VENTILATOR-ASSOCIATED PNEUMONIA DUE TO MULTIDRUG-RESISTANT ACINETOBACTER BAUMANNII: INCIDENCE OF RISK FACTORS WITH IMPACT ON EARLY AND LATE MORTALITY

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Summary
Background. This analysis is aimed to determine the incidence of risk factors for VAP, drug resistance of A.baumannii strains, 7-day (early) and 28-day (late) mortality, and association of risk factors for VAP with early and late mortality in multidrug-resistant (MDR) A.baumannii VAP patients cohort.

Materials and methods. Adult subjects with the first episode of MDR A.baumannii VAP during 2 years period were included. VAP was diagnosed using the 2005 ATS/IDSA criteria. Risk factors for VAP predicting early and late mortality were estimated using binary logistic regression analysis.

Results. The incidence of risk factors for VAP among 60 MDR A.baumannii VAP patients was: both continuous sedation and stress ulcer prophylaxis –100%, exposure to intravenous broad-spectrum antibiotics within the last 90 days – 98.3%, current hospitalization ≥ 5 days – 95.0%, red blood cell transfusion – 60.0%, sepsis – 53.3%, mechanical ventilation before VAP >8 days and SAPS II score > 50 on ICU admission – 50.0%. The resistance of A.baumannii strains to piperacillin/tazobactam and ciprofloxacin was 100%, to carbapenems, cephalosporins and ciprofloxacin but colistin. Both early and late mortality was predicted by higher severity of illness by SOFA score on ICU admission, but only late one – by the treatment with > 3 different classes of antibiotics used before the onset of VAP (OR 3.993; 95% CI 1.050-15.193, p=0.042) – late one.

Conclusions. It was estimated twice higher late versus early mortality, and marginal resistance of A.baumannii strains to piperacillin/tazobactam, carbapenems, cephalosporins and ciprofloxacin but colistin. Both early and late mortality was predicted by higher severity of illness by SOFA score on ICU admission, but only late one – by the treatment with > 3 different classes of antibiotics before VAP onset.

Introduction
Over the recent decade, Acinetobacter baumannii (A.baumannii) has become one of the most prevalent pathogens in nosocomial settings, responsible for 5–10% of nosocomial pneumonia cases in ICU patients [1-4]. The persistent increasing of multidrug resistance of A.baumannii strains has been recognized as one of the most challenging issues in modern medicine [3].

In the literature the severity of illness, invasive monitoring and treatment procedures, exposure to intravenous antibiotics, especially to carbapenems and cephalosporins, mechanical ventilation (MV), also prolonged hospital length of stay (LOS) are defined as the risk factors for ventilator-associated pneumonia (VAP) due to multidrug-resistant (MDR) A.baumannii strains [2,5-6]. Meanwhile, timeliness and appropriateness of antibacterial treatment are referred to be the one of the most important factors related to the outcomes of MDR A.baumannii pneumonia patients [1]. Due to the high level of antibacterial resistance of A.baumannii strains, the selection of appropriate treatment becomes complicated, so the broad-spectrum empirical antibiotic therapy use to be
frequently prescribed. The broad-spectrum antibiotics use is closely related to the development of antibacterial resistance, thus why the careful selection of treatment strategy is crucial to prevent further growth of it. Antibacterial resistance of *A. baumannii* strains varies in different geographical regions, countries and hospitals, and it changes over time [3]. So, the knowledge about local epidemiology, the incidence of risk factors for VAP and their interrelation with mortality, as well as the assessment of the individual patient characteristics (disease severity, underlying condition, comorbidities) are important for clinicians to suspect and diagnose VAP also to start appropriate treatment more rapidly.

A recent retrospective similarly designed study conducted by our group have evaluated risk factors for VAP and mortality interrelation with resistance profiles of *A. baumannii* also have estimated independent risk factors for overall inhospital mortality in VAP due to MDR *A. baumannii* cohort [10]. The link between risk factors for VAP and 7-day (early) also 28-day (late) mortality has not been analysed until now. Our hypothesis was that some of the risk factors for VAP could be useful for early and late mortality prognosis in VAP patients.

The main objective of this study was to identify risk factors for VAP predicting early and late mortality in MDR *A. baumannii* VAP patients. Secondary objectives were to identify: (1) incidence of risk factors for it due to MDR *A. baumannii*, (2) drug resistance of *A. baumannii* strains, also (3) early and late mortality in this cohort.

**Materials and methods**

This analysis of retrospective cohort study [10] on VAP due to MDR *A. baumannii* was performed at the largest Lithuanian University hospital Kaunas Clinics over a 24 month. The study was approved by the Kaunas Regional Biomedical Research Ethics Committee (No BEC-MF-156). Inclusion criteria: (1) age ≥ 18 years, (2) the first episode of VAP due to MDR *A. baumannii*.

If pneumonia has occurred later than 48 h after the onset of MV it was defined as ventilator-associated. 2005 ATS/IDSA criteria were used to make the clinical diagnosis of VAP [8], and the EUCAST guidelines - for the identification of *A. baumannii* isolates and testing their antibacterial susceptibility [9]. *A. baumannii* isolates were defined as MDR when they were non-susceptible to at least one agent in three or more antibacterial categories, whereas XDR – when they were non-susceptible to at least one agent in all but two or fewer antimicrobial categories [10]. Sepsis-2 criteria were used to define sepsis status, SOFA and SAPS II scores – to assess the disease severity on ICU admission [11].

Data collected for each VAP case have included age, gender, type of admission (medical/surgical), risk factors for VAP (hospital LOS, MV and antibacterial treatment d. prior to MDR *A. baumannii* VAP diagnosis, disease severity on ICU admission, sepsis status, red blood cell (RBC) transfusion, surgery, reintubation, tracheostomy, renal replacement therapy (RRT), enteral nutrition (EN), sedation, proton pump inhibitors (PPI), and classes of antibiotics received before the onset of VAP), drug resistance of *A. baumannii* strains, and outcome (discharge or death) at day 7 and 28 after the onset of VAP due to MDR *A. baumannii*.

In order to evaluate the relation between risk factors for VAP and mortality, the patients were divided into the following subgroups: based on the medians of continuous variables (disease severity by SOFA, SAPS II scores, the duration of MV and antibacterial treatment prior to VAP due to MDR *A. baumannii*), also in case of binary variables - on the presence or absence of VAP risk factors (continuous sedation, stress ulcer prophylaxis, LOS ≥ 5 d., reintubation, tracheostomy, RRT, RBC transfusion, EN).

Statistics. The variables were summarized as frequencies and percentage or medians and interquartile ranges. The normality of data was tested using the Shapiro–Wilk normality test. Mann–Whitney non-parametric, Pearson’s chi-square, or two-tailed Fisher’s exact tests were performed to detect the differences between groups as appropriate. All of the variables that were measured were subjected to univariable analysis. Binary logistic regression analysis was applied to identify the independent predictors of 7-day and 28-day mortality. In all analyses, two-sided *p* values of < 0.05 were considered to be statistically significant. Statistical analysis was carried out while using the Statistical Package for the Social Sciences SPSS, version 26 (SPSS, Chicago, IL).

**Results**

The data of 73 mechanically ventilated patients with tracheal aspirates that were positive for MDR *A. baumannii* were analyzed. Eight cases did not meet the diagnostic criteria for VAP; 5 cases were treated as *A. baumannii* colonization; therefore, 60 cases were included in the final analysis.

Data on demographics of study cohort have been reported previously [7]. MDR *A. baumannii* was more likely to cause late-onset VAP (90.0 % vs 10.0 %, *p* = 0.034). Strains of MDR phenotype caused 13.3% and of extended drug-resistance (XDR) phenotype – 86.7% of all cases of *A. baumannii* VAP, (*p* < 0.001).

The incidence of risk factors for VAP was: 100 % - stress ulcer prophylaxis with PPI and continuous sedation, 98.3% – exposure to the intravenous broad-spectrum antibiotics within the last 90 days, 95.0% – present hospitalisation ≥ 5 days, 60.0% – RBC transfusion before VAP onset, 53.3
Fig 1. Antibacterial resistance of *A. baumannii* strains, %

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistant</th>
<th>Sensible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colistin</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>43.3</td>
<td>56.7</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>66.1</td>
<td>33.9</td>
</tr>
<tr>
<td>Amikacin</td>
<td>73.3</td>
<td>26.7</td>
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<tr>
<td>Cefoperazone/ Sulbactam</td>
<td>78.3</td>
<td>21.7</td>
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<tr>
<td>Tetracycline</td>
<td>78.0</td>
<td>21.2</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>81.1</td>
<td>18.9</td>
</tr>
<tr>
<td>Ampicillin/Sublactam</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>Cefepine</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>96.7</td>
<td>3.3</td>
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<tr>
<td>Carbapenems</td>
<td>96.7</td>
<td>3.3</td>
</tr>
<tr>
<td>Trimethoprim/Sulfamethoxazole</td>
<td>98.3</td>
<td>1.7</td>
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<tr>
<td>Piperacillin/Tazobactam</td>
<td>100</td>
<td>0</td>
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<tr>
<td>Ciprofloxacin</td>
<td>100</td>
<td>0</td>
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Discussion

The knowledge about the incidence of risk factors for VAP is useful for clinicians in assessing the individual risk of mechanically ventilated patients for the development of VAP, also in selecting management strategies to diminish the risk of VAP, while together, to influence the outcomes. The associations between invasive airway procedures, stress ulcer prophylaxis also EN and the risk of VAP has previously been reported [12-17]. Data of studies comparing the interrelation between tracheostomy and VAP are controversial. Although Xie (2011) has found that tracheostomy predisposes the development of VAP, the study of Liu (2017) tracheostomy has determined as a protective factor [12-13]. The finding of Liu could be explained by more easier airway care and weaning from MV of tracheostomized patients. Reintubation and EN does increase the risk of aspiration also the acquisition of *A. baumannii* as a pathogen of VAP. It was already reflected in earlier study of Garnacho-Montero (2005) where *A. baumannii* has caused VAP in reintubated patients more often than other bacteria (46.34% vs 17.5% p < 0.001) [14]. Interestingly, the study of Ketter (2018) has determined that the human gastrointestinal tract has not the only function as a reservoir for MDR *A. baumannii* strains but also is linked to the development of drug resistance in them [15]. EN also create a favourable medium for bacterial growth and multiplication. On the other hand, EN is important for critically ill patients because...
it provides nutrition support and helps to prevent infectious complications, while the malnutrition leads to the weakening of the respiratory muscles, and complicates the weaning from MV.

The finding of previous studies on the relation between RBC transfusion and the development of VAP is also contradictory because it is difficult to confirm the causal link between RBC transfusion and VAP, as the risk of both RBC transfusion and nosocomial infections does increase with the prolonged LOS in ICU [16-17]. The earlier study of Shorr (2004) revealed the increased risk of VAP (OR 1.89), especially of late-onset one (OR 2.16) in transfused patients [16]. On the contrary, Engele (2017) didn’t identify a relationship between RBC transfusion and the development of nosocomial (51% of respiratory origin) infections [17]. The long-term treatment in the hospital was found to be related to the use broad spectrum antibiotic therapy, acquisition of MDR pathogens, and the risk of nosocomial infections. Moreover, the study of Garnacho-Montero has found a previous antibiotic use to be an independent predictor of acquisition of A.baumannii as a pathogen of VAP (OR 14, p < 0.001), though, the association of hospital LOS and VAP due to A.baumannii was observed only in univariable analysis [14]. In the small sample study of burn patients both the duration of the antibiotic therapy (OR 1.2,
p < 0.05) and the number of antibacterial classes used (OR 2.5, p<0.05) were found to be predictors of MDR *A.baumannii* infection [18]. Inchai (2015) have found that treatment with carbapenems has increased the risk of *A.baumannii* of each phenotypes of resistance (MDR, XDR, and PDR) [6]. The most prevalent risk factors for VAP in our analysis we have found to be continuous sedation, stress ulcer prophylaxis with PPI, exposure to the intravenous broad-spectrum antibiotics within the last 90 days (cephalosporins – 86.7%, penicillins with BLI –56.7%, carbapenems –30.0%), current hospitalization > 5 days, and RBC transfusion before VAP onset. These findings emphasize the importance of restrictive prescription of long-term sedative, gastric acid suppressive agents, also more responsible use of RBC transfusions in purpose to prevent VAP due to MDR *A.baumannii*. The tracheal colonisation preventive strategies (silver-impregnated tubes, subglottic suction, and oral decontamination), the early initiation of EN along with monitoring of possible delayed gastric emptying should be considered in MV patients. Overuse of multiple antibiotics should be avoided in clinical practice.

Recent VAP guidelines emphasize the role of regular generation and dissemination of local hospital antibiograms and empiric antibiotic regimens based upon the local distribution of pathogens associated with VAP and their antibacterial susceptibilities [5]. However, data on the resistance of *A.baumannii* strains actually causing VAP in different geographical regions are insufficient. We have found marginal resistance of *A.baumannii* strains to piperacillin/tazobactam, ciprofloxacin, carbapenems, and cephalosporins, but colistin. Interesting data on antibacterial resistance of bacteria belonging to *Acinetobacter calcoaceticus–Acinetobacter bauman- nii* (Acb) complex (mostly *A.baumannii*; 42.9% pneumonia cases) were presented in a long-term large-scale (259 centres) SENTRY study [3]. This study found none of the tested antibiotics to be able to inhibit the growth of all bacteria belonging to Acb complex. On all continents, they were most susceptible to colistin (from 93.9% in Europe to 98.1% in Latin America) and minocycline (from 70.1% in Europe to 91.1% in Latin America). The limited susceptibility to carbapenems was observed worldwide, and the susceptibility to meropenem (from 35.6% in Europe to 59.7% in North America) was slightly lower than to imipenem (from 39.4% in Europe to 63.9% in North America). The susceptibility to ampicillin-sulbactam ranged from 21.6% in Latin America to 54.7% in North America. Strains isolated from patients with pneumonia have been found to be less susceptible to carbapenems, minocycline, and colistin in comparison with strains isolated from blood. Moreover, pneumonia was more often caused by Acb complex bacteria of XDR phenotype (63.7%). Resistance of *A.baumannii* strains to carbapenems and ampicillin/sulbactam observed higher in our analysis than in SENTRY study might be explained by methodological differences: we have analysed selected cases of VAP due to *A.baumannii* of MDR phenotype, and most of they were of XDR phenotype, as colleagues have studied strains of all kinds of sensitivity.

VAP is characterised by high overall in-hospital mortality up to 70% in the case of MDR and XDR *A.baumannii* [19]. In our analysis, the early mortality rate was found to be almost twice higher (28.3%) than reported in Choi’s (2016) study (15%) [20]. This difference could be attributed to the fact that the treatment with colistin to which all tested *A.baumannii* strains were sensitive became available in our hospital only since March 2015, however in Choi’s study all enrolled patients have been treated with colistin for at least 3 days. The late mortality in our cohort was 55% similarly to the previous researches in this field [19]. Both early and late mortality was predicted by higher severity of illness by SOFA score on ICU admission, but only late one – by the treatment with > 3 different classes of antibiotics before VAP onset. Our findings are in line with previous studies: Choi identified the severity of illness (APACHE II score) as the prognostic factor for early mortality, Inchai and Tsioutis (SAPS II) – for a late one [6,19-20].

**Advantages and limitations of the study.** The main advantage of this study is the fact that this is, to our knowledge, the first study analysing the incidence of risk factors for VAP and their predictive relation with early and late mortality. The limitations of our study are limited sample size and single-centre retrospective design. Additionally, the incidence of other important VAP risk factors (disease severity score on VAP onset, the presence of ARDS) and their relation to mortality has been not analysed, as the analysis of patients’ data on VAP onset is the goal of our other ongoing extended study. The larger multicenter studies would be relevant.

**Conclusions**

Continuous sedation, stress ulcer prophylaxis with PPI, current hospitalisation ≥ 5 days, treatment with broad-spectrum antibiotics in preceding 90 days, and RBC transfusion were found to be the most prevalent risk factors for VAP due to MDR *A.baumannii*. Overall late mortality was found to be 55% as twice of early one. Both early and late mortality were predicted by SOFA score > 12 on ICU admission, but late one – by treatment with >3 classes of antibiotics before the onset of VAP due to MDR *A.baumannii*. Almost all strains of *A.baumannii* were resistant to piperacillin/tazo- bactam, ciprofloxacin, carbapenems and cephalosporins,
and none to colistin. Estimated twice higher late versus early mortality testifies about the aggressive nature of MDR A. baumannii in VAP. By outlook of our study, there is a clue to clinicians to increase a preventive alert and provide avoidance of risk factors for MDR A. baumannii (pathogen of VAP) as early as possible in purpose to start specific antimicrobial treatment and enable to predict high mortality more effectively. Thus recommendation for empirical use of colistin in VAP of suspected MDR A. baumannii is rational.

Declaration of interest statement. The authors report no conflicts of interest.

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https://doi.org/10.1016/j.ijantimicag.2016.07.007
https://doi.org/10.1016/j.ijantimicag.2016.05.011

DAUGINIO ATSPARUMO ANTIBIOTIKAMS ACINETOBACTER BAUMANNII SUKELTA VENTILIACINĖ PNEUMONIJA: RIZIKOS VEIKSIŲ PAPLITIMAS IR JŲ ĮTAKAANKSTYVAJAM BEI VĖLYVająM MIRŠTAMUMUI
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Raktažodžiai: A.baumannii, atsparumas antibiotikams, ventiliacinė pneumonija, rizikos veiksnių, mirštamumas.
Santrauka
Tikslas. Nustatyti ventiliacinių pneumonijos (VP) rizikos veiksniių paplitimą daugelio atsparumo antibiotikams (DAA) A.baumannii sukeltos VP pacientų, detalų A.baumannii padermių atsparumą antibiotikams, 7 d. (ankstyvaji) bei 28 d. (vėlyvaji) jų mirštamumą bei VP rizikos veiksniių gebą prognozuoti ankstyvaji bei vėlyvaji mirštamumą šiose pacientų kohortoje.
Rezultatai. VP rizikos veiksniių paplitimas 60 DAA A.baumannii VAP pacientų imtyje: nuolatinė sedacija ir stresinių opų profilaktika – 100%, gydymas antibiotikais per pastarąjį 90 d. – 98,3%, dabartinė hospitalizacija ≥ 5 d. – 95,0%, eritrocitų masės transfuzija – 60%, sepsis – 53,3%, mechaninės ventilacijos iki VP trukmė >8 d. ir SAPS II skalės įvertis > 50 hospitalizuojant į ITS – 50,0%. Visos A.baumannii padermių buvo atsparios piperaciliniui/tazobaktamui ir ciprofloksacinui, > 95% – karbapenemams ir cefalosporinams, 0% – kolistinui. Ankstyvasis mirštamumas siekė 28,3%, vėlyvasis – 55,0%. SOFA skalės įvertis >12 hospitalizuojant į ITS prognozuojant į VP ankstyvąjį (ŠS 7,5; 95% PI 2,052-27,408, p=0,002) ir vėlyvąjį (GS 4,089; 95% Pl 1,292-12,92, p=0,017) mirštamumą, o gydymas >3 klasės antibiotikais iki VP pradžios (GS 3,993; 95% PI 1,050-15,193, p=0,042) – tik vėlyvajį.
Išvados. Sunkesnė pacientų būklė pagal SOFA skalą hospitalizuojant į ITS prognozuojant tiek ankstyvaji, tiek ir vėlyvaji mirštamumą, o gydymas >3 klasės antibiotikais iki VP pradžios – tik vėlyvajį. Du kartus už ankstyvajį didesnis vėlyvasis mirštamumas užrodo agresyvų DAA A.baumannii eigą, todėl, VP sukeletų išvarta būtų A.baumannii, turėtų būti svarstoma empirinio gydymo kolistinu galimybė.
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