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Etiology and resistance patterns of bacteria causing ventilator-associated pneumonia in a respiratory intensive care unit

Uzročnici pneumonije udružene sa ventilatornom potporom bolesnika i njihova rezistencija na antibiotike u pulmološkoj jedinici intenzivnog lečenja

Vlada Injac*, Uroš Batranović†, Jovan Matijašević†‡, Marija Vukoja§, Mirjana Hadnadjev‖, Zoran Bukumirić¶, Goran Trajković¶, Slobodan Janković**

Hemofarm AD, *Research and Development Department, Belgrade, Serbia; Institute for Pulmonary Diseases of Vojvodina, Clinic for Urgent Pulmonology, †Intensive Care Unit, §Center for Pathophysiology of Breathing and Sleep Medicine, ‖Center for Microbiology, Virology and Immunology, Sremska Kamenica, Serbia; University of Novi Sad, ‡Faculty of Medicine, Novi Sad, Serbia; University of Belgrade, Faculty of Medicine, ¶Institute for Medical Statistics and Informatics, Belgrade, Serbia; University of Kragujevac, **Faculty of Medical Sciences, Kragujevac, Serbia

Abstract

Background/Aim. Ventilator-associated pneumonia (VAP) incidence, causative pathogens, and resistance patterns are different among countries and intensive care units (ICUs). In Europe, resistant organisms have progressively increased in the last decade. However, there is a lack of data from Serbian ICUs. The aims of this study were to evaluate etiology and antimicrobial resistance for pathogens causing VAP in ICU patients, to examine whether there were differences among pathogens in early-onset and late-onset VAP and to identify mortality in patients with VAP after 30 and 60 days of hospitalization. **Methods.** A retrospective cohort study was conducted in the respiratory ICU and all adult patients diagnosed with VAP from 2009 to 2014 were included. **Results.** Gram negative organisms were the major pathogens (80.3%). The most commonly isolated was *Acinetobacter* spp (59.8%). There was a statistically significant increase in the incidence of infection with *Klebsiella pneumoniae* (8.9% vs 25.6%; $p = 0.019$). Extensively drug-resistant strains (XDR) were the most common (78.7%). Late-onset VAP was developed in 81.1% of patients without differences among pathogens in comparison with early-onset VAP. *Acinetobacter* spp was susceptible to tigecycline and colistin with a significant increase in resistance to ampicillin/sulbactam (30.2% vs 58.6%; $p = 0.01$). Resistance rate of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* to carbapenems was 38% and 11%, respectively. In methicillin-resistant *Staphylococcus aureus* no resistance was observed against vancomycin and linezolid. There was no difference in mortality rate between patients with early-onset and late-onset VAP after 30 and 60 days of hospitalization. **Conclusion.** Gram negative organisms were the primary cause of bacterial VAP of which the most common was the XDR strain of *Acinetobacter* spp. Patients with early- and late-onset VAP had the same pathogens. There was no difference in mortality between this two group of patients during 60 days of hospitalization.

Key words:
pneumonia; cross infection; anti-bacterial agents; drug resistance, bacterial; respiration, artificial; mortality.

Apstrakt

Uvod/Cilj. Incidencija pneumonije udružene sa ventilatornom potporom bolesnika (VAP), njeni uzročnici i njihova rezistencija razlikuju se između zemalja i jedinica intenzivne nege (JIN). U Evropi je u poslednjih deset godina došlo do progresivnog porasta rezistentnih bakterija. Međutim, ne postoji dovoljno podataka za JIN u Srbiji. Ciljevi rada bili su da se ispita etiologija i rezistencija uzročnika VAP na antibiotike u JIN, da se ispita da li postoji razlika između uzročnika ranog i kasnog VAP i da se utvrdi letalitet kod bolesnika sa VAP nakon 30 i 60 dana hospitalizacije. **Metode.** Retrospektivno kohortno ispitivanje je bilo sprovedeno u pulmološkoj JIN. Bili su uključeni svi odrasli bolesnici sa dijagnostikovanim VAP od 2009. do 2014. godine. **Rezultati.** Glavni uzročnici VAP bili su gram negativne bakterije (80,3%). Najčešće je bio izolovan *Acinetobacter* spp (59,8%). Zabeležen je statistički značajan porast incidencije oboljevanja usled *Klebsiella pneumoniae* (8,9% vs 25,6%; $p = 0,019$). Najzastupljeniji su bili ekstremno rezistentni (XDR) sojevi bakterija (78,7%). Kasni VAP je dijagnostikovan kod 81,1% bolesnika bez razlike u patogenima u poređenju sa ranim VAP. *Acinetobacter* spp je bio osetljiv na tigeciklin i kolistin uz statistički značajan porast rezistencije na ampicilin/sulbaktam (30,2% vs 58,6%; $p = 0,01$). Rezistencija *Pseudomonas aeruginosa* i *Klebsiella pneumoniae* na karbapeneme iznosila

Correspondence to: Vlada Injac, Hemofarm AD, Research and Development Department, Prote Mateje 70, 11 000 Belgrade, Serbia.
E-mail: vlada.cekic@yahoo.com

je 38%, odnosno 11%. Kod meticilin-rezistentnog *Staphylococcus aureus* nije postojala rezistencija na vankomicin i linezolid. Nisu utvrđene razlike u letalitetu između bolesnika sa ranim i kasnim VAP posle 30 i 60 dana hospitalizacije. **Zaključak.** Gram negativne bakterije bile su glavni uzročnici VAP, od kojih je najzastupljeniji bio XDR soj *Acinetobacter* spp. Bolesnici sa ranim i kasnim VAP imali su iste uzročnike. Nije bilo razlike u letalitetu između te dve grupe bolesnika tokom 60 dana hospitalizacije.

Ključne reči:
pneumonija; infekcija, intrahospitalna; antibiotici; lekovi, rezistencija bakterija; disanje, mehaničko; mortalitet.

Introduction

Ventilator-associated pneumonia (VAP) is the most common nosocomial infection in patients receiving mechanical ventilation. VAP remains a major cause of morbidity and mortality among critically ill patients and accounts for more than one-half of all antibiotic use in an Intensive Care Unit (ICU) [1]. The estimated incidence of VAP is 9% to 27%, with a mortality rate of 25% to 50% [2]. The appropriateness of empirical antimicrobial therapy for VAP is a key determinant of patient outcome [2]. Changes in pathogen distribution and patterns of antibiotic resistance complicate antibiotic treatment and care of the patients. Duration of mechanical ventilation was found to be one of the most important factors determining the composition of offending VAP pathogens [3]. Whereas early-onset VAP is more likely to be caused by antibiotic-sensitive bacteria, late-onset VAP is more likely to be caused by multi-drug resistant (MDR) pathogens. However, studies with opposite results were published recently [4–7], which showed that bacteriology of VAP may not follow a pattern of early *versus* late infection, particularly in patients that are at risk for MDR infections [8].

The incidence of infection with specific pathogens with different susceptibility patterns causing VAP may not only vary from hospital to hospital but also within the same hospital or ICU over time [8]. This is the reason why empiric initial antibiotic treatment for VAP should be based on general guidelines, but also on up-to-date information on local epidemiology [8]. There is a lack of National registry consisting data from Serbian ICUs in regard to the local microbiological profile of pathogens causing VAP as well as to their antibiotic susceptibility and resistance patterns.

Given the scarcity of data, the primary aim of the present study was to evaluate etiology and antimicrobial resistance trends for nosocomial pathogens causing VAP in respiratory ICU patients and to examine whether there were differences among pathogens in early-onset and late-onset VAP. The aim of the study was also to identify mortality in patients with VAP after 30 and 60 days of hospitalization.

Methods

Study design

A retrospective cohort study was conducted in the 5-bed respiratory ICU of the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica (Serbia). All adult patients diagnosed with VAP from January 2009 to December 2014, were included. This study was approved by the Hospital Ethics Committee. In order to evaluate changes in the incidence of infection with pathogens and changes in their resistance pattern, 6 year period was divided into two separate periods: period I (January 2009 to December 2011) and period II (January 2012 to December 2014).

Definitions

VAP was diagnosed in the presence of a new or persistent (≥ 48 hours) and progressive radiographic infiltrate plus at least 2 of the following: temperature of ≥ 38ºC or < 35ºC; purulent tracheal secretions or a change in characteristics of sputum; or leucocytosis (> 10.000 white blood cells/mm^3) or leucopenia (< 4.000 white blood cells/mm^3) [9, 10]. After clinical diagnosis of VAP had been established, quantitative culture of endotracheal aspirate (ETA) was performed to identify VAP pathogens. Only pathogens isolated at a concentration of > 10^6 colony-forming units (CFU/mL) was considered causative of VAP [9]. VAP was classified by the onset of the disease as early-onset VAP, which occurred within the first 4 days and late-onset VAP, which developed more than 4 days after starting mechanical ventilation (MV) [3].

If patients met clinical criteria for VAP, antibiotic therapy was initiated empirically according to primary diagnosis, comorbidities, prior antibiotic exposure, duration of the previous hospitalization of the patient, and the result of the surveillance cultures.

Multidrug resistance (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. Extensively drug-resistance (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories). Pandrug-resistance (PDR) was defined as non-susceptibility to all agents in all antimicrobial categories [11]. Standardized definitions were used to determine the presence or absence of ICU complications including acute respiratory distress syndrome (ARDS) [10], severe sepsis and septic shock [12].

Data collection

Demographic data, comorbidities, Charlson comorbidity score, Simplified Acute Physiology Score II (SAPS II), Acute Physiology and Chronic Health Evaluation II (APACHE II), the Sequential Organ Failure Assessment (SOFA) score at ICU admission, admission diagnosis of the patients, reason for endotracheal intubation, prior antibiotic use, microorganisms of VAP, antibiotic susceptibility, lengths of ICU and hospital stays and duration of MV prior to VAP onset were recorded. All patients were followed-up for survival status until 60 days after the initial onset of VAP or until death (if patients died within 60 days). The overall 30-day and 60-day mortalities were recorded. Only the first VAP episode was evaluated.

Microbiology

The antibiotic susceptibility of clinical isolates was determined by the Kirby-Bauer disk diffusion method and, if required, E-test, and analyzed according to the Clinical and Laboratory Standards Institute 2013 document. Identification of pathogens and the antibiotic susceptibility was performed in the Center for Microbiology, Virology and Immunology, Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Serbia.

Statistical analysis

Data entry and analysis were performed using IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA). A descriptive analysis was used to investigate patient demographics and ICU data. The median with range was employed for not-normally distributed continuous variables, and mean ± standard deviation (SD) was used for normally distributed continuous variables. Numbers and percentages were used when applicable. The χ^2 test and Fisher's exact test were employed for testing differences in values of dichotomous variables among study groups, Kruskal-Wallis and Mann-Whitney U tests were used for continuous variables with not-normal distribution. The level of significance was set at $p < 0.05$.

Results

During the study period, 844 adult patients were admitted to the ICU. VAP developed in 144 (17%) patients during the ICU stay. Twenty two patients were excluded from the analysis (lack of data). So, the final analysis of bacterial etiology included 122 patients/ETA samples (78 males and 44 females) with 141 bacterial species isolated. The incidence of VAP was 47.3 cases per 1,000 ventilator days. The mean age of the patients was 56.8 ± 14.6 years. The most common comorbidities of the VAP patients were hypertension (50.8%), other cardiovascular diseases (41.8%) and the chronic obstructive pulmonary disease (COPD) (28.7%). The major reasons for intubation were as following: ARDS (39.3%), pneumonia (34.4%), cardiac arrest (8.2%), and exacerbation of COPD (6.6%). Clinical characteristics of analyzed patients on admission at ICU are shown in Table 1.

Median length of ICU stay was 19 days (4–85) and of hospital stay 30 (4–112) days. Out of 122 patients that developed VAP, 50 (41.0%) died. The patients who did not survive after 60 days were significantly older than the patients who survived (60.6 ± 14.5 years and 53.2 ± 13.8 years respectively; $p = 0.004$). Monomicrobial infection occurred in 103 of 122 (84.4%) patients. Isolated pathogens and mortality rates are shown in Table 2. Almost all isolate were bacteria; the fungal infection (*Candida albicans*) was reported in one patient and *Stenotrophomonas maltophilia* was isolated in one patient (0.8%) in this study (Table 2).

Early-onset VAP was diagnosed in 23 patients (18.9%) and median of onset was 4 (2–4) days of mechanical ventilation. Late-onset VAP was diagnosed in 99 (81.1%) patients and median of onset was 9 (5–27) days of MV. Mortality after 30 days (47.8% vs 39.4%; $p = 0.46$) and 60 days (47.8% vs 49.5%; $p = 0.89$) was not different in patients with early-onset and late-onset VAP, respectively. The pattern of isolates according to the type of VAP is shown in Table 3.

Figure 1 demonstrates the proportion of drug susceptibility of each microorganism.

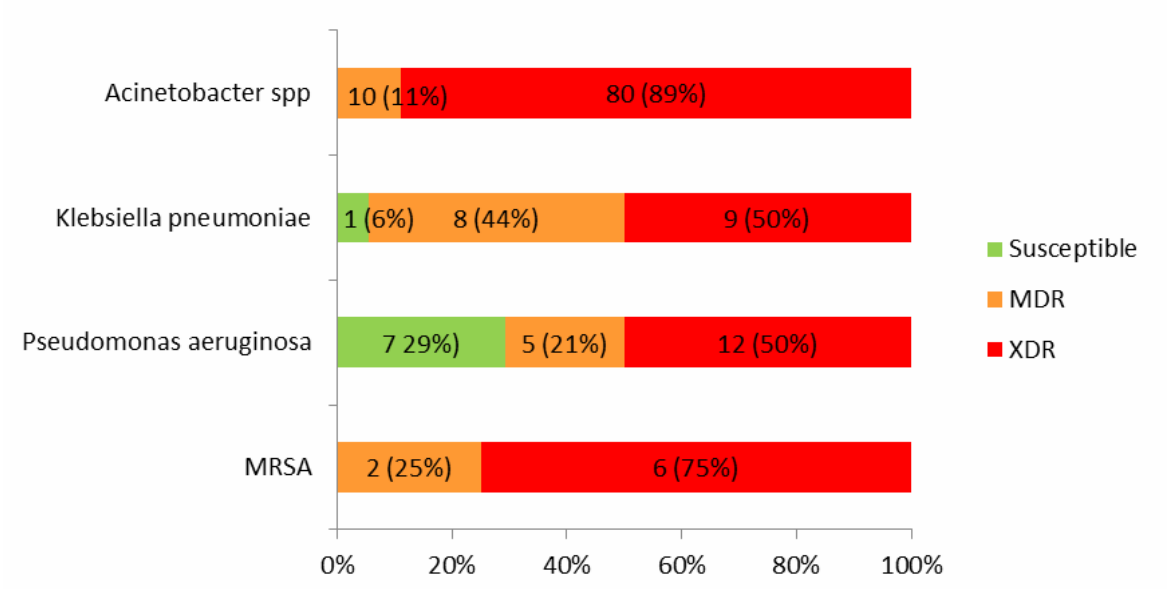

Fig. 1 – Percentage of drug susceptibility patterns for each microorganism.
MDR – multidrug resistant; XDR – extensively drug resistant; MRSA – methicillin-resistant *Staphylococcus aureus*.

Table 1

Characteristics of ventilator-associoted pneumonia (VAP) patients at intensive care unit (ICU) admission

Parameters	Value
Number of patients (n)	122
Age (year), mean ± SD	56.8 ± 14.6
Gender, n (%)	
male	78 (63.9)
Body mass index (kg/m^2), n (%)	
underweight (< 18.5)	5 (4.5)
normal weight (18.5–24.9)	44 (39.3)
overweight (25–29.9)	35 (31.3)
obese (> 30)	28 (25)
Smoking habit, n (%)	61 (63,5)
APACHE II score, mean ± SD	23.34 ± 7.03
SAPS II score, mean ± SD	47.92 ± 13.87
SOFA score, mean ± SD	8.39 ± 3.03
Patient's location before ICU, n (%)	
emergency room	22 (18.0)
ward in same hospital	55 (45.1)
other hospital	45 (36.9)
Duration of previous hospital stay if no direct ICU: admission (days), median (range)	2 (0–65)
Comorbidities, n (%)	
hypertension	62 (50.8)
ischemic heart disease	16 (13.1)
other cardiac diseases	51 (41.8)
cerebrovascular diseases	4 (3.3)
COPD	35 (28.7)
other chronic respiratory disease	22 (18)
diabetes mellitus	30 (24.6)
chronic renal disease	13 (10.7)
chronic liver disease	8 (6.6)
immunodeficiency[a] and cancer	17 (13.9)
gastrointestinal diseases	25 (20.5)
neurologic diseases[b]	10 (8.2)
psychiatric diseases and addiction disorders	26 (21.3)
other diseases	34 (27.9)
Diagnosis at ICU admission, n (%)	
CAP	68 (55.7)
HAP	15 (12.3)
HCAP	9 (7.4)
viral pneumonia	17 (13.9)
exacerbation of COPD	13 (10.7)
ARDS	53 (43.7)
pulmonary thromboembolism	4 (3.3)
severe sepsis	118 (96.7)
MODS	60 (49.2)
septic shock	44 (36.1)
acute intoxication	4 (3.3)
cardiorespiratory arrest	10 (8.2)
H1N1 infection	19 (15.6)
acute renal failure	16 (13.1)
Charlson comorbidity index, median (range)	3 (0–11)
Reason for MV, n (%)	
pneumonia	42 (34.4)
ARDS	48 (39.3)
COPD	8 (6.6)
cardiorespiratory arrest	10 (8.2)
pulmonary edema	3 (2.5)
thromboembolism	3 (2.5)
intoxication	4 (3.3)
septic shock	5 (4.1)
Prior antibiotic use[c], n (%)	79 (69.3)
cephalosporins	52 (45.6)
fluoroquinolones	24 (21.1)
macrolides	28 (24.6)
carbapenem (imipenem or meropenem)	10 (8.8)
aminoglycosides	14 (12.3)

APACHE II – Acute Physiology and Chronic Health Evaluation II; SAPS II – Simplified Acute Physiology Score II; SOFA – Sequential Organ Failure Assessment; COPD – Chronic Obstructive Pulmonary Disease; CAP – community acquired pneumonia; HAP – hospital acquired pneumonia; HCAP – health care associated pneumonia; ARDS – acute respiratory distress syndrome; MV – mechanical ventilaton; MODS – multiple organ dysfunction syndrome, SD – standard deviation.

[a]Immunodeficiency including acute rheumatoid arthritis, HIV+, Sjogren's syndrome and immunosuppressant; [b]Other commorbidities – fractures, thyroid gland diseases, osteoporosis and anemia; [c]Antibiotic treatment during the two weeks preceding ICU admission.

Table 2

Pathogens of ventilator-associated pneumonia (VAP) patients and their susceptibility pattern compared between survivor and non-survivor groups after 30 and 60 days

Organism types	Number of patients (n = 122)	Mortality after 30 days			Mortality after 60 days		
		non-survivors (n = 50)	survivors (n = 72)	*p*	non-survivors (n = 60)	survivor (n = 62)	*p*
Monomicrobial VAP, n (%)	103 (84.4)	42 (40.8)	61 (59.2)	0.91	52 (50.5)	51 (49.5)	0.50
Gram-positive (MRSA)	5 (4.1)	3 (60.0)	2 (40.0)	0.40	4 (80.0)	1 (20.0)	0.20
Gram-negative	98 (80.3)	39 (39.8)	59 (60.2)	0.59	48 (49.0)	50 (51.0)	0.93
Acinetobacter spp	73 (59.8)	31 (42.5)	42 (57.5)	0.69	38 (52.1)	35 (47.9)	0.44
Pseudomonas aeruginosa	14 (11.5)	4 (28.6)	10 (71.4)	0.32	5 (35.7)	9 (64.3)	0.28
Klebsiella pneumoniae	10 (8.2)	3 (30)	7 (70)	0.46	4 (40)	6 (60)	0.54
Polymicrobial VAP, n (%)	19 (15.6)	8 (42.1)	11 (57.9)	0.91	8 (42.1)	11 (57.9)	0.50
Acinetobacter spp *plus Pseudomonas aeruginosa*	8 (6.6)	3 (37.5)	5 (62.5)	1.00	3 (37.5)	5 (62.5)	0.72
Acinetobacter spp *plus Klebsiella pneumoniae*	5 (4.1)	3 (60.0)	2 (40.0)	0.40	3 (60.0)	2 (40.0)	0.68
Acinetobacter spp *plus* MRSA	3 (2.5)	1 (33.1)	2 (66.7)	1.00	1 (33.1)	2 (66.7)	1.00
Klebsiella pneumoniae plus Pseudomonas aeruginosa	2 (1.6)	0 (0)	2 (100)	0.51	0 (0)	2 (100)	0.50
Susceptibility pattern							
susceptible	5 (4.1)	2 (40.0)	3 (60.0)	1.00	2 (40.0)	3 (60.0)	1.00
MDR organisms	21 (17.2)	6 (28.6)	15 (71.4)	0.20	9 (42.9)	12 (57.1)	0.52
XDR organisms	96 (78.7)	42 (43.8)	54 (56.2)	0.23	49 (51.0)	47 (49.0)	0.43

MRSA – methicillin-resistant *Staphylococcus aureus;* MDR – multi-drug resistant pathogens; XDR – extensively drug-resistant pathogenus.

Table 3

Bacterial species isolated from endotracheal aspirate (ETA) samples in ventilator-associated pneumonia (VAP) patients

Bacterial species	Total (n = 122)	Early-onset VAP (n = 23)	Late-onset VAP (n = 99)	*p*
Monomicrobial VAP, n (%)	103 (84.4)	18 (78.3)	85 (85.9)	0.37
Gram-positive (MRSA), n (%)	5 (4.1)	1 (4.3)	4 (4.0)	0.95
Gram-negative, n (%)	98 (80.3)	17 (73.9)	81 (81.8)	0.39
Acinetobacter spp	73 (59.8)	13 (56.5)	60 (60.6)	0.72
Pseudomonas aeruginosa	14 (11.5)	1 (4.3)	13 (13.1)	0.23
Klebsiella pneumoniae	10 (8.2)	3 (13.0)	7 (7.1)	0.35
Polymicrobial VAP, n (%)	19 (15.6)	5 (21.7)	14 (14.1)	0.37
Acinetobacter spp *plus Pseudomonas aeruginosa*	8 (6.6)	0 (0)	8 (8.1)	0.16
Acinetobacter spp *plus Klebsiella pneumoniae*	5 (4.1)	3 (13)	2 (2)	0.05
Acinetobacter spp *plus MRSA*	3 (2.5)	1 (4.3)	2 (2)	0.47
Klebsiella pneumoniae plus Pseudomonas aeruginosa	2 (1.6)	0 (0)	2 (2.0)	1.00

MRSA – methicillin-resistant *Staphylococcus aureus.*

The resistance of all *Acinetobacter* isolates was high to piperacillin/tazobactam (99%), ciprofloxacin (97%), carbapenems (95% to imipenem and 96% to meropenem), ceftazidime (92%), cotrimoxazole (88%) and gentamicin (87%). Among the 4th generation of cefalosporins, the resistance was lower to cefepime (72%) and among aminoglycosides to netilmicin (67%). *Acinetobacter* spp was not tested for amikacin in 52 (58%) cases, however, all isolates which were tested (38/90) were resistant to amikacin (42%). Thirty six percent of *Acinetobacter* spp isolates were resistant to ampicillin-sulbactam. Monitoring of total resistance of isolates showed that there was no resistance to colistin, while resistance to tigecycline was 7%. Seventy three percent of *Pseudomonas aeruginosa* isolates were resistant to gentamicin, 67% to ciprofloxacin, 57% to meropenem, 55% to piperacillin, 42% to piperacillin/tazobactam, 52% to ceftazidime, 41% to amikacin, 38% to cefepime and 35% to imipenem. The highest resistance of *Klebsiella pneumoniae* (*K. pneumoniae*) was observed against β-lactams (100% isolates were resistant to ampicillin and 94% to ampicilin/sulbactam); among 3rd and 4th generation cephalosporins 93% isolates were resistant to ceftriaxone, 89% to cefotaxime, 94% to ceftazidime and cefepime), and then against ciprofloxacin (94%) and cotrimoxazole (93%). However, it was relatively less resistant to piperacillin-tazobactam (47%), gentamicin (44%) and amikacin (22%). Among carbapenems, 38% isolates were resistant to ertapenem, 11% to imipenem and 11% to meropenem. In methicillin-resistant *Staphylococcus aureus* (MRSA) isolates no resistance was observed against vancomycin, teicoplanin and linezolid.

There were no differences in the incidence of infection with *Acinetobacter* spp, *Pseudomonas aeruginosa* and MRSA between period I (2009–2011) and period II (2012–

2014), but there was a statistically significant increase in incidence of infection with *K. pneumoniae* from 8.9% in period I to 25.6% in period II ($p = 0.019$). A significant increase in resistance rates of *Acinetobacter* spp to ampicillin/sulbactam in period II (2012–2014) was also found (30.2% vs 58.6%; $p = 0.01$).

The resistance of Acinetobacter spp to other tested antibiotics did not change significantly between the two studied periods. The resistance of *Pseudomonas aeruginosa* to cefepime, piperacillin/tazobactam and amikacin increased between the two studied periods, without a statistically significant difference, but it increased significantly to imipenem (20% vs 71.4% respectively; $p = 0.052$). The resistance of *K. pneumoniae* increased, especially to carbapenems (ertapenem: 0% vs 54.5%; $p = 0.09$), but due to the small sample statistical significance could not be detected. During the two studied periods there was no difference in incidence of infection with XDR (75.9% vs 83.7%; $p = 0.317$) and MDR strains (19% vs 14%; $p = 0.482$) in all isolated bacteria.

Discussion

Multiresistant bacteria pose a great threat and challenge in everyday clinical practice. That is why regular monitoring of susceptibility of isolated bacteria to antibiotics in each ICU is essential. These data, as well as data regarding patient's characteristics on admission to ICU will help determine the most efficient empirical therapy for VAP. Empirical therapy based on data on local resistance has an impact on lowering morbidity and mortality, shortening of hospitalization, lowering of treatment expenses, and prevents the development of MDR bacteria in patients with VAP [13, 14].

The overall incidence of VAP was 47.3 per 1,000 ventilator-days. The incidence rate of VAP ranges from 13.2 to 51 per 1,000 ventilator days [9]. In a study performed in surgical and medical ICUs in a tertiary hospital in China it was found that the incidence of VAP in the medical ICU was 29.7 cases per 1,000 ventilator days [15]. Differences in infection control practices and lack of established infection control programs may account for higher rates.

VAP caused mortality varies (25%–50%) and greater mortality has been noticed in infections caused by *Pseudomonas* and *Acinetobacter*, in medical ICUs compared to surgical ones, and with the administration of inadequate empirical antibiotic therapy [3]. In our study, mortality in patients with VAP was 41.0% after 30 days, and 49.2 % after 60 days. In a study by Song et al. [15] mortality in VAP patients in medical ICU was 41.9% after 30 days, and 53.5% after 60 days. One of the significant characteristics of our patients on admission to ICU was a high incidence of ARDS (43.7%). A number of clinical studies have shown that pulmonary infection is very frequent in patients with ARDS (34–70%) and often leads to sepsis, multiorgan dysfunction and death [16]. In a study by Forel et al. [17], mortality in patients with ARDS and VAP was 41.8%.

According to American Thoracic Society, empirical antibiotic therapy in VAP treatment should be based on VAP onset time (early-onset *vs* late-onset VAP) and on the presence of the risk factors for the development of MDR bacteria [3]. In our study, most of the patients had late-onset VAP (81.1%) and there was no difference among pathogens causing early-onset and late-onset VAP. These results were expected since most of our patients had a number of risk factors which predispose to the colonization with MDR bacteria (69.3% of patients had received antibiotic treatment prior to admission, 82% was previously hospitalized). Evidence suggests that resistance is a problem also in patients with early-onset VAP is growing. In a study conducted in Serbia in multidisciplinary ICU, no difference was shown among isolated bacteria in patients with early-onset and late-onset VAP [18]. It is interesting that prospective multicenter study in 27 ICUs in 9 European countries showed that even in patients who do not have classic risk factors for MDR bacteria, in 50.7% VAP was caused by multiresistant bacteria [5]. These results speak in favor of the fact that microbiology does no longer follow model "early-onset *vs* late-onset VAP" what presents a new problem for empirical therapy [19]. Our study also showed there was no difference in mortality in patients with early-onset and late-onset VAP after 30 days and 60 days suggesting that VAP onset time is not mortality predictor. Other researchers who compared mortality in patients with early-onset and late-onset pneumonia, came to the similar results [20–23].

In our study, gram negative bacteria were the main pathogens of VAP (80.3%) which is in correlation with the results from other recently published studies, especially from the developing countries. In a study by Chittawatanarat et al. [24] gram negative bacteria caused VAP in 94.7% of cases. During the past ten years, the significance of *Acinetobacter* spp has increased due to the rapid spreading of the strains resistant to the most of the antibiotics, which was noticed throughout the world [25]. One of the important features of *Acinobacter baumannii* (*A. baumannii*) is its ability to survive for a longer time period on the surfaces around patient from where is transmitted to patients in the direct or indirect way. In our study, *Acinetobacter* spp was the most frequent VAP pathogen (59.8%), and the most common XDR strain (89%). Studies in Asian countries have shown that *A. baumanii* was the most frequent pathogen in mixed medical-surgical ICUs where the incidence of infection was between 25% and 50% [24, 26]. In the neighboring countries, a high incidence of infection with MDR *A. baumannii* (85.6%) and XDR (14.4%) was also noticed [27]. Having in mind risk factors for infections caused by multiresistant *Acinetobacter* spp (ARDS, septic shock, severity of the disease, previous use of broad spectrum antibiotics, previous hospitalisation, duration of ICU stay and contamination of patient's surroundings) [28, 29], significant presence of *Acinetobacter* spp in our ICU (frequency of ARDS – 43.7% and septic shock on admission – 36.1%, previous antibiotic therapy – 69.3%, previous hospitalisation – 82%) can be partially explained. Although carbapenems are considered to be a basic therapy in the treatment of *A. baumannii*, during the last years reporting of strains resistant to carbapenems is increased throughout the world [30]. In our study, *Acinetobacter* spp showed a high degree of resistance to carbapenems (more than 95%). According to the Annual Report of the European Antimicrobial Resistance Surveillance Network for 2014, resistance to carbapenems was from

0% (the Netherlands) to 93.2% (Greece) [31]. Šuljagić et al. [32] in their study analyzed and compared the surveillance data on *Acinetobacter* spp nosocomial colonization/infection collected during the wartime with the data collected in peacetime in surgical clinics of the Military Medical Academy in Belgrade (Serbia). Their data showed that resistance of *Acinetobacter* spp to imipenem was 0% during wartime (1999) and 18.6% during peacetime (2001), and to meropenem 4.6% and 27.1%, respectively. Having in mind these data, pronounced increase of *Acinetobacter* spp resistance to carbapenems in our region during the last 15 years is obvious. In the study by Šuljagić et al. [32] *Acinetobacter* spp resistance to ampicillin/sulbactam was 30% what is similar to the results of our study. However, in our study the significant increase of resistance to ampicillin/sulbactam was noticed between two study periods (30.2% vs 58.6%; $p = 0.012$). The increase of resistance was also marked in the neighboring countries. In Croatia, resistance of *Acinetobacter* spp to ampicillin/sulbactam increased from 13% (2009) to 19% (2012) [33]. In our ICU resistance to colistin was not detected, although it was noticed during the last years in Europe (4%), and countries with the highest resistance to colistin were Greece and Italy [31]. The assumed reason that resistance to colistin was not detected in this study is that the colistin was just approved in Serbia for hospital use in 2013, and in this paper data analyzed was ending with the year 2014.

In our study the resistance of *Pseudomonas aeruginosa* to piperacillin/tazobactam, ciprofloxacin, ceftazidime and aminoglycosides was high in comparison with the latest data on resistance in other European countries. The highest resistance of *Pseudomonas aeruginosa* was marked in Romania (62.2% to piperacillin/tazobactam, 55.4% to ciprofloxacin, 59.1% to ceftazidime, 63.4% to aminoglycosides), and the lowest in Denmark, Iceland and Luxembourg (0%–4.4%) [31]. Besides, the resistance of *Pseudomonas aeruginosa* to imipenem was grown significantly between the two studied periods. The trend of resistance increase to carbapenems from 2011 to 2014 was also noticed in Germany, Hungary and Slovakia [31].

In our study significant increase in the incidence of infection with *K. penumoniae* as a pathogen of VAP between the two study periods was noticed (3.8% vs 16.3%; $p = 0.02$). This increase was due to the statistically significant increase of XDR strain of *K. pneumoniae* (3.8% vs 14.0%; $p = 0.040$). For the first time, XDR strain of *K. pneumoniae* (sensitive only to aminoglycosides and carbapenems) was noticed in December 2010. The resistance of *K. pneumoniae* has also increased to imipenem, meropenem i ertapenem between the two studied periods (0% vs 18.2%; 18.2% vs 54.5%, respectively), but statistically significant difference could not be detected. The three European countries with the highest marked resistance to carbapenems in 2014 were Greece (62.3%), Italy (32.9%) and Romania (31.5%). In these countries the greatest percent of *K. pneumoniae* resistant to polymyxins was noticed, indicating that the situation is very worrying [31].

All cases of MRSA caused VAP (5/122) were marked in 2009 and all were sensitive to linezolid and vancomycin. Reports from other European countries also show a decrease of MRSA from 2011 to 2014 from 18.6% to 17.4%, respectively [31].

Empirical treatment of VAP with the high probability of MDR pathogens is one of the greatest challenges met by the intensive care specialists. Having in mind the results of our study, adequate empirical therapy of VAP would be colistin (because of the high prevalence of XDR *Acinetobacter* spp) plus imipenem (because of *Pseudomonas aeruginosa* and *K. pneumoniae*) plus vancomycin (because of MRSA). But, due to the increased use of colistin PDR strains of *Acinetobacter* spp can develop, and that is why its use must be rational [29]. After the short duration of broad spectrum antibiotic therapy and after the antibiogram is obtained, quick deescalation of antibiotic therapy should follow [34].

The significance of this study is in a long monitoring period and therefore obtaining insight in resistance changes of VAP pathogens during the time. Besides, this is the first analysis of VAP pathogens in our ICU.

There are several limitations of this study. The first one is that the bacterial isolates from the VAP cases may not reflect the true etiologic pathogens because more specific diagnostic procedures, such as bronchoalveolar lavage, were not performed. The second limitation is that it was performed in one ICU of the tertiary health facility specialized in treating pulmonary patients. Therefore, the microbiological profile of the isolated strains of VAP pathogens reflects the local epidemiologic situation and it can not be extrapolated to other centres. That is why multicentric research that would help forming general recommendation is necessary. Also, it is necessary to continue regular monitoring of microbiological profile and susceptibility of the pathogens in our ICU in order to timely detect changes, especially today when PDR strains are marked in southeast Europe.

Conclusion

According to results obtained in this study, gram negative bacteria were the main pathogens of ventilator associated pneumonia, out of which the most common was XDR strain of *Acinetobacter* spp with a high resistance to all tested antibiotics except for colistin and tigecycline. There was no difference in pathogens in patients with early-onset and late-onset ventilator associated pneumonia. Mortality in patients with VAP was 41.0% after 30 days and 49.2 % after 60 days of hospitalization, and there was no difference in mortality between patients with early-onset and late-onset VAP.

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