolates from a non-respiratory sample. The model shows an association between MDR-PA infection and PIRO score >2 when sampling (OR 2.06), vasopressors at infection onset (OR 4.40), previous antipseudomonal cephalosporins (OR 6.31), and fever/hypothermia (OR 9.09) (Table 3). A similar model was identified when ICU stay over 2 weeks was entered into the model. Finally, no differences in the predictors between respiratory and non-respiratory episodes were identified.

Discussion

This study provides novel, potentially useful information for the clinical management of patients harboring MDR-PA and

Table 3 Multivariate analysis showing the predictors for MDR-PA infection from clinical samples

Independent variable	*p*-Value	OR (95% CI)	Hosmer and Lemeshow test
PIRO score >2 at sample	<0.05	2.06 (1.18–3.58)	$p = 0.196$
Vasopressors at infection onset	<0.05	4.40 (1.05–18.39)	
Previous antipseudomonal antibiotic	<0.01	6.31 (1.59–25.13)	
Fever/hypothermia	<0.01	9.09 (2.81–34.89)	

for the control of the spread of this organism. Our findings suggest that MDR-PA isolation in critically ill patients is not terminal; indeed, more than 50% of episodes occurred in patients younger than 50 years of age with a predicted survival of at least 10 years. Half of them were immunocompromised patients with multiple organ dysfunction at ICU admission, who presented MDR-PA isolation with a tracheostomy in situ and prolonged ICU stay. *Pseudomonas* clinical samples were obtained in 10% of our ICU patients, with MDR-PA representing 25% of isolates. MDR-PA was identified particularly in clinical samples from blood and the respiratory tract, and was associated with true infection in two-thirds of these isolates. More than 60% of MDR-PA were highly carbapenem-resistant, more than 80% were only susceptible to amikacin and colistin, and an additional 10% were susceptible only to colistin (an agent used in the SDD protocol in this ICU and the only antibiotic to which all of the strains were susceptible). Interestingly, prior exposure to antipseudomonal cephalosporins was associated with a 4-fold increased risk of MDR-PA infection, a finding which stresses the implications of antibiotic stewardship in ICU patients. Our predictive model identified different variables associated with infection or colonization among positive clinical samples. These findings may be of help in improving appropriate empiric antimicrobial prescriptions in the ICU.

A recent report [18] published the global epidemiology of *P. aeruginosa*, but specific studies of patients harboring MDR-PA remain scarce [19, 20] and great uncertainty exists regarding the management implications in specific ICU patients harboring MDR or extensively-resistant strains. Indeed, in our cohort, there were not many differences in the clinical samples harboring MDR-PA with regard to whether patients developed infection or merely remained colonized. Our predictive model identified different risk factors for samples associated with infection versus colonization, but not for colonization per se. The model identified PIRO score >2 at sampling, fever/hypothermia, shock, and previous exposure to antipseudomonal antibiotic as independent predictors of MDR-PA infection. These findings may help to increase the choice of appropriate empiric antibiotic prescription. The PIRO score is a severity assessment score for CAP and VAP, based on the PIRO concept [11, 21]. It is a simple, practical clinical tool for predicting ICU 28-day mortality. It allows an easy risk stratification of patients at different levels of severity with progressive rates of mortality, and it is associated with progressive healthcare resources utilization. The PIRO concept considers the predisposing conditions, the nature and extent of insult, the nature and magnitude of the host response, and the degree of concomitant organ dysfunction. So, taking into account the different features of infection with a pathophysiological focus; it is reasonable to be associated with respiratory as well with non-respiratory infection. Additionally, we found that patients coming from post-surgical recovery or with Rh-negative type of blood were more likely to present multiple infections, as reported elsewhere for other bacterial, viral, and parasitic infections [22–24].

The main limitation of this study is its single-center retrospective design, which means that the data should be treated with caution. Given the inherent limitations of current diagnostic definitions, a misclassification bias is possible (i.e., in the colonization group, there may be undiagnosed infections). However, misclassification is probably minimized, since all cases were assessed by the same investigator (BB) and since cultures were retrieved based on suspected infection and simultaneously from all possible infection sites, a design that reflects the reality of clinical practice and provides results that can be applied to bedside decision-making. In fact, this counts as a strength, since it lowers the probability of false-positive infections. The sample size is relatively small for the multivariate model and some variables, like duration of prior antibiotic exposure, were not recorded. Thus, further studies might identify additional variables. Finally, clonal analysis of MDR-PA was not performed; nevertheless, it is highly likely that these strains belonged to an epidemic high-risk clone (ST175, ST111, and ST235) currently reported in other hospitals in Barcelona [24, 25].

Conclusions

Multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA) infections represented two-thirds of clinical isolates of MDR-PA in the intensive care unit (ICU), with an incidence of 25.0 per 1000 hospitalized adults, in spite of high compliance with hand hygiene and infection control. The typical index case was a non-terminal adult who underwent 2 weeks of ventilation via a tracheostomy. The respiratory tract was the most common clinical site. Restriction of antipseudomonal cephalosporin use might help to reduce its incidence of MDR-PA. High levels of carbapenem resistance are common and worrisome for therapy. Thus, early use of colistin and amikacin in patients at risk might help to reduce delays in providing effective treatment. Further research is warranted to assess the implications of MDR-PA for outcomes and identification of newer antimicrobial agents susceptible to these isolates.

Compliance with ethical standards

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Conflict of interest J.R. is on the speaker's bureau for Cubist, AztraZeneca, MedImmune, Kenta, Pfizer, Genentech, and Paratek. The other authors have no conflicts of interest to report.

Ethical approval The study was approved by the local institutional review board, with the identification "PR_AG_247-2012".

Informed consent Informed consent was waived due to the observational nature of the study.

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VII. MANUSCRIPT No.2:

The clinical significance of pneumonia in patients with respiratory specimens harbouring multidrug-resistant Pseudomonas aeruginosa: a 5-year retrospective study following 5667 patients in four general ICUs

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ORIGINAL ARTICLE

The clinical significance of pneumonia in patients with respiratory specimens harbouring multidrug-resistant *Pseudomonas aeruginosa*: a 5-year retrospective study following 5667 patients in four general ICUs

B. Borgatta[1,2,3] · S. Gattarello[2] · C. A. Mazo[1,2,3] · A. T. Imbiscuso[4] · M. N. Larrosa[5] · M. Lujàn[6] · J. Rello[2,3,7]

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Abstract *Pseudomonas aeruginosa* is the leading cause of pneumonia in intensive care units (ICUs), with multidrug-resistant (MDR) strains posing a serious threat. The aim of this study was to assess the clinical relevance of MDR *Pseudomonas* isolates in respiratory clinical specimens. A 5-year retrospective observational study in four medical-surgical ICUs from a referral hospital was carried out. Of 5667 adults admitted to the ICU, 69 had MDR-PA in respiratory samples: 31 were identified as having pneumonia (HAP/VAP): 21 ventilator-associated pneumonia (VAP) and ten hospital-acquired pneumonia (HAP). Twenty-one (67.7%) adults with MDR-PA HAP/VAP died after a median of 4 days (18 of the 21 deaths within 8 days), compared with one (2.6%) without pneumonia at day 8. In a Cox proportional regression model, MDR-PA pneumonia was an independent variable [adjusted hazard ratio (aHR) 5.92] associated with 30-day ICU mortality. Most strains (85.1%) were susceptible to amikacin and colistin. Resistance to beta-lactams (third-generation cephalosporins and piperacillin–tazobactam) ranged from 44.1% to 45.3%. Meropenem showed poor overall activity ($MIC_{[50/90]}$ 16/32 mg/dL), with 47.0% having a minimum inhibitory concentration (MIC) breakpoint >8 mg/L. Twenty-four (77.4%) HAP/VAP episodes received inappropriate empirical therapy. Although empirical combination therapy was associated with less inappropriate therapy than monotherapy (16.7% vs. 88.3%, $p < 0.01$), there was no difference in survival (30% vs. 33.3%, $p = 0.8$). Pneumonia was identified in one-third of adult ICU patients harbouring MDR-PA in respiratory clinical specimens. These patients have a 6-fold risk of (early) death compared to ventilator-associated tracheobronchitis (VAT) and respiratory colonisation. New antibiotics and adjuvant therapies are urgently needed to prevent and treat MDR-PA HAP/VAP.

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✉ B. Borgatta
bborgattab@gmail.com

1 Critical Care Department, Vall d'Hebron University Hospital, Pg Vall d'Hebron, 119–129, 08035 Barcelona, Spain

2 CRIPS, Vall d'Hebron Institute of Research (VHIR), Barcelona, Spain

3 Universitat Autònoma de Barcelona, Barcelona, Spain

4 Anesthesiology Department, Institut Hypnos, Hospital General de Catalunya, Barcelona, Spain

5 Microbiology Department, Vall d'Hebron University Hospital, Barcelona, Spain

6 Respiratory Medicine Department, Fundació Sanitària Parc Taulí, Sabadell, Spain

7 CIBERES, Madrid, Spain

Background

The spread of *Pseudomonas aeruginosa* (PA) multidrug-resistant (MDR) strains over the last decade is a matter of profound concern in the critically ill setting [1, 2] and poses a serious threat. We already know that ventilator-associated pneumonia (VAP) is associated with increased days on mechanical ventilation (MV) [3], and that PA pneumonia is associated with poorer outcomes [4–7]. When compared to susceptible strains, resistant PA infections have increased mortality [8]. Furthermore, it has been shown that MDR-PA isolation and infection in the critically ill is associated with poor prognosis [2, 6, 8], though with conflicting results [9]. Despite extensive

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research, the spectrum of outcomes in patients with MDR-PA isolated in different respiratory infections in the intensive care unit (ICU) has not been studied to date. Isolates in respiratory specimens correspond to pneumonia, tracheobronchitis or colonisation, depending on the inflammatory response.

The aim of the study was to assess the clinical relevance of MDR *Pseudomonas* isolates in respiratory specimens. We hypothesised that MDR-PA respiratory infections would be associated with excess morbidity and mortality, and that specific predictors of mortality would emerge for this group of infections in the critically ill. Our research questions were: Is the risk of death due to pneumonia independent of other variables in a cohort of ICU patients harbouring MDR-PA in respiratory specimens? Is combination empirical therapy superior to monotherapy in a cohort of MDR *Pseudomonas* pneumonia? Finally, how should the presence of an MDR-PA clinical isolate in a respiratory specimen influence clinical practice?

Methods

Study design

We conducted a retrospective case–control study in four medical-surgical ICUs at Vall d'Hebron University Hospital, a major teaching hospital in Barcelona, Spain. The study population comprised all consecutive adult patients admitted to the ICU between January 2010 and April 2015. Selective digestive decontamination (SDD) with tobramycin and colistin was used in all intubated patients. Patients with MDR-PA isolation previous to ICU admission were excluded. Details of the global epidemiology have been reported elsewhere [10]. For the purposes of these analyses, only respiratory clinical samples harbouring MDR-PA were included. These samples were taken when infection was suspected and were requested by the attending physician. Samples associated with surveillance studies were excluded. In patients with multiple infections, only the last episode was retained. Patients with MDR-PA respiratory samples without evidence of pneumonia and without subsequent MDR-PA infection were included as controls, in accordance with the research questions. All computerised patient records were reviewed up to death or ICU discharge by a consultant intensive care physician (BB). Statistical analysis was performed in the pneumonia and control cohorts. To identify predictors of mortality among ICU adults harbouring MDR-PA in respiratory samples, a univariate analysis of survivors versus non-survivors was performed in patients with pneumonia (hospital-acquired pneumonia, HAP plus ventilator-associated pneumonia, VAP). The local institutional review board approved the study, and the need for written consent was waived due to its observational nature.

Variables included were demographic data, comorbidities and risk factors for MDR-PA isolation [6]. Baseline comorbidity was assessed with the Charlson comorbidity index [11]. Severity scores (APACHE-II [12] and SOFA [13]) were recorded at admission and at the time of sample collection (SOFA and PIRO [14]). Antibiotic exposure in the 30 days prior to culture was recorded. Clinical, microbiological and laboratory data associated with infection state, severity of illness and the appropriateness of initial antibiotic therapy were also collected. Epidemiological, clinical and microbiological data were recorded for each patient. The outcomes analysed were attributable 30-day ICU mortality, excess ICU length of stay (LOS) and excess MV days. Complications included shock, moderate-severe hypoxaemia, acute respiratory distress syndrome (ARDS) and acute kidney injury (AKI), which were recorded if the onset was within a time frame of ±6 days of culture.

Microbiology

Pseudomonas aeruginosa was isolated from respiratory samples (sputum, endotracheal aspirate and bronchoalveolar lavage) and blood. MDR-PA strains were identified in the microbiology laboratory using the VITEK MS automated system (bioMérieux, Marcy l'Etoile, France). The antimicrobial susceptibility of isolates was tested using disk diffusion, and resistant strains were verified using the gradient diffusion method. Minimum inhibitory concentrations (MICs) were classified according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [15]. Multidrug resistance was defined as non-susceptibility to at least one agent in ≥3 antimicrobial categories, and extensively drug-resistant (XDR) as non-susceptibility to at least one agent in all but ≤2 antimicrobial categories [16]. Adequate initial antibiotic treatment was defined, regardless of lung penetration, as in vitro susceptibility to at least one antibiotic administered at adequate dose [17]. Breakpoints of resistance were >8 mg/L for meropenem, imipenem, ceftazidime and cefepime and >16 mg/L for piperacillin–tazobactam and amikacin.

Definitions

- *Pneumonia cases*: patients who met VAP or HAP diagnostic criteria according to the 2005 ATS/IDSA guidelines [18].
- *Controls*: patients who met diagnostic criteria for ventilator-associated tracheobronchitis (VAT) and colonisation. VAT was diagnosed according to the definition proposed by Craven and Hjalmarson [19]. Colonisation was diagnosed in positive specimens with MDR-PA that did not meet the criteria for infection.
- *Shock*: new presence of sustained hypotension and/or initiation of vasopressors (noradrenaline) or increase of ≥20% in less than 24 h.

- *Moderate-severe hypoxaemia*: PaO_2/FiO_2 or $SpO_2/FiO_2 \leq 200$ only if FiO_2 was increased by ≥10% in less than 24 h.
- *ARDS*: according to the Berlin definition [20].
- *AKI*: according to the KDIGO criteria [21].
- *ΔSOFA*: SOFA at culture minus SOFA at admission, in order to assess the patient's evolution from admission to culture.
- *Crude mortality*: all deaths occurring during ICU stay.
- *Attributable 30-day ICU mortality*: the difference between observed 30-day ICU mortalities in the pneumonia and the control groups [22].

Statistical analysis

Continuous variables were checked with the Kolmogorov–Smirnov test to assess deviations from normality. Discrete variables were summarised as frequency (%) and continuous variables as median and interquartile range (IQR). Univariate analysis was performed using Pearson's Chi-squared test, two-tailed Fisher's exact test or the Mann–Whitney *U*-test, as appropriate. Analysis of variance (ANOVA) and the Kruskal–Wallis test were performed in variables with more than two categories. Kaplan–Meier curves were generated for the survival analysis in the different cohorts. A Cox proportional hazards model using the enter method was applied to analyse predictors of ICU mortality. Variables investigated as predictors of mortality were included if they reached statistical significance in the univariate analysis and if they were considered clinically relevant according to current knowledge with a *p*-value <0.10. Adjusted hazard ratios (aHRs) were expressed with 95% confidence intervals (CIs). Statistical analysis was performed using Stata for Mac version 13 (StataCorp LP, College Station, TX, USA) and SPSS for Mac version 18 (SPSS, Chicago, IL, USA). Statistical significance was set at $p < 0.05$.

Results

Patients

During the study period, 5667 patients were admitted to the ICU. Of the 69 adults with MDR-PA isolated in respiratory samples, 31 were identified as having pneumonia (21 VAP) and were compared with the remaining 38 patients (16 of whom had tracheobronchitis), who served as the control group without pneumonia. Bacteraemia was present in only 8 (25.8%) HAP/VAP patients. However, the 30-day mortality rates for HAP, VAP, colonisation and VAT were 90% (9/10), 57.1% (12/21), 22.7% (5/22) and 12.5% (2/16), respectively. Among these HAP/VAP patients, 7 (22.5%) had at least one previous MDR-PA infection and one-third (10, 32.3%) had previous PA colonisation. No differences were observed in the median Charlson scores between the pneumonia and non-pneumonia cohorts: 3 (IQR: 1 to 4) vs. 2 (IQR: 1 to 3), $p = 0.5$.

Patients with MDR-PA HAP/VAP were predominantly male (20; 64.5%), with a median age of 60 years (IQR: 48 to 68), a median APACHE-II at admission of 24 (IQR: 17 to 29) and a median ICU stay of 18 days (IQR: 1 to 41). There was no difference in ΔSOFA between groups: 0 (median, IQR: −1 to 4) in HAP/VAP vs. 0 (median, IQR: −2 to 2) in controls ($p = 0.16$). Twenty-one were immunosuppressed (67.7%), of whom 11 (35.5%) were solid organ transplant patients and 7 (22.6%) had an active malignancy. Table 1 shows the patients' characteristics and compares controls vs. patients with HAP/VAP.

None of the ten HAP patients had 'do not resuscitate' orders. Their primary diagnoses were respiratory failure in six, (respiratory) septic shock in three and urinary sepsis in one. Seven were immunocompromised (three solid organ transplant, two with active malignancy and two neutropaenic). Online Resource 3 compares the causes of immunosuppression between HAP, VAP and controls.

MDR-PA HAP/VAP was significantly associated ($p < 0.05$) with development of organ injury: shock ensued in 20 (64.5%), moderate-severe hypoxaemia also in 20 (64.5%), AKI in 17 (54.8%) and ARDS in 6 (19.4%). HAP was more severe, presenting with as many as 8 patients (80%) presenting at least one organ dysfunction, compared with almost 60% of VAP patients. See Table 2 for a detailed comparison of outcomes and complications between HAP, VAP, VAT and colonisation. The time from culture to organ injury development was very short; injury was usually present at onset and most injuries developed within 48 h (Fig. 1).

Mortality

In total, 28 out of 69 patients died (40.6%) and the overall 30-day mortality was 37.7% (26/69). HAP/VAP crude and 30-day mortality were the same, rising to 67.7% (21/31). The crude mortality of the control group was 18.4% (7/38) and the 30-day mortality was 13.2% (7/38). The estimated attributable 30-day ICU mortality for HAP/VAP was 54.5% (67.7% vs. 13.2%, $p < 0.01$). The median time to death was significantly shorter in the HAP/VAP group: 4 days [IQR: 3 to 8] vs. 17 days [IQR: 9 to 64] in the controls ($p < 0.01$). Within 8 days of infection, 85.7% (18/21) of deaths in the HAP/VAP group had already ensued and all deaths occurred by day 14. The comparison of time-to-ICU-mortality between HAP/VAP and controls is shown in Fig. 2. Online Resources 1 and 2 show the patients' characteristics and compare them between survivors and non-survivors.

Table 1 Demographic data and risk factors in patients with multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA) ventilator-associated pneumonia (VAP) and hospital-acquired pneumonia (HAP). Univariate analysis between the pneumonia group (HAP/VAP) and the control group (respiratory colonisation and ventilator-associated tracheobronchitis, VAT)

Demographics, epidemiological variables and risk factors	All, *n* = 69	Pneumonia (HAP/VAP), *n* = 31	Controls, *n* = 38
Age, years, median [IQR]	59 [49; 66]	60 [48; 68]	57.5 [49; 65]
Sex			
Male	47 (68.1%)	20 (64.5%)	27 (71.1%)
Female	22 (31.9%)	11 (35.5%)	11 (28.9%)
Charlson comorbidity index, median [IQR]	2 [1; 3]	3 [1; 4]	2 [1; 3]
Severity			
APACHE-II at admission, median [IQR]	22 [17; 28]	24 [17; 29]	22 [17; 27]
SOFA at admission, median [IQR]	5 [3; 8]	5 [4; 9]	5 [3; 7]
SOFA at culture, median [IQR]	6 [3; 10]	8 [5; 12]**	5 [3; 7]**
ΔSOFA, median [IQR]	0 [−2; 3]	0 [−1; 4]	0 [−2; 2]
PIRO at culture, median [IQR]	2 [1; 3]	3.2 [3; 4.2]*	1 [0; 2]*
Origin			
Ward	22 (31.9%)	12 (38.7%)	10 (26.3%)
Emergency room	17 (24.6%)	6 (19.4%)	11 (28.9%)
Operating theatre	12 (17.4%)	6 (19.4%)	6 (15.8%)
Recovery room	7 (10.1%)	3 (9.7%)	4 (10.5%)
Other centre	11 (15.9%)	4 (12.9%)	7 (18.4%)
Surgical patients	19 (32.2%)	12 (46.2%)	7 (18.4%)
ICU admission diagnosis			
Infectious	20 (28.9%)	12 (38.7%)	8 (21.1%)
Respiratory	19 (27.5%)	11 (35.5%)	8 (21.1%)
Neurological	10 (14.5%)	2 (6.5%)	8 (21.1%)
Gastrointestinal	7 (10.1%)	4 (12.9%)	3 (7.9%)
Cardiovascular	6 (8.7%)	0**	6 (15.8%)**
Other	4 (5.8%)	1 (3.2%)	3 (7.9%)
Oncological/haematological	3 (4.4%)	1 (3.2%)	2 (5.3%)
Comorbidities			
Immunocompromised	35 (50.7%)	21 (67.7%)*	14 (36.8%)*
Chronic lung disease (not COPD/CF)	25 (36.2%)	12 (38.7%)	13 (34.2%)
COPD or CF	16 (23.2%)	7 (22.6%)	9 (23.7%)
Diabetes mellitus	20 (28.9%)	9 (29%)	11 (28.9%)
Chronic heart disease	14 (20.3%)	5 (16.1%)	9 (23.7%)
Chronic renal disease	13 (18.8%)	5 (16.1%)	8 (21.1%)
Chronic liver disease	10 (14.5%)	4 (12.9%)	6 (15.8%)
Neurological squeal	5 (7.2%)	1 (3.3%)	4 (10.5%)
Skin ulcers (chronic or previous pressure ulcers)	4 (5.8%)	3 (9.7%)	1 (2.9%)
Current admission			
Hospital LOS pre-ICU, days, median [IQR]	1 [0; 12]	2 [0; 17]	0.5 [0; 8]
ICU LOS pre-culture, days, median [IQR]	23 [6; 45]	18 [1; 41]	25.5 [8; 58]
Previous PA colonisation	18 (26.1%)	10 (32.3%)	8 (21.1%)
Previous MDR-PA infection	14 (20.3%)	7 (22.6%)	7 (18.4%)
Previous mechanical ventilation	53 (76.8%)	20 (64.5%)*	33 (86.8%)*
Tracheostomy	50 (72.5%)	20 (64.5%)	30 (78.9%)
Previous RRT	14 (20.3%)	8 (25.8%)	6 (15.8%)
Parenteral nutrition	25 (36.2%)	15 (48.4%)	10 (26.3%)

CF = cystic fibrosis, COPD = chronic obstructive pulmonary disease, IQR = interquartile range, LOS = length of stay, RRT = renal replacement therapy

*$p < 0.01$

**$p < 0.05$

Other outcomes

Only ten patients with HAP/VAP were alive at the time of ICU discharge. Although the difference did not reach statistical significance, HAP/VAP survivors had triple the post-culture duration of mechanical ventilation compared to control survivors, 20.5 days [median, IQR: 5 to 46] vs. 7.5 days [median, IQR: 3 to 22], $p = 0.13$, and double the post-culture ICU stay, 27 days [median, IQR: 13 to 55] vs. 15 days [median, IQR: 10 to 33], $p = 0.31$ (see Table 2 for detailed outcomes in each group).

Predictors of ICU 30-day mortality

A Cox proportional regression model with the enter method was performed in all patients, using SOFA at culture, immunosuppression, VAT, pneumonia, shock and inadequate initial antibiotic therapy (IIAT) as independent variables. The model identified only MDR-PA pneumonia (HAP + VAP) as independently associated with ICU mortality, with an aHR of death of 5.92 (95% CI 1.19–29.57). See Table 3.

Table 2 Outcomes in patients with MDR-PA VAP, HAP and VAT compared to controls

Outcomes	All, *n* = 69	Respiratory colonisation, *n* = 22	VAT, *n* = 16	HAP, *n* = 10	VAP, *n* = 21	*p*-Value
Complications						
Shock	25 (36.8%)	4 (18.2%)	1 (6.67%)	8 (80.0%)	12 (57.1%)	<0.01
Moderate-severe hypoxaemia	27 (39.1%)	6 (27.3%)	1 (6.25%)	8 (80.0%)	12 (57.1%)	<0.01
ARDS	6 (8.7%)	0	0	2 (20.0%)	4 (19.0%)	<0.05
AKI	22 (31.9%)	5 (22.7%)	0	8 (80.0%)	9 (42.9%)	<0.01
MV post-culture, days, median [IQR]	11 [3; 23.5]	7.5 [4.5; 16.5]	12 [2; 24]	–	19 [5; 33]	0.85
ICU LOS post-culture, days, median [IQR]	20 [11; 32]	15 [11; 26]	17.5 [10; 32]	–	25 [13; 43]	0.42

ICU length of stay and mechanical ventilation days analysis was performed only in survivors; HAP was not analysed since there was only one survivor

ARDS = acute respiratory distress syndrome, AKI = acute kidney injury, HAP = hospital-acquired pneumonia, LOS = length of stay, MV = mechanical ventilation, VAP = ventilator-associated pneumonia, VAT = ventilator-associated tracheobronchitis

Antibiotic exposure and susceptibility

Meropenem showed poor overall activity ($MIC_{[50/90]}$ 16/32 mg/L), with 47.0% having an MIC breakpoint >8 mg/L. Only 7 patients (22.6%) with HAP/VAP had prior exposure (during the prior 30 days) to carbapenems. The vast majority of isolates (85.1%) were susceptible only to amikacin and colistin, while 3 (6.4%) were XDR (susceptible only to colistin). Online Resource 4 shows the overall susceptibility of *P. aeruginosa* from ICU respiratory samples. Resistance to beta-lactams (third-generation cephalosporins and piperacillin–tazobactam) ranged from 44.1% to 45.3%. Indeed, differences between meropenem and anti-pseudomonal cephalosporins were lower than 3%. There were no differences in susceptibility between controls and HAP/VAP (see Online Resource 2).

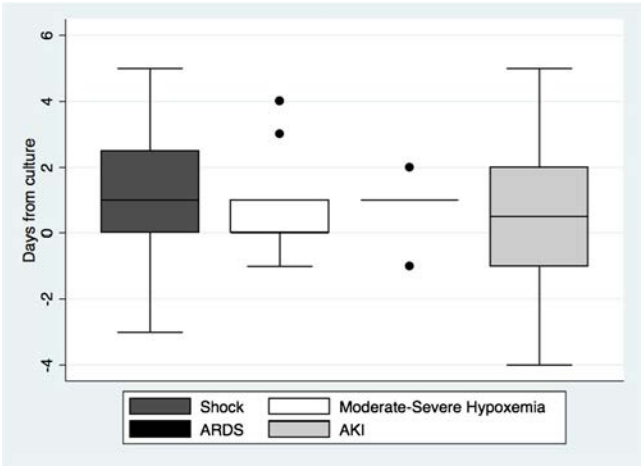

Fig. 1 Time from culture to organ injury development in patients with multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA) pneumonia (hospital-acquired pneumonia, HAP and ventilator-associated pneumonia, VAP). **a** Shock: 1 day (median, IQR: −1; 2.5); **b** moderate-severe hypoxaemia: 0 days (median, IQR: 0; 1); **c** acute respiratory distress syndrome (ARDS): 1 day (median, IQR: 1; 2); **d** acute kidney injury (AKI): 0.5 days (median, IQR: −1; 2)

Prior systemic exposure to amikacin and colistin was present in 1 (3.2%) and 6 (19.4%) patients, respectively, although all VAP patients had prior exposure to SDD with tobramycin and colistin. In addition, 13 (41.9%) and 5 (16.1%) patients with HAP/VAP had prior exposure to beta-lactams and quinolones, respectively.

As a consequence, 24 (77.4%) HAP/VAP episodes received inappropriate empirical therapy, which was not associated with mortality. The seven subjects who received a susceptible agent were treated with amikacin (five patients) and IV colistin (two patients). Interestingly, these patients with adequate empirical therapy were more ill at the time of culture than those with inappropriate empirical therapy (median SOFA score of 11 [IQR: 8 to 15] vs. 5 [IQR: 3 to 9], $p < 0.05$). Indeed, combination therapy was prescribed in 7/15 patients with SOFA score >8 and in 3/16 patients with SOFA score in the range 0–8. Moreover, no differences in severity scores at ICU admission or in ΔSOFA were seen between the groups.

Thirteen (41.9%) patients received empirical therapy with a beta-lactam, 11 (35.5%) with a carbapenem, 5 (16.1%) with amikacin and 2 (6.5%) with IV colistin. Quinolones were not used. Survival was 1 in 11 patients (9.1%) with carbapenems as empirical therapy and 9 in 20 patients (45%) without carbapenems ($p < 0.05$). Although empirical combination therapy was associated with less IIAT than monotherapy (16.7% vs. 88.3%, $p < 0.01$), there were no differences in survival (30% vs. 33.3%, $p = 0.8$). Details of antibiotic use are summarised in Table 4.

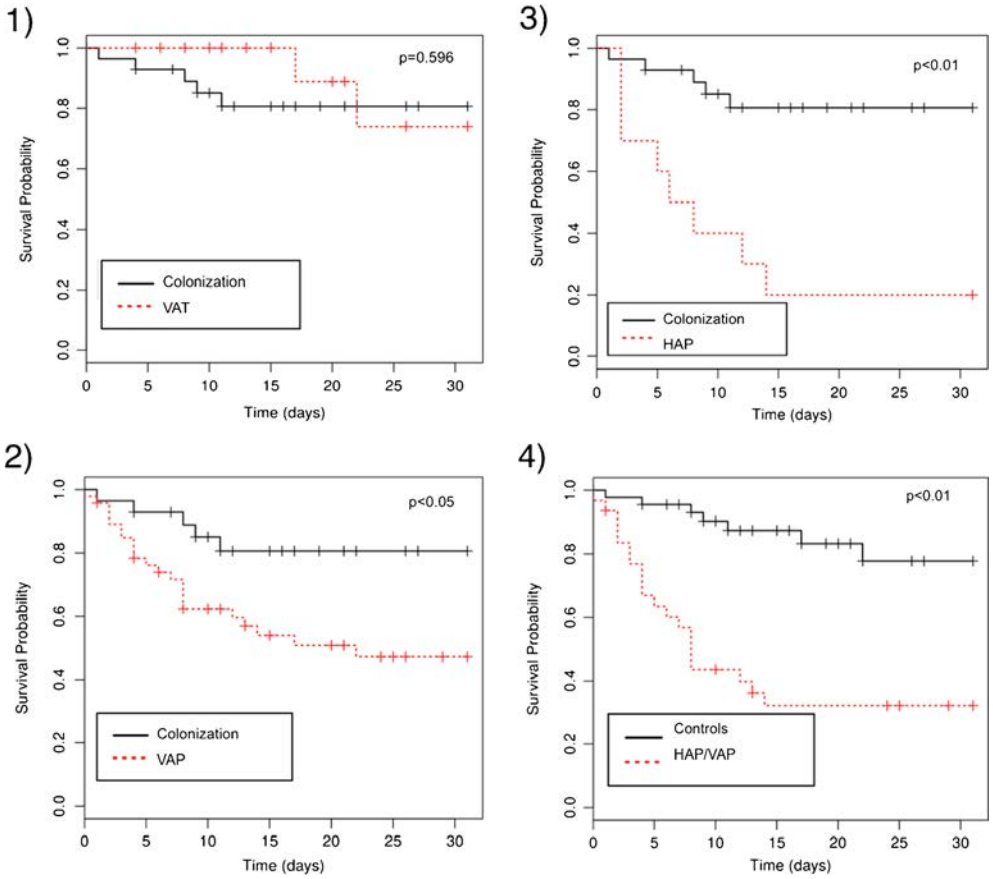

Fig. 2 Time-to-ICU-mortality compared between patients with MDR-PA respiratory infection or colonisation. Kaplan–Meier curves are shown and groups were compared using the log-rank test; statistical significance is expressed with *p*-values. Data were censored at 30 days after infection onset. (*1*) Time-to-ICU-mortality in patients with MDR-PA ventilator-associated tracheobronchitis (VAT) vs. respiratory colonisation. (*2*) Time-to-ICU-mortality in patients with MDR-PA VAP vs. respiratory colonisation. (*3*) Time-to-ICU-mortality in patients with MDR-PA HAP vs. respiratory colonisation. (*4*) Time-to-ICU-mortality in patients with MDR-PA pneumonia: HAP plus VAP vs. controls

Conclusions

MDR-PA HAP/VAP is associated with increased mortality. MDR-PA pneumonia was associated with a high percentage of IIAT due to the presence of very high resistance to meropenem. Death ensued within 8 days in at least 3 out of 4 patients. We emphasise that new antibiotics and therapies aimed to reduce infection, as well as adjuvant treatments, are urgently needed to deal with the challenge of MDR-PA HAP/VAP.

Table 3 Cox proportional hazards model for ICU mortality in patients with MDR-PA pneumonia (HAP + VAP, $n = 31$) and controls (respiratory colonisation + VAT, $n = 38$), sample total $n = 69$

Risk factors	aHR	95% CI	*p*-Value
SOFA at culture	0.96	0.81–1.14	0.67
Immunosuppression	1.78	0.67–4.74	0.25
VAT	0.79	0.11–5.84	0.81
Pneumonia (HAP + VAP)	5.92	1.19–29.57	<0.05
Shock	2.1	0.59–7.54	0.26
Inadequate empirical therapy	1.56	0.36–6.79	0.55

aHR = adjusted hazard ratio

Discussion

This study is the first comprehensive assessment of the clinical implications of pneumonia in adult ICU patients harbouring MDR-PA in respiratory isolates. Most strains were only susceptible to colistin and amikacin, and the MIC for meropenem is reported. Our results suggest that HAP/VAP is associated with extremely high, rapid mortality and alarming morbidity rates. In contrast, a low and similar 30-day mortality and outcomes was seen in VAT and in respiratory colonisation. After adjusting for known risk factors for increased mortality and comparing with our controls, we identified MDR-PA pneumonia as a predictor of 30-day ICU mortality.

Patients with purulent respiratory secretions can be characterised as being colonised, having tracheobronchitis or having pneumonia, depending on the inflammatory response, biomarkers and alternative collected specimens. The triggers of pneumonia in patients harbouring an organism in the respiratory tract are unknown. We were not surprised to find associations between higher severity, longer MV or more immunosuppression and a higher incidence of pneumonia; this is common in opportunistic organisms like *P. aeruginosa*. Our findings also show that pneumonia was associated with more complications than non-pneumonia. In contrast with what happens with other MDR organisms (e.g. *Acinetobacter*

Table 4 Details of antibiotic therapy administered in patients with MDR-PA pneumonia (HAP/VAP)

Antimicrobial agent	HAP, *n* = 10		VAP, *n* = 21	
	Empirical	Directed	Empirical	Directed
Piperacillin–tazobactam	3	0	2	0
Cephalosporins*	0	3	2	3
Carbapenems*	6	4	5	3
Amikacin	4	4	1	4
Colistin	0	2	2	4
Quinolones	0	0	0	0

*Includes only agents with anti-*P. aeruginosa* spectrum. Some patients received multiple antibiotics

baumannii) in which death seems to be a terminal event, MDR-PA was associated with a rapid (median 4 days) fatal outcome. In addition, our data showing that VAT is closer to colonisation than VAP represents an addition to the literature.

A novel method [22] was used to estimate attributable mortality, which compares patients with MDR-PA pneumonia and patients with MDR-PA respiratory colonisation or VAT. This approach allows the analysis of patients with MDR-PA pneumonia with a real matched and comparable cohort, and, thus, reduces bias. Indeed, the results of the outcomes (in Table 2) and the Kaplan–Meier analysis in the two groups show that VAT and respiratory colonisation have similar effects on patient mortality and complications, providing support for our methodology. We estimated an attributable mortality for MDR-PA HAP/VAP of 54.5%, whereas the figures for susceptible strains reported in previous studies ranged between 13% and 18% [4–6].

It should be noted that 68% of the pneumonia cohort were immunosuppressed, which may explain the elevated mortality. However, the Cox regression model did not identify immunosuppression as an independent predictor of mortality. Although previous studies have associated MDR-PA pneumonia and VAT with increased LOS [13, 23], we were unable to validate these findings, even though LOS and MV days were both increased. We believe that our results failed to reach statistical significance due to a type II error (i.e. only ten patients with MDR-PA HAP/VAP survived). The severity of the MDR-PA pneumonia was another striking finding: two-thirds of MDR-PA pneumonia patients had at least one organ injury, a proportion that increased to 80% in HAP. Similarly, 20% of both HAP and VAP patients developed ARDS.

Our findings illustrate the complexity of MDR-PA pneumonia, with different implications for outcomes compared with other non-fermentative organisms. Remarkably, a low percentage of meropenem-resistant strains were previously exposed to carbapenems. In contrast, all ventilated patients received SDD with colistin and amikacin, with susceptibility to these agents remaining above 85%. With a difference of resistance lower than 3% with cephalosporins and piperacillin–tazobactam, in an area with low prevalence of *Klebsiella pneumoniae* carbapenemase (KPC) and high prevalence of extended-spectrum beta-lactamase (ESBL), this raises concerns about the appropriateness of de-escalating to beta-lactams as a streamlining strategy. Our findings demonstrate how challenging it is to treat infections caused by MDR organisms and indirectly suggest that infection control should be the cornerstone of prevention.

Interestingly, and contrary to previous evidence [13, 24], we did not find an association between inadequate therapy and increased mortality. Indeed, previous work has shown the most important predictor of mortality in patients with *P. aeruginosa* pneumonia to be illness severity [14], which was included as an adjusting variable to the Cox model. Moreover, we found that the inadequate therapy was due to the presence of high carbapenem resistance in the MDR strains. In contrast with non-MDR *Pseudomonas* pneumonia where initial combination therapy has presented superior survival [25], our cases had high MICs to carbapenems. A recent multicentre study testing ceftolozane/tazobactam as a treatment for carbapenem-resistant *P. aeruginosa* infections supports this [26]. It demonstrated effectiveness in 3 out 4 cases, except when isolates had MICs >8 mg/L, where 100% treatment failure was observed. Similarly, ward patients with positive cultures for meropenem-resistant *P. aeruginosa* had increased mortality, increased ICU admission and increased healthcare costs [27]. This problem is further complicated by the fact that the therapy that is currently considered as 'appropriate', systemic administration of amikacin or colistin as monotherapy, has been proved to be suboptimal in clinical practice due to limited lung penetration.

Our findings also have important practical considerations. In contrast to carbapenem-resistant Enterobacteriaceae or *A. baumannii*, in which carbapenem still plays an important therapeutic role, MDR-PA pneumonia requires a different approach. Finally, our findings in a cohort with extensive carbapenem resistance do not support the recommendation in the 2016 IDSA/ATS guidelines [28] to treat *Pseudomonas* pneumonia with combination therapy. Alternative agents are urgently needed.

The main limitations of the present study are the small sample size and its single-centre retrospective design, which means that the data should be treated with caution because the study is susceptible to an attrition bias. Similarly, given the inherent limitations of the current definitions, a misclassification bias is possible (i.e. there may have been undiagnosed infections in the colonisation group). However, the risk of bias is limitated, since the same investigator assessed all cases and the results are clinically consistent. The decision to consider HAP (*n* = 10) and VAP (*n* = 21) together might also be questioned, even though HAP may be as severe as VAP. Similarly, mixing respiratory specimens associated with

colonisation and VAT is a debatable step. However, the outcomes are consistent, as reported in the Kaplan–Meier survival curves (Fig. 2) and outcomes (Table 2). Regulatory agencies and recent studies [29] on MDR *Pseudomonas* support this classification. Different variables might be identified if other control groups (such as susceptible strains) were used. Our findings concerned the clinical significance of pneumonia in patients with respiratory specimens harbouring MDR-PA. Thus, assessing prognostic factors in a general cohort or when compared with susceptible strains or other organisms would require a different control group.

In summary, it has been demonstrated that MDR-PA HAP/VAP is an extremely severe entity associated with very high mortality and without any modifiable variables that might improve it. This is a matter of particular concern, given that *P. aeruginosa* is one of the main causes of respiratory infections in the ICU setting, the rising resistance rates worldwide and the lack of new antibiotics to effectively control and cure this infection. Efforts should be directed toward finding new effective antibiotics, but also, and probably more importantly, toward developing therapies that reduce the colonisation and infection by MDR-PA and adjuvant treatments that reduce its virulence.

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Compliance with ethical standards

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Conflict of interest J.R. is on the speaker bureau for Cubist, AstraZeneca, MedImmune, Kenta, Pfizer, Genentech and Paratek. The other authors have no conflicts of interest to report.

Ethical approval The study was approved by the local institutional review board, with the identification 'PR_AG_247-2012'.

Informed consent Informed consent was waived due to the observational nature of the study.

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VIII. DISCUSSION

This doctoral study provides novel, potentially useful information for the clinical management of patients harbouring MDR-PA and for the control of the spread of this organism. It is also the first comprehensive assessment of the clinical implications of pneumonia in adult ICU patients harbouring MDR-PA in respiratory isolates.

1. Epidemiological, Microbiological and Clinical Characteristics of Multidrug-resistant *P. aeruginosa* isolates

Our findings illustrate the complexity of MDR-PA pneumonia, with different implications for outcomes compared with other non-fermentative organisms. They suggest that MDR-PA isolation in critically ill patients is not terminal; indeed, above 50% of episodes occurred in patients younger than 50 with a predicted survival of at least 10 years. Also, that pneumonia is associated with alarming morbidity rates and extremely high, rapid mortality; whereas VAT is associated with a low morbidity and mortality, as seen in cases of respiratory colonisation. Finally, we identified MDR-PA pneumonia as a predictor of 30-day ICU mortality. Half of the cohort was immunocompromised, with multiple organ dysfunction at ICU admission, which presented MDR-PA isolation with a tracheostomy in situ and prolonged ICU stay. Pseudomonas clinical samples were obtained in 10% of our ICU patients, with MDR-PA representing 25% of isolates. MDR-PA was identified particularly in clinical samples from blood and respiratory tract, and was associated with true infection in two-thirds of these isolates.

Above 60% of MDR-PA were highly carbapenem-resistant, above 80% were only susceptible to amikacin and colistin (with a MIC breakpoint >8 mg/L) and an additional 10% were susceptible only to colistin (an agent used in the selective digestive decontamination [SDD] protocol in this ICU and the only antibiotic to which all of the strains were susceptible).

We were not surprised to find associations between higher severity, longer MV or more immunosuppression and a higher incidence of pneumonia; this is common in opportunistic organisms like as *Pseudomonas aeruginosa*. Our findings also show that pneumonia was associated with more complications than non-pneumonia. In contrast

with what happens with other MDR organisms (e.g., *Acinetobacter baumannii*) in which death seems to be a terminal event, MDR-PA was associated with a rapid (median 4 days) fatal outcome. In addition, our data showing that VAT is closer to colonisation than VAP represents an addition to the literature.

2. Implications in prevention, management and infection control

Prior exposure to anti-pseudomonal cephalosporins was associated with a 4-fold increased risk of MDR-PA infection, a finding that stresses the implications of antibiotic stewardship in ICU patients. Our predictive model identified different variables associated with infection or colonization among positive clinical samples. These findings may be of help in improving appropriate empiric antimicrobial prescriptions in the ICU.

A recent report published the global epidemiology of *P. aeruginosa* **[24]**, but specific studies of patients harbouring MDR-PA remain scarce and great uncertainty exists regarding the management implications in specific ICU patients harbouring MDR or extensively resistant strains. Indeed, in our cohort, there were not many differences in the clinical samples harbouring MDR-PA with regard to whether patients developed infection or merely remained colonized. Our predictive model identified different risk factors for samples associated with infection versus colonization, but not for colonization *per se*. The model identified PIRO score> 2 at sampling, fever/hypothermia, shock and previous exposure to antipseudomonal antibiotic as independent predictors of MDR-PA infection. These findings may help to increase the choice of appropriate empiric antibiotic prescription. The PIRO score is a severity assessment score for community-acquired pneumonia and VAP, based on the PIRO concept. It is a simple, practical clinical tool for predicting ICU 28-day mortality. It allows an easy risk stratification of patients in different levels of severity with progressive rates of mortality; and it is associated with progressive health-care resources utilization. The PIRO concept considers the predisposing conditions, the nature and extent of insult, the nature and magnitude of the host response, and the degree of concomitant organ dysfunction. So, taking into account the different features of infection with a pathophysiological focus; it is reasonable to be associated with

respiratory infection as well with non-respiratory. Additionally, we found that patients coming from post-surgical recovery or with Rh-negative type were more likely to present multiple infections, as reported elsewhere for other bacterial, viral and parasitic infections.

Interestingly and contrary to previous evidence**,** we did not find an association between inadequate therapy and increased mortality. Indeed, previous work has shown the most important predictor of mortality in patients with PA pneumonia to be illness severity, which was included as an adjusting variable to the Cox model. Moreover, we found that the inadequate therapy was due to the presence of high carbapenem resistance in the MDR strains. In contrast with non-MDR *Pseudomonas* pneumonia where initial combination therapy has presented superior survival, our cases had high MIC to carbapenems. A recent multicentre study testing ceftalozone/tazobactam as a treatment for carbapenem-resistant PA infections supports this **[25]**. It demonstrated effectiveness in 3 out 4 cases, except when in isolates had MIC >8 mg/L, where 100% treatment failure was observed. Similarly, ward patients with positive cultures for meropenem-resistant PA had increased mortality, increased ICU admission and increased health-care costs. This problem is further complicated by the fact that the therapy that is currently considered as "appropriate", systemic administration of amikacin or colistin as monotherapy has been proved to be suboptimal in clinical practice due to limited lung penetration.

Our findings also have important practical considerations. In contrast to carbapenem-resistant Enterobacteriaceae or *A. baumannii,* in which carbapenem still plays an important therapeutic role, MDR-PA pneumonia requires a different approach. A low percentage of meropenem-resistant strains were previously exposed to carbapenems. In contrast to all ventilated patients that received SDD with colistin and amikacin, with susceptibility to these agents remaining above 85%. With a difference of resistance lower than 3% with cephalosporins and piperacillin-tazobactam, in an area with low prevalence of *Klebsiella pneumoniae* carbapenemase (KPC) and high prevalence of extended-spectrum beta-lactamase (ESBL), this raises concerns about the appropriateness of de-escalating to betalactams as a streamlining strategy. Our findings demonstrate how challenging it is to treat infections caused by MDR organisms and indirectly suggest that infection control should be the cornerstone of prevention.

Finally, our findings in a cohort with extensive carbapenem-resistance do not support the recommendation in the 2016 IDSA/ATS guidelines to treat *Pseudomonas* pneumonia with combination therapy. Alternative agents are urgently needed.

3. Implications in research models

A novel method was used to estimate attributable mortality, which compares patients with MDR-PA pneumonia and patients with MDR-PA respiratory colonisation or VAT. This approach allows the analysis of patients with MDR-PA pneumonia with a real matched and comparable cohort, and thus reduces bias. Indeed, the results of the outcomes (in Table 2) and the Kaplan-Meier analysis in the two groups show that VAT and respiratory colonisation have similar effects on patient mortality and complications, providing support for our methodology. We estimated an attributable mortality for MDR-PA pneumonia of 54.5%, whereas the figures for susceptible strains reported in previous studies ranged between 13% and 18%.

The decision to consider HAP (n= 10) and VAP (n=21) together might also be questioned even though HAP may be as severe as VAP. Similarly, mixing respiratory specimens associated with colonisation and VAT is a debatable step. However, the outcomes are consistent, as reported in Kaplan-Meier survival curves (Figure 2) and outcomes (Table 2). Regulatory agencies and recent studies [**26**] on MDR *Pseudomonas* support this classification. Different variables might be identified if other control groups (such as susceptible strains) were used. Our findings concerned the clinical significance of pneumonia in patients with respiratory specimens harbouring multidrug-resistant *Pseudomonas aeruginosa.* Thus, assessing prognostic factors in a general cohort or when compared with susceptible strains or other organisms would require a different control group.

4. Limitations

The main limitations of the present study are the small sample size and its single-centre retrospective design, which means that the data should be treated with

caution because the study is susceptible to an attrition bias. Nevertheless, the sample is derived from a surveillance protocol and more than 5000 patients were screened during the study period. Similarly, given the inherent limitations of the current definitions, a misclassification bias is possible (i.e., there may have been undiagnosed infections in the colonisation group). However, the risk of bias is limited since the same investigator assessed all cases and the results are clinically consistent. Also, specimens were retrieved based on suspected infection and simultaneously from all possible infection sites, a design that reflects the reality of clinical practice and provides results that can be applied to bedside decision-making. In fact, this counts as a strength since it lowers the probability of false positive infections. Even though clonal analysis of MDR-PA was not performed; it is highly likely that these strains belonged to an epidemic high-risk clone (ST175, ST111 and ST235) currently reported in other hospitals in Barcelona **[27].**

IX. CONCLUSIONS

1. The typical index case was a non-terminal adult who underwent two weeks of ventilation via a tracheostomy. Half of the cohort was immunocompromised, one-third with chronic lung disease, on top of multiple organ dysfunction at ICU admission.
2. MDR-PA infections represented two-thirds of clinical isolates of MDR-PA in the ICU with an incidence of 25.0 per 1,000 hospitalized adults, in spite of high compliance with hand hygiene and infection control. The respiratory tract was the most common clinical site of isolation.
3. On a logistic regression analysis we identified independent predictors for MDR-PA infection: fever/hypothermia (OR 6.65), PIRO score>2 (OR 5.81), vasopressors at infection onset (OR 5.89) and recent antipseudomonal cephalosporin therapy (OR 5.71). So, restriction of anti-pseudomonal cephalosporin use might help reduce MDR-PA isolation in ICU.
4. MDR-PA pneumonia was identified in one-third of adult ICU patients harbouring MDR-PA in respiratory clinical specimens. Pneumonia was associated with alarming morbi-mortality. Shock, moderate-severe hypoxemia and/or AKI ensued in two-thirds of cases, and ARDS in 19.4%. MDR-PA pneumonia 30-day mortality was 67.7% and the estimated attributable-mortality 54.5%. More than two-thirds of adults with MDR-PA pneumonia died after a median of 4 days and by 14 days all deaths had ensued.
5. A Cox proportional regression model identified MDR-PA pneumonia as a predictor of 30-day ICU mortality (aHR 5.92). New antibiotics and adjuvant therapies are urgently needed to prevent and treat MDR-PA pneumonia.

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XI. APPENDIX No.1. Review Article

Risk factors for Pseudomonas aeruginosa pneumonia in the early twenty-first century.

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Jordi Rello
Bárbara Borgatta
Thiago Lisboa

Risk factors for *Pseudomonas aeruginosa* pneumonia in the early twenty-first century

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J. Rello (✉) · B. Borgatta
Critical Care Department, Hospital Vall d'Hebron, Barcelona, Spain
e-mail: jrello@crips.es

B. Borgatta
e-mail: bborgattab@gmail.com

J. Rello
CIBERES, Barcelona, Spain

J. Rello · B. Borgatta
Vall d'Hebron Institute of Research, Barcelona, Spain

J. Rello
Universitat Autonoma de Barcelona, Barcelona, Spain

T. Lisboa
Critical Care Department and Infection Control Committee, PPG Pneumologia, Hospital de Clinicas, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

T. Lisboa
Rede Institucional de Pesquisa e Inovação em Medicina Intensiva (RIPIMI), Complexo Hospitalar Santa Casa, Porto Alegre, Brazil

Abbreviations

COPD	Chronic obstructive pulmonary disease
ICU	Intensive care unit
MDR	Multidrug-resistant
OR	Odds ratio
PSA	*Pseudomonas aeruginosa*
RR	Relative risk
VAP	Ventilator-associated pneumonia

Introduction

Along with *Staphylococcus aureus*, *Pseudomonas aeruginosa* (PSA) heads the list of pathogens associated with Hospital-acquired pneumonia [1–3]. Indeed, even in patients with appropriate empiric antibiotic administration [3], the attributable mortality has been estimated to be 13.5 %. In survivors, PSA increases ICU length of stay and use of healthcare resources. In the early twenty-first century, the increase and dissemination of clones with progressive resistance are a cause of concern. In an international study of over 1,200 ICUs in 75 countries, the risk of infections, including those due to *Pseudomonas* species, was found to increase with duration of ICU stay; moreover, infection was also associated with an increased risk of mortality [1]. Therefore, understanding the epidemiology of this pathogen and identifying the risk factors the development of pneumonia should be a priority in research, and has obvious implications for infection control/prevention and therapy.

In 1994, a seminal article published in *Intensive Care Medicine* [4] reported that the risk of ventilator-associated pneumonia (VAP) due to PSA was increased in patients with chronic obstructive pulmonary (COPD) disease [relative risk (RR) 29.9], a mechanical ventilation period longer than 8 days (RR 8.1), and prior use of antibiotics (RR 5.5). The analysis addressed risk factors for PSA versus other types of VAP, but not for PSA VAP incidence per se. Because respiratory tract infections by this organism are associated with high mortality, empiric treatment of VAP episodes in this population must have a strong anti-pseudomonal activity until culture data become available [5]. Seventy-seven articles have referenced this manuscript over the past 20 years (Scopus, 15 May 2013), 32 of them reviews or guidelines. In the light of the current challenges posed by the increasing resistance to antibiotics among pathogens [6], we decided to

reassess the contribution of the *Intensive Care Medicine* study to PSA pneumonia [4].

Pseudomonas aeruginosa and VAP

The analysis by the French national nosocomial pneumonia surveillance group [7] reported an association between PSA and advanced age and length of mechanical ventilation, antibiotics at admission, transfer from a medical unit or ICU, and admission to a ward with a high incidence of patients with *Pseudomonas* infection. PSA was less frequent in trauma patients and in those admitted to a ward with high admission turnover.

The incidence of PSA VAP may differ in patients who are exposed to selective digestive decontamination (SDD) and those who are not [7]. The influence of specific risk factors in both cohorts has not been analyzed. However, the association between prolonged antibiotic exposure and PSA pneumonia is well confirmed [8]. Therefore, reducing the duration of antibiotic use should be promoted if feasible as a stewardship strategy that may reduce the risk of PSA pneumonia.

Pseudomonas aeruginosa has been reported to be one of the top three organisms causing respiratory infection which are resistant to carbapenems [9]. Independent risk factors associated with imipenem-resistance in PSA, *S. aureus*, *Acinetobacter baumannii*, or *Stenotrophomonas* pneumonia are previous use of a fluoroquinolone or aminoglycoside, use of invasive blood pressure monitoring (probably a surrogate for vasopressor use), and bilateral chest X-ray involvement.

Bonten et al. [10] assessed how risk factors for VAP identified in epidemiologic studies have provided a basis for testable interventions in randomized trials. Their study hypothesized that in hospital settings with low baseline levels of antibiotic resistance, approaches to prevent VAP may be different from those in place in settings with high levels of antibiotic resistance. Indeed, a recent report [11] evaluating prescriptions of antibiotics for pneumonia in 27 European ICUs confirmed that baseline incidence of resistance in a specific ICU/ward should be taken into account in the decision-making process for prescribing antibiotics for pneumonia. Moreover, recent studies [12] suggested that even in the absence of classical risk factors, PSA may cause pneumonia, sometimes of early-onset VAP.

A specific group of interest is the subset of patients with mechanical ventilation and concomitant antibiotic exposure. In these patients, PSA was independently associated with an ICU stay of 5 days or longer (RR 3.59), and absence of coma (RR 8.36) [13]. The risk of pathogens other than PSA in early-onset pneumonia associated with coma was estimated to be 87.5 %. This observation emphasizes that the risk factors in patients receiving recent antibiotic treatment caused by PSA or other organisms are not the same, and may have implications for preventive and therapeutic approaches for pneumonia.

Interestingly, Flanagan et al. [14] showed that bacterial diversity decreased following the administration of antibiotics and that communities became dominated by a single pulmonary pathogen. PSA became the dominant species in six of seven patients studied, even though five of these six were treated with antibiotics to which it was sensitive in vitro. These data demonstrate that the loss of bacterial diversity under antibiotic selection is strongly associated with the development of pneumonia in ventilated patients colonized with PSA.

Trouillet et al. [15] sought to determine the epidemiologic characteristics of ICU patients who developed VAP caused by piperacillin-resistant PSA or piperacillin-susceptible PSA. Multivariate analysis identified the following significant independent factors for piperacillin-resistant PSA: presence of an underlying fatal medical condition [odds ratio (OR) 5.6], previous fluoroquinolone use (OR 4.6), and initial disease severity (OR 0.8). This study suggested that restricted fluoroquinolone use was the sole independent risk factor susceptible to intervention.

Summary

Multiple studies have confirmed the association of risk factors for exogenous cross-contamination (>5 days and prior antibiotic exposure) and endogenous colonization (such as COPD) (Table 1). Recently, PSA pneumonia has been identified in patients without these risk factors. Updated information on risk factors has implications for infection management and control, suggesting that the classical paradigm of restricting anti-pseudomonal agents to patients with hospitalization longer than 1 week or prior antibiotic exposure may lead to a substantial delay

Table 1 Summary of risk factors for isolation of multidrug-resistant *Pseudomonas aeruginosa*

Admission to chronic facilities
Prolonged hospitalization
Prolonged ICU stay
Mechanical ventilation
Candida albicans airway colonization
High severity of illness
Invasive blood pressure monitoring
Bilateral chest X-ray involvement
Previous antibiotic exposure
Multiple agents
Broad spectrum agents
Fluoroquinolones: levofloxacin > ciprofloxacin
Aminoglycoside
Cephalosporins: broad-spectrum/anti-pseudomonic
Carbapenems: especially imipenem; except ertapenem

in appropriate therapy in some patients and may compromise outcomes. The emergence of newer multidrug-resistant (MDR) clones with susceptibility only to amikacin and colistin represents a potential challenge that requires further epidemiological, molecular, and experimental research.

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XII. APPENDIX No.2. Review Article

Pneumonia in immunocompetent patients: combination antibiotic therapy.

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Pneumonia in immunocompetent patients: combination antibiotic therapy

S. SALVA [1], B. BORGATTA [2], J. RELLO [2, 3, 4]

[1]Department of Anesthesia, Vall d'Hebron University Hospital, Barcelona, Spain; [2]Department of Intensive Care, Vall d'Hebron University Hospital, Barcelona, Spain; [3]CIBERES, Barcelona, Spain; [4]Universitat Autonoma de Barcelona, Barcelona, Spain

ABSTRACT

Pneumonia's burden is still important worldwide not only because of its high incidence and mortality, but also for the elevated costs related to it. Despite the concerted efforts to reduce the incidence of sepsis-related complications, they continue to represent a major human and economic burden. The cornerstone of sepsis management is early appropriate empiric broad spectrum antibiotics, resuscitation, and source control. The association between inappropriate use of antibiotics and increased mortality is the rationale for the use of empiric antibiotic combination therapy in critically ill patients. The aim of this manuscript was to discuss recent literature regarding the management of severe pneumonia, both community-acquired and hospital-acquired/ventilator-associated, in critically ill patients. Use of combination therapy is warranted in severe infections with shock; considerations should be made on the importance of optimal antibiotic administration and adverse reactions, thus providing guidance for a rational use of antibiotics. *(Minerva Anestesiol 2014;80:495-503)*

Key words: Sepsis - Shock - Combined modality therapy - Pneumonia.

Severe sepsis and especially septic shock are responsible for high rates of intensive care unit (ICU) morbidity and mortality that had remained almost unchanged at around 40% [1, 2] before the introduction of the surviving sepsis campaign bundles. The cornerstone of its management is early appropriate empiric broad spectrum antibiotics, resuscitation, and source control. The vast majority (>75%) of hospitalized patients receive antibiotics at least once during their stay,[3] a practice which has contributed to the generation of antibiotic resistance all over the world.[4] Initial inappropriate therapy and treatment failure in severe sepsis and septic shock is associated with worse outcomes, such as increased mortality and length of stay (LOS).[5] The high variability in clinical practice and the current need to contain healthcare costs deserve the design of guidelines for adequate antimicrobial treatment.

In this article we aim to establish whether the use of combination therapy is helpful in critically ill patients with pneumonia, through an analysis of the most relevant recent research. We hope that the results will contribute to optimizing the use of antibiotics, which continue to be the most important weapons in the Intensive Care Unit (ICU) armory.

Community acquired pneumonia

Pneumococcal infection is the leading cause of community acquired pneumonia (CAP). Atypical pathogens of CAP include *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*. Co-infection of *S. pneumoniae* with atypical pathogens occurs in up to one third of cases.[6-8] Epide-

miological data regarding CAP in Europe vary widely, but recent studies have focused on giving a global view of its burden. The incidence of CAP ranges from 4.5% to 24.8%; it rises notably with age and is especially high in people over 75 years. Its mortality varies from <1% to 48%, and is not associated with antibiotic resistance. Severe forms are associated with increased hospital length of stay. Annually in Europe the direct costs of CAP reach € 16 billion, while indirect costs are around € 3.5 billion. Quality of life and degrees of disability depend on disease severity.[9] Treatment failure in hospitalized patients is relatively frequent and increases costs even more.[10] A very small proportion of hospitalized patients with CAP have bacteremia, but when present it is associated with increased severity of presentation and consequently greater mortality.[6, 7, 11, 12] Furthermore, in pneumococcal CAP, high bacterial loads (≥10^3 copies/mL) are associated with a higher risk of septic shock, need for MV and mortality (OR=5.4).[13] In spite of improvements in critical care management and the availability of new therapies, severe CAP (SCAP) morbidity and mortality rates have remained unchanged over the last decade. Inappropriate empiric therapy in patients with severe sepsis and septic shock also increases mortality and length of stay (LOS).[12, 13] Thus adequate antibiotic treatment remains the cornerstone in the management of SCAP and can help reduce the use of resources.[7]

The 2007-IDSA/ATS CAP guidelines recommend a beta-lactam plus either azithromycin or a fluoroquinolone for the treatment of patients admitted to the ICU for SCAP.[11] Unfortunately, ICU patients are routinely excluded from trials, and these recommendations are extrapolations from non-severe cases, rendering standard guidelines inappropriate for optimal treatment. Furthermore, it should be borne in mind that some ICU patients with severe sepsis and multiorgan dysfunction have inadequate plasma levels of antibiotics, which may be exacerbated if they receive continuous renal replacement or ECMO therapies.[3, 14] As highlighted by Ulldemolins *et al.*,[14, 15] hypoalbuminemia is present in more than half of critically ill patients and poses a major problem for the optimal and efficient antibiotic management of severe infections in this population. This is because hypoalbuminemia modifies the pharmacokinetics and pharmacodynamics of antibiotics, especially protein-bound ones (such as cefotaxime, ertapenem, aztreonam, amongst others), which reduces antibacterial exposure. Unfortunately, clinical trials do not take this situation into account.

Combination therapy vs. *monotherapy*

Kumar *et al.* demonstrated the beneficial impact of combination therapy (CT) for both Gram-positive and Gram-negative infections in septic shock patients treated with B-lactam plus aminoglycosides or fluoroquinolones or macrolides or clindamycin.[16] CT is not only a safe strategy, but is superior to monotherapy (MT) for patients with SCAP, intubated CAP, CAP with shock and bacteremic pneumococcal CAP.[17-21] In fact, in the subset of patients with septic shock, a beneficial effect on survival is seen with CT even when the B-lactam is the appropriate therapy,[7] and in SCAP (class V pneumonia severity index) when compared with levofloxacin.[21] In addition, in children aged >6 years with CAP, CT (beta-lactam plus macrolide) decreased LOS and hospital readmission.[22]

The main benefits of CT include increased survival and ventilator-free days with lower morbidity and LOS. The association is stronger at higher levels of severity and, in fact, is not seen in patients with mild CAP. Mortality-adjusted OR for death may be as high as 6.4 with effective MT, compared with 0.4 for CT at 90 days of follow up.[21, 23-25] Some studies have found no superiority of CT over MT, but most of them included patients with non-severe CAP or were underpowered.[7, 12, 24]

CT is also beneficial for the management of atypical SCAP. In patients admitted to the ICU for SCAP due to *Legionella pneumophilia* infection, CT with rifampicin plus clarithromycin was beneficial; the odds ratio for death was even lower in the presence of shock.[26]

Combination therapy: are there differences among combinations? The community setting

Another interesting issue in the optimal antibiotic management of SCAP is whether there are differences between antibiotic combinations.

Initially, the improvement in outcomes achieved with CT was attributed to the adequate coverage obtained by the extended spectrum of activity and/or a synergistic effect between antibiotics. The superiority of a macrolide combination for SCAP, bacteremic pneumococcal infection and septic shock has been well established in numerous studies [23-25, 27] even with macrolide-resistant strains.[25]

In a multicenter, randomized, double-blind non-inferiority trial comparing MT with moxifloxacin *vs*. CT with ceftriaxone and levofloxacin, no statistically significant differences were found in terms of clinical cure rate, bacteriological success, adverse events or mortality.[21] Two aspects should be borne in mind: First, although statistical significance was not achieved, there was a trend towards greater mortality in the moxifloxacin arm with PSI (Pneumonia Severity Index) IV and V. Second, only 10% of the population had SCAP (PSI class V) and almost half had class IV PSI.

Comparison of the use of macrolides *vs.* fluoroquinolones or tetracyclines as add-on therapy shows that macrolides are systematically associated with better outcomes in critically ill patients with CAP.[27] Many mechanisms have been proposed to explain this. The extended spectrum with coverage of atypical pathogens is an improbable explanation, since SCAP due to atypical pathogens is rare.[17] Nonetheless, empiric CT with macrolides has been associated with lower relapse rates than targeted therapy. Another proposed mechanism is a theoretical synergy between mechanisms of actions, where the macrolide inhibits protein synthesis and the beta-lactam the cell wall; however, this hypothesis is not supported by *in vitro* studies.[28] Interestingly, the immunomodulatory properties of macrolides reduce the release of proinflammatory products and probably the production of virulence factors as well; this also applies to macrolide-resistant strains.[12, 29, 30] Recent studies suggest a potential immunomodulatory effect for some fluoroquinolones.[11]

Healthcare-associated pneumonia, ventilator-associated pneumonia

One of the best known bacteria in this clinical entity is *Pseudomonas aeruginosa*. The use of combination therapy in patients infected with *P. aeruginosa* has been common practice in different countries for over 30 years.[31-33] Recent antibiotic exposure and innapropriate antibiotic therapy in Gam-negative sepsis is associated with increased length of stay and hospital costs.[34, 35] The American Thoracic Society's guidelines for the management of healthcare-associated pneumonia (HAP) recommend judicious use of combination therapy, with the addition of a short course (5-day) combination of an aminoglycoside and a beta-lactam to treat pneumonia caused by *P. aeruginosa*.[36] A suitable combination could be meropenem and amikacin. Both are active in front of ampC beta-lactamases and have highest susceptibility rates for *Pseumononas aeruginosa.* This practice is based on the fact that the bacteremia secondary to *P. aeruginosa* is generally associated with a high rate of resistance. Recent reports describe an increased resistance to various antibiotics like carbapenems (9%), quinolones (15%) and cephalosporins (20%).[37] An important characteristic of *P. aeruginosa* is the ease with which it develops or acquires mechanisms of resistance. Patients infected by *P. aeruginosa* have a mortality rate close to 15%,[38] as well as an increase in hospitalization, days on MV and ICU LOS, representing a significant increase in costs.[39] Another interesting feature is its resilience and ability to develop biofilms in the ICU environment as well as in the patient, in sites such as invasive devices, wounds, and so on. A very recent study found that in ICU units with a high prevalence of multidrug resistant microorganisms, patients with early onset pneumonia and septic shock who are not exposed to potentially resistant microorganisms benefit from empiric treatment that is probably effective against these organisms.[40]

The decision to use more than one antibiotic should be based primarily on the severity of the disease and should take into account local data on antimicrobial susceptibility.[40, 41] In 2004 Safdar [42] published a meta-analysis of 17 studies, five of which dealt directly with *P. aeruginosa* infections. They found a clear benefit with the use of a combination therapy in this group of patients, which decreases the mortality rate by approximately 50%. In another study of patients with bacteremia secondary to *P. aeruginosa* infection, combination therapy was found to be

more effective than monotherapy (27% *vs*. 47%, P<0.02) with a significant reduction in the mortality rate at ten days.[43]

The benefit of combination therapy has been highlighted by several studies of the association of beta-lactams and aminoglycosides for severely ill patients. CT helps to reduce the possibility of inadequate initial therapy, resulting in a greater chance of survival.[44]

Garnacho-Montero reported in CCM [44] that the administration of one effective agent for *P. aeruginosa* ventilator-associated pneumonia (VAP) obtained outcomes comparable to those achieved with a combination. However, the initial use of combined therapy was associated with better survival as it reduced the risk of inappropriate therapy. Therefore, initial combination therapy followed by reduction to one effective agent once susceptibility is established should be the preferred strategy.

Other authors do not support combination therapy. Bliziotis *et al.*[31] found that all-cause mortality did not differ between monotherapy (8/19 [42%]) and combination therapy groups (aminoglycoside or quinolone plus beta-lactam) (6/31 [19%]) P=0.11. However, the same authors accept that their study lacks sufficient statistical strength to identify small or moderate differences.

Treatment of patients with suspicion of antibiotic-resistant SCAP caused by Gram-positive cocci, mainly methicillin-resistant *Staphylococcus aureus* (MRSA), lacks robust data; the current guidelines support the use of either vancomycin or linezolid, although the latter has shown superior clinical outcomes. In addition, linezolid reduces the expression of MRSA toxins which although an infrequent event, has devastating consequences. We suggest the use of linezolid as first agent in this setting; vancomycin's nephrotoxicity is of special concern in this group of patients receiving empirical treatment with multiple agents. This was shown in the Zephyr study [45] and confirmed by a secondary analysis of patients enrolled in Spain.[46]

Combination therapy: are there differences among combinations? The health care setting

Martin-Loeches *et al.*[27] reported a secondary analysis from a prospective observational multicenter study that included 280 patients at 27 ICUs in nine European countries. The authors concluded that the combination of antibiotic therapy with macrolides improves survival rates in intubated patients with severe community-acquired pneumonia.

Multiple studies suggest that the patients who may benefit most from combination therapy are the severely ill, as demonstrated by the study by Kumar [16] and the meta-analysis/meta-regression study by the same author.[47] Another study conducted by Restrepo [23] also assessed the impact on mortality of patients with severe sepsis secondary to pneumonia treated with macrolides (N.=237). Multivariate analysis reported a decrease in mortality at 30 days (hazard ratio [HR] 0.3, 95% confidence interval (CI) 0.2 to 0.7) and at 90 days (HR 0.3, 95% CI 0.2-0.6) even in cases reporting macrolide resistance.

Studies of combination therapy use a beta lactam associated with a macrolide, a quinolone or an aminoglycoside, based on the local microbiology and the suspected microorganism. Studying the influence of the addition of an aminoglycoside to combination therapy on the prognosis of patients with gram-negative bacteremia, Martínez *et al*. found that in the subgroup of patients with shock or neutropenia combination therapy was an independent protective factor (shock OR=0.6; 95% CI, 0.4 to 0.9 - neutropenia OR, 0.5; 95% CI, 0.3 to 0.9). For this reason they stressed that combined therapy is justified in patients in these clinical situations.[48]

Metersky *et al.*[25] conducted a very interesting study which included a cohort of patients with bacteremia secondary to pneumonia. Excluding patients with atypical primary infection, they found that the use of macrolides was associated with a lower risk of hospital mortality (OR, 0.59, 95% CI, 0.40 to 0.88, P=0.01), and 30-day mortality (OR=0.61, 95% CI, 0.43 to 0.87, P=0.007).

This finding is also supported by the CAPUCI network in Spain. In their study of a sample of 270 patients with shock the group that included macrolides showed a greater survival (HR 1.44, 95% CI 1.01 to 2.07). Using quinolones, there was no difference in cumulative survival.[17]

Are we mixing apples and oranges? Patients with and without shock

Should CAP be treated in the same way as ventilator-associated pneumonia? The answer seems clear. Non-severe infections without shock or mechanical ventilation and with low risk scores should receive monotherapy in accordance with local antimicrobial susceptibility. In more severe infections, however, patients should be treated with combination therapy.

Comparing patients with severe pneumonia and bacteremia treated with single *vs*. combination therapy, Baddour *et al.*[20] found that the mortality rate at 14 days did not differ significantly between the two groups. However, in the subgroup of critically ill patients, combination therapy was associated with a lower mortality at 14 days (23.4% *versus* 55.3%, P<0.01). This finding was independent of the country of origin, ICU, type of antibiotic received or even activity *in vitro*. The benefit of combination therapy in patients with low risk or no shock is not clear.

One of the key differences between the studies is the percentage of patients with MV or shock. In patients with serious conditions, combination therapy reduced mortality. Kumar's study,[16] which included patients with shock, supports combination therapy. Table I shows several studies supporting CT, althoughthey include critically ill patients with a high percentage of shock. A meta-analysis of 50 studies published by the same author concluded that combination therapy increases survival, particularly in patients at high risk or with shock, but is counterproductive in low risk patients.[47] The differentiation can be defined as follows: possibility of death or clinical failure is <15% with an OR of 1.53, 15-25% with an OR of 1.05 and >25% with an OR of 0.54.

Table II shows the studies that do not support the use of combination therapy. Patients with high severity scores or patients with shock are not included here. In studies with high percentages of patients with shock or more severe conditions, such as the CAPUCI study where 270 patients with shock were studied, a clear benefit of CT was observed.[17]

Conclusions

The decision to apply combination therapy should be a patient-based decision, and the se-

Table I.—*Published studies that support the combination therapy.*

Author	Year	Patients (N.)	Site	Study design	Cohort	Outcome	Patients with shock (%)
Rello *et al.*[26]	2012	25	ICU	Prospective multicenter study	CAP caused by *L. pneumophilia*	Difference in mortality	60%
Ambroggio *et al.*[21]	2012	20,743	Ward	Retrospective, multicenter cohort study	CAP	Hospital LOS, readmission	N/A
Kumar *et al.*[15]	2010	4662	ICU	Retrospective, multicenter	All causes sepsis	Combination therapy was associated with decreased 28-day mortality	100%
Martín-Loeches *et al.*[28]	2010	280	ICU	Multicenter, prospective	Intubated CAP	ICU Lower ICU mortality IDSA/ATS combination plus macrolide	75.7%
Kumar *et al.*[44]	2010	N/A Fifty studies	Ward/ ICU	A meta-analytic/ meta-regression study	All causes sepsis	Difference in Mortality/ Clinical Failure (%)	Up to 100%
Rodriguez *et al.*[16]	2007	529	ICU	Prospective observational, cohort study	CAP	Higher adjusted 28-day in-ICU survival with combination therapy	51%
Baddour *et al.*[19]	2004	844	Ward/ ICU	Prospective, multicenter, observational	Pneumococcal bacteremia	Lower hospital mortality with combination	N/A

NA: not available.

Table II.—*Published studies that have not shown a benefit for combination therapy.*

Author	Year	Patients (N.)	Site	Study design	Cohort	Outcome	Patients with shock (%)
Brunkhorst *et al.*[46]	2012	551	ICU	A randomized, open-label, parallel-group trial	Severe sepsis or septic shock	PE: sepsis-related organ dysfunction, SE: rates for 28-day and 90-day mortality also were not statistically significantly different	N/A
Bliziotis *et al.*[33]	2011	92	Ward/ ICU	Retrospective cohort study	Bacteremia *P. aeruginosa*	All-cause mortality did not differ	10
Hee-Chang *et al.*[48]	2009	35	Ward	Retrospective	MRSA bacteremia	No difference	N/A
Torres *et al.*[7]	2008	733	Ward/ ICU	Prospective, multicenter, randomized, double-blind noninferiority trial	CAP	Difference in clinical cure rate, bacteriological success, adverse event nor mortality	10% (PSI V)
Garnacho-Montero *et al.*[42]	2007	183 episodes	ICU	Retrospective, multicenter, observational, cohort study	VAP for *P. aeruginosa*	One effective antimicrobial or combination therapy provides similar outcomes	N/A
Dwyer *et al.*[47]	2006	340	Ward	Multicenter, retrospective	Bacteremic pneumococcal CAP	No significant effect on case fatality rate	N/A

PE: primary endpoint; SE: secondary endpoint; PSI: Pneumonia Severity Index; NA: not available.

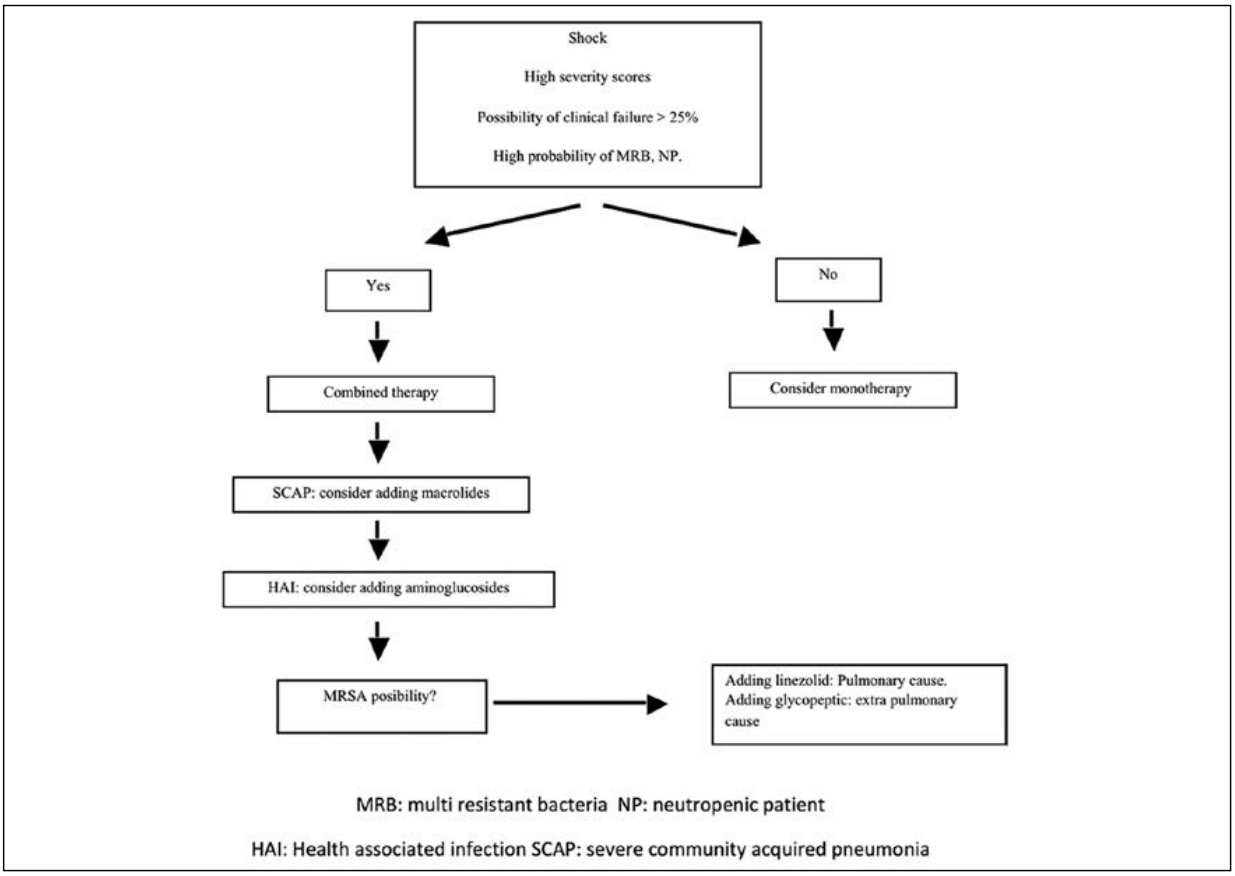

Figure 1.—Algorithm of combination therapy used in the ICU. MRB: multiresistant bacteria; NP: neutronic patients. HAI: health associated infection; SCAP: severe community acquired pneumonia.

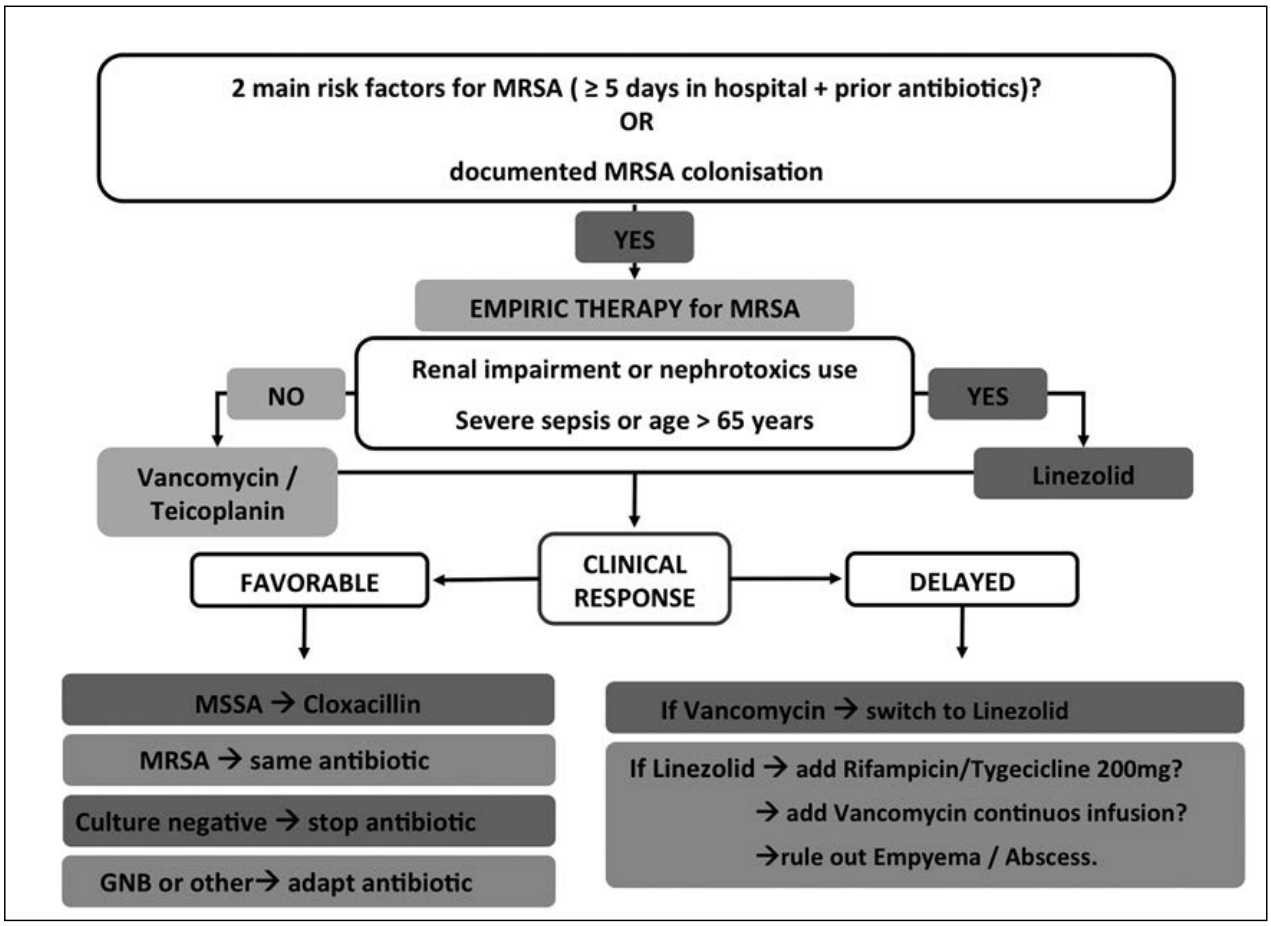

Figure 2.—Algorithm of therapeutic management of MRSA pneumonia.

verity of illness, the probability of infection by specific microorganisms and the degree of inflammatory response should be taken into consideration (Figures 1, 2). Patients with shock may not benefit from monotherapy. In the presence of severe sepsis or shock, several studies support the addition of a second agent in empiric therapy. In cases of pneumonia due to *P. aeruginosa*, the outcome obtained using one effective agent is comparable to that obtained with a combination, but there is an increased risk of initial inappropriate therapy; therefore, the preferred approach would be two initial potential effective agents, followed by de-escalation once susceptibility is known. No studies support combining two agents for fungal infections.

Specific agents should be customized to specific situations. In patients with severe CAP, adding a macrolide to cefotaxin/ceftriaxone seems optimal.[17, 27] For patients at risk of VAP caused by *P. aeruginosa* we recommend the combination of meropenem and amikacin, since both are more active than ampC betalactamases and have the highest susceptibility rates for this particular bacterium.[44, 48] In patients with *Klebsiella pneumoniae* carbapenemase (KPC), high dose tigecycline (100 mg/12 h),[49] meropenem and colistin are good options.[50] For *Acinetobacter baumannii* with MIC above 8 to tobramycin, a combination of carbapenem plus colistin or rifampicin is recommended based on experimental models.

Key messages

— Combination therapy is warranted when vasopressors are required, but it may be detrimental in milder forms of disease. In patients at risk of resistant phenotypes, empiric combination therapy reduces the risk of suboptimal therapy.

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Conflicts of interest.—Dr. Rello servers in the advisory Board and Speakers Bureau of Astellas, Pfizer, Merck, KENTA and Roche.

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Corresponding author: J. Rello, Department of Intensive Care, Vall d'Hebron University Hospital, P. Vall d'Hebron 129, Area General-5a planta, 08035 Barcelona, Spain. E-mail: jrello@crips.es

XIII. APPENDIX No.3. Review Article

Pseudomonas aeruginosa ventilator-associated pneumonia management

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REVIEW

Pseudomonas aeruginosa ventilator-associated pneumonia management

Sergio Ramírez-Estrada[1]
Bárbara Borgatta[1,2]
Jordi Rello[3,4]

[1]Critical Care Department, Vall d'Hebron University Hospital, [2]CRIPS, Vall d'Hebron Institute of Research (VHIR), [3]Department of Medicine, Universitat Autònoma de Barcelona (UAB), Barcelona, [4]Centro de Investigación Biomédica en Red Enfermedad Respiratoria – CIBERES, Madrid, Spain

Abstract: Ventilator-associated pneumonia is the most common infection in intensive care unit patients associated with high morbidity rates and elevated economic costs; *Pseudomonas aeruginosa* is one of the most frequent bacteria linked with this entity, with a high attributable mortality despite adequate treatment that is increased in the presence of multiresistant strains, a situation that is becoming more common in intensive care units. In this manuscript, we review the current management of ventilator-associated pneumonia due to *P. aeruginosa*, the most recent antipseudomonal agents, and new adjunctive therapies that are shifting the way we treat these infections. We support early initiation of broad-spectrum antipseudomonal antibiotics in present, followed by culture-guided monotherapy de-escalation when susceptibilities are available. Future management should be directed at blocking virulence; the role of alternative strategies such as new antibiotics, nebulized treatments, and vaccines is promising.

Keywords: multidrug-resistant, ICU, new-antibiotics, adjunctive-therapies, care-bundles

Background

Ventilator-associated pneumonia (VAP) is the most common infection among the critically ill and the first cause of antibiotic prescription in intensive care units (ICUs), with an incidence of five to 20 cases per 1,000 mechanical ventilation (MV)-days and a global prevalence of 15.6%[1–5] that has not changed significantly despite the implementation of care bundles. Episodes caused by multidrug-resistant (MDR) organisms, such as *Pseudomonas aeruginosa* are associated with significant attributable mortality;[3,6] VAP represents a major clinical and economical problem in critically ill patients due to its associated morbidity, prolonged MV-days, and ICU length of stay (LOS), which translates to elevated health care costs as high as US$40,000 per episode.[7,8]

P. aeruginosa (with *Staphylococcus aureus*) is one of the most common bacteria causing VAP,[5,9] with a prevalence of approximately 4%,[2] and its attributable mortality is as high as 13.5%, even with adequate antibiotic treatment.[3] In MDR strains, mortality rises up to 35.8%, and the presence of MDR strains has been identified as an independent predictor of hospital death (adjusted odds ratio [AOR] 1.634, 95% confidence interval [CI]: 1.124–2.374) and is the single strongest predictor of initial inadequate antibiotic therapy (AOR 5.706, 95% CI: 3.587–9.077).[5,9] A recent study by Micek et al demonstrated that *P. aeruginosa* VAP mortality has increased to 41.9%, with increased age and Charlson comorbidity score, inappropriate initial antibiotic therapy, and vasopressor use as independent predictors of mortality.[10] Antibiotic resistance has been on the rise in the last decade,[5,11–13] which is worrisome since *P. aeruginosa* is one of the three top microorganisms causing health care respiratory infection and is resistant

Correspondence: Bárbara Borgatta
Critical Care Department, Vall d'Hebron University Hospital, Pssg Vall d'Hebron, 119-129, 08035 Barcelona, Spain
Tel +34 932 746 209
Fax +34 932 746 062
Email bborgattab@gmail.com

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to carbapenem,[14] and, even in patients with early-onset VAP and no risk factors, MDR *P. aeruginosa* is frequent.[15,16] Among known risk factors for MDR *P. aeruginosa* in MV patients, the most frequent are antimicrobial therapy within 90 days (51.9%) and current hospitalization of more than or equal to 5 days (45.3%).[2] Infection by MDR *P. aeruginosa* is associated with worse outcomes with an excess mortality rate of 12 with a more than twofold increased risk of mortality (relative risk [RR] 2.34, 95% CI: 1.53–3.57) and ICU LOS, compared to susceptible strains.[11] In VAP caused by MDR *P. aeruginosa*,[10,17] both prior antibiotic use and delayed effective antibiotic therapy in infection also negatively affect mortality and cost.[5,18,19]

P. aeruginosa serotypes causing VAP have different behavior; O6 and O11, the most common, are associated with a clinical resolution of 60%, and serotypes O1 and O2, represent less common strains, with higher mortality.[16] Vallés et al performed an analysis of pulsed-field electrophoresis on more than 1,700 isolates of *P. aeruginosa* in ICU patients, identifying different genotypes. Clones that were responsible for colonization (skin, gut, and respiratory) least frequently caused pneumonia, and VAP's resolution was frequent and uncomplicated. However, clones that were not related to prior colonization were associated with very high mortality rates.[20] This observation may be associated with the expression of virulence factors in *P. aeruginosa*, such as type III secretory proteins.[21]

Most clonally related isolates caused gastric colonization before skin or respiratory tract colonization, suggesting an association with instillation of tap water used for medication by the oral route. A similar study conducted in two different ICUs in a single hospital in France[4] identified an MDR clone of *P. aeruginosa* in the sinks of 12 rooms. As a whole, from 26 cases of colonization/infection by *P. aeruginosa*, five were related to an exogenous colonization (environmental colonization in four patients and cross-infection in one). These findings emphasize the fact that different risk factors may be implicated depending on whether the clone is from exogenous contamination or carried as endogenous colonization. Therefore, different infection control strategies should be applied to prevent colonization of patients with *P. aeruginosa*, including strategies to limit the potential of sinks to act as potential reservoirs.

Risk factors

Risk factors for *P. aeruginosa* in VAP are mainly prior antibiotic exposure and MV longer than 5 days.[22–24] Patients with chronic obstructive pulmonary disease and other chronic respiratory diseases may carry endogenous colonization and can develop a severe respiratory infection following intubation and MV. Interestingly, risk factors in patients with *P. aeruginosa* and prior antibiotic exposure are different.[25] *P. aeruginosa* is the first cause of pneumonia in the postoperative period of lung transplant[26] and in intubated patients with a prior episode of pneumonia.[27] *P. aeruginosa* is also the most common pathogen in patients with health care-associated pneumonia who required ICU admission and further MV.[28]

Current management

Latest guidelines for the antibiotic treatment of *P. aeruginosa* VAP are the 2005 American Thoracic Society/Infectious Diseases Society of America guidelines, which recommend combination therapy with antipseudomonal cephalosporin (cefepime, ceftazidime) or carbapenem (imipenem, meropenem, or β-lactam/β-lactamase inhibitor [piperacillin–tazobactam]) plus antipseudomonal fluoroquinolone (ciprofloxacin or levofloxacin) or aminoglycoside.[29] However, since their publication a decade ago, many findings have been made in the field of antibiotic management in the critically ill, highlighting inappropriate treatment due to insufficient dosing and suboptimal antibiotic exposure, which are associated with increased mortality and worse outcomes.[30–33] Furthermore, the rise of MDR strains in nosocomial pneumonia renders this approach outdated.[12,34] It is important to bear in mind that it is critical to avoid antibiotics to which the patient has been exposed over the last 30 days, since the new episodes usually are relapses of a strain with phenotypic variations and not reinfection. Also, recently, a multicenter study has shed some light regarding treatment failure in *P. aeruginosa* VAP. With an occurrence rate of approximately 30% of episodes, the study identified risk factors for failure, including age, chronic illness, limitation of life support, severity of illness, previous use of a fluoroquinolone, and bacteremia. Interestingly, neither antibiotic susceptibility patterns nor combination therapy influenced failure rates; on the other hand, treatment with a fluoroquinolone did decrease it.[35] Figure 1 outlines initial *P. aeruginosa* VAP management.

To avoid suboptimal antibiotic management, we believe that a composite approach has to be made, taking into account variables other than the classic microbiological paradigm of appropriate antibiotic therapy based only in minimum inhibitory concentration (MIC)'s susceptibility patterns and tailoring treatment to each patient, assessing specific risk factors especially for MDR (Figure 1).[36] The cornerstone for improving outcomes is timing; early effective therapy as

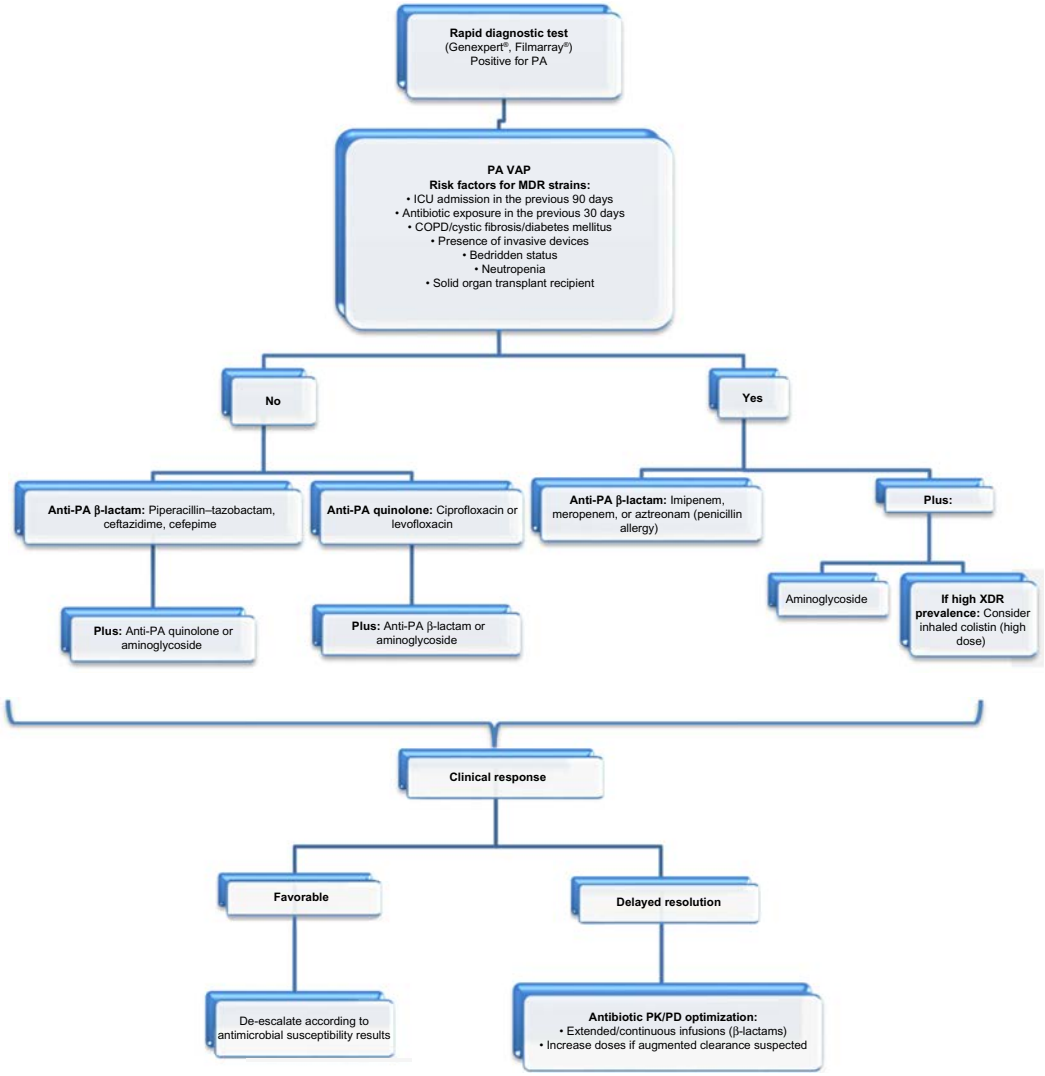

Figure 1 Management of PA VAP.
Notes: Carbapenems are usually reserved for MDR or polymicrobial infections. Aminoglycosides should be avoided as monotherapy despite antimicrobial susceptibility given its poor performance in lung tissue. High-dose inhaled colistin: 5 million units every 8 hours.
Abbreviations: COPD, chronic obstructive pulmonary disease; MDR, multidrug-resistant; PA, *Pseudomonas aeruginosa*; PK/PD, pharmacokinetic/pharmacodynamic; VAP, ventilator-associated pneumonia; XDR, extensively drug resistant; ICU, intensive care unit.

soon as possible might be the difference between death and successful treatment, especially when shock is present.[37,38] Appropriate empirical choice of agent is fundamental, as is the use of a broad-spectrum antibiotic based on local ecology followed by reassessment of clinical response and microbiological data at 48–72 hours.[39,40] In *P. aeruginosa* VAP, empiric combination therapy with a β-lactam plus an aminoglycoside has proved to be superior to monotherapy, reducing mortality up to 50% in many studies and meta-analyses, mainly due to appropriate initial therapy.[40–42] However, there is no difference between one or two effective antibiotics, which is the rationale for de-escalating to monotherapy once microbiological results are available.[42] De-escalation is a safe strategy and has to be done when possible, even in neutropenic patients.[43] Regarding duration of therapy, many studies have demonstrated that 8 days of antibiotic for VAP is safe, reduces emergence of MDR and costs, and avoids unnecessary toxicity to the patient.[44–47] However, in VAP caused by gram-negative bacilli, 8 vs 15 days of antibiotic is associated with increased pulmonary infection recurrence.[45] Since the aim of antibiotic therapy is pneumonia resolution and not *P. aeruginosa* eradication from the endotracheal tube/tracheostomy biofilm, antibiotic courses longer than 10 days in patients with clinical cure only add MDR-strain selection. In *P. aeruginosa* VAP, patients with inappropriate empirical antibiotic therapy, clinical resolution (fever and hypoxemia)

is delayed 8 days (median), as happens with other MDR bacteria.[46] Furthermore, longer antibiotic courses may be recommended for immunosupressed patients with initial inappropriate empirical therapy VAP caused by MDR/extensively drug-resistant strains without clinical resolution.[47] Recently, biomarkers' roles in antibiotic duration guidance have been the subject of multiple studies, with procalcitonin being the only one that has proved to be safe and reduce antibiotic days in VAP. When procalcitonin concentration is <0.5 ng/mL or has decreased by ≥80% (compared with the first peak concentration), antibiotics can be discontinued even in very short-course therapy (3 days), irrespective of the severity of the infectious episode; however, in bacteremic patients, at least 5 days of therapy is recommended.[48–50]

Another point to consider is optimizing the choice of antimicrobial according to pharmacokinetic (PK)/pharmacodynamic parameters. It is important to bear in mind that the antibiotic we choose has to reach therapeutic concentrations at the site of infection, where the bacteria–antibiotic interaction takes place, in order to obtain bacterial clearance as soon as possible.[51] Also, administration of a loading dose and administration of β-lactams in extended and continuous infusions increases antibiotic exposure and the probability of PK target attainment, which is essential in cases of septic shock, obesity, burn patients, and intermediate-resistant *P. aeruginosa* strains,[32] and it is associated with decreased 14-day mortality, faster recovery, and shorter ICU LOS and duration of treatment.[52–64] With this in mind, nebulized antibiotic administration in MV may increase alveolar penetration compared with IV administration.[47] Nebulized colistin (high dose) in monotherapy has been studied in a small-randomized trial and a retrospective study, and noninferiority to IV combination therapy has been reported.[65–67] This approach is very interesting since it enables delivery of high concentrations of the antibiotic with minimal absorption and marginal systemic levels, which could be a turning point in cases of MDR strains where available drugs are highly toxic. Effective treatment of VAP caused by MDR organisms such as *P. aeruginosa* and *Acinetobacter baumannii* has been reported with high-dose nebulized colistin, even achieving airway eradication.[65] Currently, a few agents are available for nebulization (colistin, tobramycin, aztreonam, ceftazidime, and amikacin) but are required to be tested in randomized clinical trials to know the safety and what adds to standard therapy. Further research and evidence-based guidelines are required. Other nebulized agents such as hypertonic saline and *N*-acetylcysteine, sometimes used as coadjutant therapy in the treatment of *P. aeruginosa* lung infection in cystic fibrosis patients, are still controversial, without strong evidence supporting or advice against its use in VAP treatment.[68–72]

New antibiotic treatments

Cephalosporins

Proven efficacy, broad spectrum (some of them including *P. aeruginosa*), and a well-characterized PK/pharmacodynamic profile, in addition to a favorable safety profile, make this antimicrobial class play an important role in nosocomial infection treatment, including VAP.[73] In response to the emergence of nosocomial infections due to β-lactam-resistant gram-negative bacteria in recent years, two strategies have been developed to improve their coverage: the development of new β-lactam molecules with the capacity to evade some mechanisms expressed by resistant bacteria and the addition of novel compounds capable of inactivating β-lactamases.[74]

Ceftobiprole (BAL9141)

Ceftobiprole medocaril has enhanced activity against gram-negative pathogens, including *Escherichia coli*, *Klebsiella pneumoniae*, *A. baumannii*, and other Enterobacteriaceae; its antipseudomonal in vitro activity is similar to that of cefepime, and *P. aeruginosa* cross-resistance between ceftobiprole and other antipseudomonal cephalosporins has been reported.[75,76] Also, it is inactive against bacteria expressing extended-spectrum β-lactamase (ESBL).[75,76] Its bactericidal activity also acts against gram-positive bacteria, including resistant *Streptococcus pneumoniae*, methicillin-resistant *S. aureus*, and *Enterococcus faecalis*, but not against *Enterococcus faecium*.[78] Its activity against some of the ESKAPE pathogens and its stability against a wide range of β-lactamases (not KPC) make it an attractive option for hospital-acquired pneumonia treatment. A total of 781 patients were included in a Phase III study, 210 of whom had VAP. Clinical cure rates overall were 49.9% and 52.8% for ceftobiprole and ceftazidime/linezolid, respectively. However, while the cure rates were not different in nosocomial pneumonia, ceftobiprole performed worse on VAP (23.1% vs 36.5 cure rate). In contrast, those patients who had to be ventilated because of worsening of the pneumonia had a better outcome with ceftobiprole than with ceftazidime/linezolid (Table 1).[77] These findings might be associated with increases in distribution volume in septic patients receiving sedation to start MV, which cannot be anticipated using Monte Carlo simulation.

Ceftazidime–avibactam

Ceftazidime is a well-known antipseudomonal cephalosporin, also active against other gram-negative bacilli and

Table 1 Studies regarding the effect of new antibiotics on *Pseudomonas aeruginosa* infection

Authors/ sponsors	Year	Type of study	Number of patients	Interventions	Results
Awad et al[77]	2014	Randomized, double-blind, multicenter	781	Ceftobiprole vs ceftazidime + linezolid	Clinical cure, % (95% CI): HAP (excluding VAP): 77.8% vs 76.2% (–6.9 to 10) VAP: 37.7% vs 55.9% (–36.4 to –0)
Vazquez et al[84]	2012	Prospective, Phase II, randomized, investigator-blinded	135	Ceftazidime–avibactam vs imipenem–cilastatin	Favorable microbiological response, % (95% CI): 70.4 vs 71.4 (–27.2 to 25)
AstraZeneca[86]	Ongoing	Phase III, randomized, multicenter, double-blind, double-dummy, parallel-group, comparative	Recruiting*	Ceftazidime–avibactam vs meropenem	Ongoing
Solomkin et al[92]	2015	Prospective, randomized, double-blind	993	Ceftolozaneztazobactam + metronidazole vs meropenem	Clinical cure, % (95% CI): 83% vs 87.3% (–8.91 to 54)
Calixa Therapeutics, Inc[94]	2009	Multicenter, double-blind, randomized, Phase II	127	Ceftolozane–tazobactam vs ceftazidime	Favorable microbiological response, % (95% CI): 83.1 (71.7–91.2) vs 76.3 (59.8–88.6)
Cubist Pharmaceuticals Holdings LLC[95]	Ongoing	Multicenter, open-label, randomized	Recruiting*	Ceftolozane–tazobactam vs piperacillin–tazobactam	Ongoing
Polyphor Ltd[100]	Ongoing	Phase II, open-label, multicenter	Recruiting*	POL7080 coadministered with standard of care	Ongoing

Notes: *Patient numbers for the ongoing studies are not yet available, however, the estimated patient enrollment numbers are 850 for the AstraZeneca trial, 728 for the Cubist trial, and 25 for the Polyphor Ltd trial.
Abbreviations: CI, confidence interval; HAP, hospital-associated pneumonia; VAP, ventilator-associated pneumonia.

gram-positive cocci and playing an important role in the treatment of nosocomial infections; however, it is susceptible to degradation due to β-lactamases, especially those of Ambler class A and C. Avibactam (NXL 104), recently added to the three approved β-lactamase inhibitors, is a molecule capable of avoiding the activity from A-, B-, and some D-class β-lactamases, including AmpC, KPC (*Klebsiella pneumoniae* carbapenemase), and ESBL.[73,74,78,79] Despite not having antibacterial activity, its union with ceftazidime protects it from degradation from β-lactamases, enhancing its activity against Enterobacteriaceae producing β-lactamases, including *P. aeruginosa*.[79,80] In a murine model, ceftazidime–avibactam has shown good penetration of epithelial lining fluid and effectiveness against *P. aeruginosa* with an MIC up to 32 μg/mL.[81] Ceftazidime–avibactam exhibits a great in vitro MIC50/90 reduction against *P. aeruginosa* producing β-lactamases compared with ceftazidime alone and also shows activity against some meropenem-non-susceptible strains in catheter-associated urinary tract infection.[74,82,83] Phase II trials with ceftazidime avibactam have shown favorable results, a good safety profile, and have been well tolerated when used alone for complicated urinary infections, and when used with metronidazol for intra-abdominal infections.[84,85] Its role in nosocomial pneumonia is actually being analyzed in a Phase III study (Table 1).[86] Caution should be taken into account in countries/institutions where the main resistance problem is OXA-48, and consideration given to the need for initial loading dose, to avoid the potential risk of initial underdosing, particularly in those patients with decreased creatinine clearance.

Ceftolozane–tazobactam (CXA-201)

Like other cephalosporins, ceftolozane develops its bactericidal activity by inhibiting the cell wall synthesis via penicillin-binding proteins; particularly, ceftolozane has shown an enhanced affinity for these proteins in comparison with β-lactams.[87] In vitro studies suggest it is not affected by some β-lactam resistance mechanisms expressed by *P. aeruginosa*, such as efflux pumps or reduced wall permeability due to porin channel mutations,[88,89] making it the most active antipseudomonal β-lactam.[90,91] However, by itself it does not have activity against β-lactamase-producing strains. Tazobactam's activity against β-lactamases bring to ceftolozane the potential to eliminate many resistant strains of *P. aeruginosa* and other β-lactamase-producing gram-negative bacteria.[92] A Phase III trial has shown ceftolozane–tazobactam's efficacy in complicated intra-abdominal infections in combination with metronidazole, including those caused by MDR

pathogens,[93] and a Phase II trial also demonstrated its efficacy in complicated urinary tract infection treatment.[94] Currently, a Phase III study is evaluating its safety and efficacy in VAP (Table 1).[95]

Arbekacin

Arbekacin is an aminoglycoside discovered in the 1970s and has been used in many countries for more than 2 decades. Usually indicated in the treatment of infections caused by methicillin-resistant *S. aureus*, it has also shown activity against gram-negative pathogens, including *Pseudomonas* spp. Its capacity to be unaltered by many of the aminoglycoside-modifying enzymes, one of the most frequent ways by which aminoglycosides are inactivated, confers to arbekacin enhanced activity against *P. aeruginosa* resistant to amikacin, gentamicin, and tobramycin.[93,96] In vitro analysis suggests that arbekacin in combination with aztreonam is an effective regimen against MDR *P. aeruginosa*, including metallo-β-lactamase-producing strains;[93] however, further studies are needed to show its applicability and safety in clinical practice. In PK studies, arbekacin has shown acceptable pulmonary tissue distribution and an adequate safety profile;[97] however, therapeutic plasma level monitoring is recommended to optimize its efficacy and minimize adverse effects, mainly nephrotoxicity.[93]

POL7080

POL7080 is a novel peptidomimetic antibiotic with proven activity against *P. aeruginosa* in murine models.[98] Its mechanism of action is not totally clear, but it is known that it modifies the lipopolysaccharide-assembling of the bacterial outer membrane via the lipopolysaccharide-assembling protein LptD.[98] A Phase I study has shown POL7080 to be safe and well tolerated,[99] and actually a Phase II study is evaluating its safety and efficacy in patients with VAP due to *P. aeruginosa* (Table 1).[100] Nephrotoxicity is a major concern with this drug.

Pathogenicity and newer adjunctive therapies

Pathogenicity

P. aeruginosa's pathogenicity is very complex,[101–103] and a detailed analysis is far from the objective of this report. During a host's infection process, *P. aeruginosa* uses pili, flagella, and fimbriae, a series of functional elements, to move and adhere on living and nonliving surfaces, such as different tissues and medical devices,[104,105] and also employs these mobile elements to form bacterial communities based on an intricate intercellular communication mechanism (ie, quorum sensing), many times surrounded by a polysaccharide-based structure known as biofilm. This structure is produced by the bacterial colony and acts as a barrier against different chemical factors and physical forces (eg, immune system response and antibiotics), providing a favorable environment for colony survival and playing an important role in its permanency and in the chronic colonization/infection process.[21,104,106,107]

Many of the steps in the biofilm formation process are being highly investigated as treatment targets, with many others not being completely understood yet.[21]

Alginate is a very important virulence variable, affecting children with cystic fibrosis.[108,109] However, cystic fibrosis patients carry mucosal strains[110] which are uncommon in patients with VAP, requiring different therapeutic considerations.

Quorum sensing

Quorum sensing is an evolved adaptive strategy expressed in several gram-negative and gram-positive bacteria species, based on a highly complex cell-to-cell communication mechanism, which allows a group of bacteria to exchange information and make dynamic and coordinated changes in response to different environmental stimuli, thus playing an important role in host infection and the bacterial permanence.[111] This system is based on signal molecules expressed by bacteria in a density-dependent way and released to the environment; these molecules are called autoinducers and are recognized by other cells, in some cases from different species (eg, between *P. aeruginosa* and *Burkholderia cepacia*), inducing genomic changes and giving to a population of bacteria the ability to deploy coordinated responses to affront different environmental assaults.[111–113] With three known autoinducers from the acyl-homoserine lactone (AHL) family, Las, Rhl, and the *P. aeruginosa* quinolone signal, *P. aeruginosa* has one of the most classical and understood quorum sensing models, involved in many defense mechanisms such as biofilm formation and production of antimicrobial substances and bacterial virulence factors.[21,111,112,114] This communication system facilitates host infection, ensures the permanency of colonies, and makes eradication of these colonies difficult, making it a highly attractive target for novel treatments. Three targets in this communication circuit have been identified as susceptible to pharmacological intervention: the inhibition of both Las and Rhl synthesis, the autoinducers' degradation, and the blockage of AHL receptor function,[21,111,115] with several in vitro and animal model trials demonstrating

the blockade of the quorum sensing as a feasible strategy to reduce the bacterial virulence and restore some *P. aeruginosa* susceptibility to classical antibiotics. However, further investigations are needed to evaluate its role in the treatment of human infections due to MDR *P. aeruginosa*.

Monoclonal antibodies

Current research in the management of *P. aeruginosa* infection has been directed toward prevention of infection in high-risk patients with vaccines and modulation of virulence with monoclonal antibodies instead of focusing on bacterial clearance attainment. Its main appeal relies on multidrug therapy with one molecule targeting mechanisms of action of bacteria covering MDR strains and probably active in different infection models.

Monoclonal anti-type three secretion system antibodies

Type three secretion system, known as TTSS or T3SS, is a complex system expressed by some bacteria which allows intoxication of host cells. This system is present in many gram-negative bacteria, including *P. aeruginosa*, and is based in several groups of proteins (more than 20) exhibited in the bacterial wall, which acts as a syringe, making the bacteria capable of injecting modulation factors and cytotoxins into other eukaryotic organisms, including the immune host apparatus and epithelial cells, inducing cellular death and playing an important role in *P. aeruginosa* virulence and in the inflammatory response.[116–119] TTSS is a marker of virulence in *P. aeruginosa* penumonia[110] and its presence in patients with VAP is associated with worse outcomes.[119,120] TTSS plays an important role in VAP, since worse clinical outcomes are seen when TTSS is present. An obvious implication of this is that adjunctive therapies targeting these proteins, such as antibodies, may improve outcomes of patients under MV and *P. aeruginosa* respiratory isolation (both colonization and infection).[119,121] The PcrV is a needle-tip protein involved in many steps of the TTSS-mediated infection process, sensing the outside environment and helping bacteria to recognize the strange cells. It also plays a role in translocation and secretion control of some proteins involved in functional molecular syringe assembling and facilitating the union into the molecular needle and the host membrane, which makes an attractive target in TTSS-mediated virulence control, with studies showing loss of virulence capacity in bacteria with an unfunctional PcrV, both in in vitro and in vivo animal models.[116,121] Based on this idea, antibodies have been developed for the blockage of PcrV protein function, with many studies reporting a decrease in blood bacterial colonies and a less severe inflammatory response in various animal models treated with anti-PcrV immunoglobulins.[117,122–124] One of the most successful is the KB001, a high-affinity PEGylated Fab antibody, which, in a Phase II study, has been well tolerated and showed a safety profile in mechanically ventilated patients colonized by *P. aeruginosa*, also showing a nonstatistically significant tendency to reduce *P. aeruginosa* pneumonia episodes in the intervention group (Table 2).[125]

Monoclonal anti-alginate antibodies

Alginate is involved in many processes during *P. aeruginosa* infection, providing protection against a variety of host defense mechanisms and environmental factors such as antimicrobial agents; it also is highly present in mucoid biofilms and facilitates medical device colonization.[105,107,126] This exopolysaccharide, principally exhibited by mucoid strains of *P. aeruginosa*, is capable of reducing the host immune response by interfering with the activation of complements and polymorphonuclear chemotaxis, and also was shown to play a role in decreasing the phagocytosis of *Pseudomonas* spp., both those that are planktonic and those that form biofilm structure guaranteeing the *P. aeruginosa* survival during the first steps of primary infection, its permanency, and its chronic colonization development.[105,107,127]

Different monoclonal antibodies against alginate have been developed, showing an increase in *P. aeruginosa* phagocytosis. In some cases, as with the monoclonal antibody F429,

Table 2 Studies regarding the effect of adjunctive therapies on *Pseudomonas aeruginosa* infection

Authors	Year	Type of study	Number of patients	Interventions	Results
François et al[125]	2012	Multicenter, randomized, placebo-controlled, double-blind, Phase IIa	39	Single intravenous infusion of KB001 PEGylated Fab antibody (3 and 10 mg/kg)	Was well tolerated and not immunogenic
Zaidi and Pier[128]	2008	Experimental, murine model	NA	Immunoglobulin G1 monoclonal antibody	Reduction of bacterial levels in the eye and the associated corneal pathology
Lu et al[131]	2011	Multicenter, open-label, pilot, Phase IIa	18	Panobacumab in three doses of 1.2 mg/kg	Was well tolerated and not immunogenic

Abbreviations: Fab, fragment antigen-binding; NA, not applicable.

this improvement in immune response against *P. aeruginosa* infection was also reported in different models of infection such as pneumonia, sepsis, and keratitis in animal models,[109,128] being promising as an adjunctive strategy in *P. aeruginosa* infection management (Table 2).

Panobacumab (AR-101)

Panobacumab is an IgM-type human monoclonal antibody that is directed against IATS 011 serotype *P. aeruginosa*, one of the most prevalent serotypes associated with nosocomial pneumonia.[16,129,130] A multicenter Phase II study using panobacumab in combination with different antipseudomonal antibiotics in critical patients with nosocomial pneumonia due to *P. aeruginosa* serotype O11, almost all with VAP, showed a good safety profile with good PKs (Table 1).[131,132]

Vaccines

P. aeruginosa's infection mechanism and its interaction with the host immunity is highly studied and well known. With the advances in antimicrobial therapy and many sites identified as possible targets to improve the acquired immunity response and block the *P. aeruginosa* infection and biofilm formation, different types of vaccines are being designed to improve the immune response against many substances involved in this process. The most common targets are components of the bacterial surface, such as outer membrane proteins (Opr) and different polysaccharides (lipopolysaccharides, mucoid exopolysaccharide, and O-polysaccharides), structures involved in *P. aeruginosa* adhesion and movement, such as flagella, pili, and several virulence factors, such as TTSS, exotoxin A, or proteases.[133,134] Development of an effective vaccine is difficult due to the high variability between *Pseudomonas* species and the complexity of its infection process and its interaction with the host immune response. In many cases during phase I, II and III studies, some molecules failed to provide an adequate coverage against different *P. aeruginosa* strains, or showed a low inmunogenicity capacity or an unsecure profile.[133–135]

One of the most promising targets to induce an acquired immune response are the Opr, showing an improved immune response against *P. aeruginosa* infection in murine models previously exposed to modified epitopes from Opr.[133,135,136] From this group, the Opr-based vaccine IC43 has been used in healthy individuals and in different groups with increased risk to develop *P. aeruginosa* infection, including critical patients under MV, showing a good safety profile and being well tolerated,[137–140] and there is an ongoing Phase II/III study designed to show its effect on mortality in mechanically ventilated ICU patients.[141]

Conclusion

P. aeruginosa VAP management requires prompt and adequate antibiotic exposure. Initial empiric therapy should be done with broad-spectrum antibiotics in combination therapy followed by de-escalation with one effective antibiotic since its effectiveness equals two antibiotics. Immunotherapy, including strategies with monoclonal antibodies, might be a new approach to treat (and perhaps prevent) *P. aeruginosa* infections. Future research should focus on optimizing outcomes with strategies of blocking virulence and vaccination.

Disclosure

Jordi Rello has served in the Advisory Boards and Speakers Bureau of Cubist. The authors report no other conflicts of interest in this work.

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XIV. APPENDIX No.4. Editorial Article

Management of Pseudomonas aeruginosa pneumonia: one size does not fit all.

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CRITICAL CARE

COMMENTARY

Management of *Pseudomonas aeruginosa* pneumonia: one size does not fit all

Jordi Rello[1,2,3,4*], Bárbara Borgatta[1,3] and Leonel Lagunes[1,3]

See related research by Lu *et al.*, http://ccforum.com/content/18/1/R17

Abstract

In view of the mortality associated with *Pseudomonas aeruginosa* (PSA) ventilator-associated pneumonia (VAP) and the frequency of inadequate initial empiric therapy, recent findings underscore the need for a different management paradigm with effective anti-pseudomonal vaccines for prophylaxis of patients at risk. The association of virulence factors is a variable that splits PSA in two phenotypes, with the possibility of adjunctive immunomodulatory therapy for management of virulent strains. We comment on recent advances in and the state of the art of PSA-VAP management and discuss a new paradigm for tailored and optimal management.

In the previous issue of *Critical Care*, Lu and colleagues [1] reported a visionary study assessing the distribution of *Pseudomonas aeruginosa* (PSA) serotypes in patients with ICU pneumonia and suggested differences in outcomes depending on serotypes. In this report, serotype O6 predominated, being associated with better clinical outcomes than serotype 011, which were frequently producing toxins secreted by the type III secretion system (TTSS). These findings have important implications for both clinical practice and future studies.

In an international study of over 1,200 ICUs in 75 countries, the risk of infections, including those due to *Pseudomonas* species, was found to increase with duration of ICU stay; in addition, infection was associated with an increased risk of mortality [2]. In 2014, at a time when multidrug-resistant clones are emerging and represent a strong risk of dissemination, we have much more information on *Pseudomonas* pneumonia management. We know that one effective agent is equivalent to two [3,4] but that initial combination followed by de-escalation improves survival by reducing the risk of delay in appropriate therapy. We know that resolution of episodes with appropriate therapy is similar to core pathogens [5] but that wrong initial therapy is associated with a resolution similar to that of methicillin-resistant *Staphylococcus aureus* [6].

Pulsed-field electrophoresis analysis performed in an ICU with a high prevalence of PSA identified the genotypes of more than 1,700 isolates [7]. Interestingly, the most frequently isolated clones were responsible for gut or skin colonization, in addition to respiratory colonization, but were only rarely associated with pneumonia. When ventilator-associated pneumonia (VAP) was present, most patients achieved clinical resolution without major consequences. On the other hand, non-related clones suggestive of prior colonization were associated with a very high mortality rate [7]. Most clonally related isolates caused gastric colonization before skin or respiratory tract colonization, suggesting an association with the tap water used in the administration of medication. These findings emphasize that different risk factors may be implicated depending on whether the clone is due to exogenous contamination or or as endogenous colonization from being a carrier. Therefore, conventional identification provided by the microbiology laboratory results is insufficient for assessing the patient and effective management.

Indeed, recent advances have demonstrated the importance of virulence factors in PSA infections. Although several different mechanisms such as quorum sensing and biofilm formation have been reported [8], the TTSS, encoded by PSA, has become one of the most important and widely studied virulence factors. After the microorganism has come into contact with the cell, the needle-like TTSS mechanism allows the bacteria to inject toxins directly into the cytoplasm of the host cell

* Correspondence: jrello@crips.es
[1]Critical Care Department, Hospital Vall d'Hebron, Passeig de la Vall d'Hebron, 119-129, 08035 Barcelona, Spain
[2]CIBERES, Recinto Hospital Joan March, Carretera Soller Km 1207110, Mallorca, Bunyola, Illes Balears, Spain
Full list of author information is available at the end of the article

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[9], evading direct recognition by the host's immune system [10]. Recent studies suggest that failure to eradicate PSA in patients with VAP may be linked to TTSS. Patients infected with *Pseudomonas* sp. strains which express at least one type of TTSS protein ($TTSS^+$) at the onset of VAP are more likely to have recovered at day 8 post-VAP, whereas eradication is achieved in patients with undetectable levels of TTSS proteins [11]. The transfer of our knowledge of the virulence factors to the clinical setting is crucial in order to evaluate the potential of virulence factor-directed therapies.

In view of the mortality associated with PSA-VAP [3,5,12] and the frequency of inadequate initial empiric therapy [13-15], these findings underscore the need for a different management paradigm with effective anti-pseudomonal vaccines for prophylaxis of patients at risk and the need for rapid diagnostic test methods and monoclonal-specific antibodies blocking virulence factors in patients with VAP.

We have also learned that association of virulence factors is a variable that splits *P. aeruginosa* in two phenotypes, with the possibility of adjunctive immunomodulatory therapy for management of virulent strains [16]. A combination of general risk factors and molecular diagnosis techniques may identify suitable candidates for intervention. As in invasive pneumococcal infections [17], further research is required to identify potential associations of comorbidities and serotypes as well as of serotypes and specific complications.

Abbreviations

PSA: *Pseudomonas aeruginosa*; TTSS: Type III secretion system; VAP: Ventilator-associated pneumonia.

Competing interests

JR has served on advisory boards or speakers bureau (or both) for Kenta Biotech (Zürich-Schlieren, Switzerland), Astellas (Tokyo, Japan), Pfizer Inc. (New York, NY, USA), KaloBios (South San Francisco, CA, USA), Clinigen (Burton-on-Trent, Staffordshire, UK), Roche (Basel, Switzerland), and Bayer (Leverkusen, Germany) and has received research grants from Sanofi Pasteur (Paris, France) and Cubist (Lexington, MA, USA). The other authors declare that they have no competing interests.

Author details

[1]Critical Care Department, Hospital Vall d'Hebron, Passeig de la Vall d'Hebron, 119-129, 08035 Barcelona, Spain. [2]CIBERES, Recinto Hospital Joan March, Carretera Soller Km 1207110, Mallorca, Bunyola, Illes Balears, Spain. [3]Vall d'Hebron Institute of Research, Passeig de la Vall d'Hebron, 119-129, 08035 Barcelona, Spain. [4]Universitat Autònoma de Barcelona, Plaça Cívica, Campus de la UAB,Sardañola del Vallés, 08193 Barcelona, Spain.

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XV. APPENDIX No.5. Editorial Article

How to approach and treat VAP in ICU patients.

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BMC Infectious Diseases

REVIEW Open Access

How to approach and treat VAP in ICU patients

Bárbara Borgatta[1,2*] and Jordi Rello[1,2,3,4]

Abstract

Background: Ventilator-associated pneumonia (VAP) is one of the most frequent clinical problems in ICU with an elevated morbidity and costs associated with it, in addition to prolonged MV, ICU-length of stay (LOS) and hospital-length of stay. Current challenges in VAP management include the absence of a diagnostic gold standard; the lack of evidence regarding contamination *vs.* airway colonization *vs.* infection; and the increasing antibiotic resistance. We performed a Pubmed search of articles addressing the management of ventilator-associated pneumonia (VAP). Immunocompromised patients, children and VAP due to multi-drug resistant pathogens were excluded from the analysis. When facing a patient with VAP, it's important to address a few key questions for the patient's optimal management: when should antibiotics be started?; what microorganisms should be covered?; is there risk for multirresistant microorganisms?; how to choose the initial agent?; how microbiological tests determine antibiotic changes?; and lastly, which dose and for how long?. It's important not to delay adequate treatment, since outcomes improve when empirical treatment is early and effective. We recommend short course of broad-spectrum antibiotics, followed by de-escalation when susceptibilities are available. Individualization of treatment is the key to optimal management.

Keywords: Ventilator-associated pneumonia, Nosocomial pneumonia, Treatment, Antibiotic, Management

Background

Ventilator-associated pneumonia (VAP) is one of the most frequent clinical problems in ICU. With an estimated incidence from 5–20 cases per 1.000 mechanical ventilation (MV) days; which has decreased over the last decade with the implementation of care bundles. However it still remains as the most frequent infection amongst critically ill patients and as the main cause of antibiotic prescription in ICU [1-4]. Despite presenting a low attributable mortality (less than 10%); its burden relies on the elevated morbidity and costs associated with it, such as an estimated excess of cost as high as $40,000 per patient's episode, in addition to prolonged MV, ICU-length of stay (LOS) and hospital-length of stay [2,5,6].

VAP represents 80% of hospital-acquired pneumonia (HAP) and is defined as pneumonia developing after 48-72 h of MV. Many screening and diagnostic criteria have been used in order to an early identification of VAP and differentiation from ventilator-associated tracheobronquitis (VAT), with suboptimal results since radiological findings in the critically ill lack sufficient sensitivity and specificity. Recently, Centers for Disease Control and Prevention (CDC) and Klompas *et al.* have established a new surveillance strategy for the screening of infection-related ventilator-associated complications (IVAC) [7] that represents a major change in VAP diagnosis paradigm, and focusing on sustained hypoxemia (lasting 2 calendar days) as a *sine qua non* characteristic of VAP, even in the absence of clear findings in the Rx. IVAC include all patients with 3 or more days of MV; with worsening of oxygenation lasting 2 calendar days, identified as increase in FiO2 or PEEP; which can be classified as *possible VAP* and *probable VAP*, depending on the criteria they meet.

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VAP management

When facing a patient with VAP, it's important to address a few key questions for the patient's optimal management: when should antibiotics be started?; what microorganisms should be covered?; is there risk for multirresistant microorganisms?; how to choose the initial agent?; how microbiological tests determine antibiotic changes?; and lastly, which dose and for how long? See Figure 1.

* Correspondence: bborgattab@gmail.com
[1]Critical Care Department, Hospital Universitario Vall d'Hebron, Barcelona, Spain
[2]Vall d'Hebron Institute of Research, Barcelona, Spain
Full list of author information is available at the end of the article

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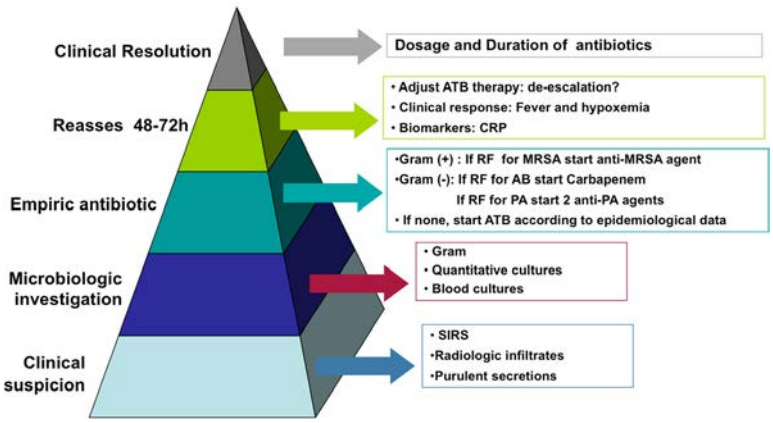

Figure 1 VH-ICU Paradigm for VAP. AB: *Acinetobacter baumanii*, ATB: antibiotic, CRP: C-reactive protein, MRSA: methicilin-resistant *Staphilococcus aureus*, PA: *Pseudomonas aeruginosa*, SIRS: systemic inflammatory response syndrome.

Antibiotic start and choice

It has been well documented that delayed effective therapy increases morbidity and mortality rate among patients with VAP [3,8]. Indeed, changing to an active agent after microbiology reports may not improve patient's outcomes [9]. Initial antibiotic should be active against likely pathogens; therefore its choice should be based on prior antibiotic exposure, patient co-morbidities, length of hospitalization and local epidemiology. Special considerations should be taken with patients with Health-care associated pneumonia (HCAP), since causative organism differs with higher probability for multi-drug resistant (MDR) pathogens. This subset of patients include recent hospitalization in acute care facility (<90 days), resides in a nursing home or long-term care facility; received recent intravenous antibiotic therapy, chemotherapy, or wound care within the past 30 days of the current infection; or attended a hospital or hemodialysis clinic [10].

We support prompt antibiotic initiation with a short course of broad spectrum antibiotics, followed by de-escalation when susceptibilities are available [11]; stressing that initial narrow-spectrum antibiotic should not be used. Certainly, besides microbiological sensitivities, lung penetration of active agents is a crucial matter that has to be considered.

Combined therapy is a long established practice in ICU, especially in VAP caused by *P. aeruginosa* because of its high rates of resistance and initial ineffective antibiotic therapy [12]. Many studies support that in bacteremic infections and VAP due to *P. aeruginosa,* combination therapy improves appropriate empirical therapy [10,13-15]; moreover a meta-analysis was able to detect reduced mortality in this subset of patients (OR 0.50, 95% CI 0.30-0.79), and not in infection due to other gram-negative bacilli [13]. When analyzed by severity-of-illness, combined therapy in patients at high risk of death is significantly associated with reduced mortality only in the subset of patients with shock, whereas patients without shock have worse outcomes, probably due to toxicity [16,17].

Predicting causative organism

Overall, VAP's main causative microorganisms are *Pseudomonas aeruginosa* and *Staphylococcus aureus* [3]. When considered by the time of onset, early-VAP (within the first 4 days of MV) is usually associated with normal oropharynx flora; such as *S. pneumoniae, S. aureus and H. influenzae.* However, a multicenter study showed a high prevalence (50.7%) of potentially resistant microorganisms (PMR) in this subset of patients with no risk factors for PMR [18]. Late-VAP is largely caused by aerobic Gram-negative bacilli, of which up to 70% of cases are due to *P. aeruginosa*, *Acinetobacter baumannii*, or methicilin-resistant *S. aureus* (MRSA). Also differences are seen in surgical and neurological patients, where *S. aureus* is the main pathogen [1-3].

Strategies based on guidelines are accurate for predicting causative microorganism and thus appropriate initial antibiotic in VAP (97.9%, $p < 0.05$), but also are endotracheal aspirates from samples retrieved 2 days before the onset of VAP [19].

Tailoring antibiotic treatment

Standard antibiotic dosage has proven to be insufficient (under-dosage) in critically ill patients with severe sepsis, especially when they undergo continuous renal replacement or ECMO therapies [20,21]. A recent multicenter study addressing antibiotic levels in ICU patients treated with standard doses of betalactams, showed that 16% of them do not meet adequate levels and that it was associated with worse outcomes, while patients who achieved

50% and 100% ratios of free antibiotic concentrations above the minimum inhibitory concentration of the pathogen were associated with positive clinical outcome (OR: 1.02 and 1.56, respectively, $p < 0.03$) [22]. Suggesting that antibiotic dosage and form of administrations should be personalized in order to improve patient's outcomes.

A new approach in VAP treatment is the use of nebulized antibiotics. Its main appeal is that allows achieving high local concentration of antibiotics, with fast clearance, which reduces risk for development of resistance, and with minimal absorption that translates into less toxicity. Even though many issues have to be improved, such as effective delivery systems and optimal formulations that are able to reach the alveoli and are well tolerated by the patients [23]; it poses as a desirable strategy for VAP antibiotic treatment, especially in multirresistant strains where active agents have elevated risk of toxicity. Disadvantages include frequent ventilator's filter obstruction, which some groups solve by routinely change after each administration [24].

Recent studies are lacking of robust data, in spite of which, nebulized therapy has shown to be effective. Nebulized monotherapy has proven to be non-inferior to IV therapy; and as adjunctive to IV regimens is associated with higher antibiotic concentrations at target tissue and less number of antibiotics per patient per day [24-28], and in some cases respiratory eradication of the microorganism [24,29]. Available formulations for nebulization include tobramycin, aztreonam, ceftazdime, amikacyn and colistin.

Duration of treatment

Optimal duration of antibiotic therapy is still controversial. Until recently, it was standard practice antibiotic regimens of minimum 15 days for uncomplicated infections [3]. Current trends favor short courses of antibiotics of 7–8 days if patient's response is satisfying; always individualizing to resolution. This approach is has equivalent clinical cure rates than long courses [30] and enables the reduction of side effects, costs and development of resistant phenotypes [3]. A recent meta-analysis concluded that short courses are associated with more antibiotic free days without any detrimental effect on mortality, besides the fact that prolonged antibiotic courses do not prevent recurrences [30,31]. Not to mention that in patients with VAP and negative bronchoalveolar lavage cultures, early antibiotic discontinuation does not affect mortality and is associated with fewer respiratory and multidrug resistant superinfections (10.0% vs. 28.6% and 7.5% vs. 35.7%, $p < 0.05$ respectively) [32].

Optimization of antibiotics

Optimization of antibiotics does not mean strictly following guidelines; instead it means empowerment, stewardship and team working. Antibiotic stewardship is a simple and cost-effective way to improve clinical outcomes while minimizing antibiotic side effects and its negative consequences; maintaining quality of care [33,34].

What's next?

Research should be directed towards the development of ultra-fast diagnostic techniques that can immediately predict causative microorganism, without the need of specimen processing and also detect multirresistance mechanisms to avoid inadequate initial antibiotic treatment.

Conclusions

Getting it right the first time: It's important not to delay adequate treatment, since outcomes improve when empirical treatment is early and effective. *Prompt appropriate therapy, then step-down:* we recommend short course of broad-spectrum antibiotics, followed by de-escalation when susceptibilities are available. *Individualize always!:* regarding dosage, way of administration and duration based on clinical response.

Abbreviations

CDC: Centers for disease control and prevention; HAP: Hospital-acquired pneumonia; HCAP: Health-care associated pneumonia; LOS: Length of stay; MDR: Multi-drug resistant; MRSA: Methicilin-resistant *S. aureus*; MV: Mechanical ventilation; PMR: Potentially resistant microorganisms; VAP: Ventilator-associated pneumonia; VAT: Ventilator-associated tracheobronquitis.

Competing interest

Jordi Rello has served in the as board and speaker bureaus for Pfizer, Astellas, and Cubicin. Bárbara Borgatta has no competing interest.

Authors' contribution

BB and JR contributed equally to the writing of this manuscript. All authors read and approved the final manuscript.

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Author details

[1]Critical Care Department, Hospital Universitario Vall d'Hebron, Barcelona, Spain. [2]Vall d'Hebron Institute of Research, Barcelona, Spain. [3]CIBERES, Barcelona, Spain. [4]Universitat Autònoma de Barcelona, Barcelona, Spain.

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