ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF ACINETOBACTER SPP IN THE PERIOD 2012 - 2015

Sandra STEFAN MIKIĆ

Summary

Introduction. Representatives of the genus Acinetobacter have become an important cause of hospital-acquired infections due to their great ability to survive and spread in a hospital environment, as well as rapid development of resistance to many antibiotics. The aim of this study was to determine the incidence of nosocomial infections caused by Acinetobacter spp among patients hospitalized at the Clinic of Infectious Diseases of the Clinical Center of Vojvodina; determine the presence and prevalence of resistance to antimicrobials in strains Acinetobacter spp, isolated from patient materials routinely sampled at the Clinic of Infectious Diseases of the Clinical Center of Vojvodina in the period January 1, 2012 to December 31, 2015. Material and Methods. A retrospective study included 1.673 patients with infectious diseases of bacterial etiology treated at the Clinic of Infectious Diseases of the Clinical Center of Vojvodina. The analysis of blood cultures, urine, cerebrospinal fluid culture, wound/decubitus swab, throat swabs, cannula/tube swabs, and bronchial aspirate was performed to establish the incidence of infections caused by Acinetobacter spp and antimicrobial resistance. Results. During the four-year research, Acinetobacter spp was isolated from blood samples in 14/260 (5.4%), urine in 6/198 (3.0%), cerebrospinal fluid in 2/43 (4.7%), wound/decubitus swabs in 33/128 (25.8%), throat swabs in 14/124 (11.3%) cannula/tube swabs and bronchial aspirate in 32/72 (44.4%) samples. The isolates of Acinetobacter spp showed the highest susceptibility to colistin (100%). Resistance to carbapenems and piperacillin/tazobactam accounted for nearly 100% in all tested isolates, while resistance to other antibiotics was over 63.6%, except to tobramycin whose resistance accounted for 11.1%. Conclusion. Representatives of the genus Acinetobacter are a common cause of nosocomial infections in hospitalized patients. Acinetobacter spp is only sensitive to colistin (100%), while it is resistant in various percentages to all other tested antibiotics. Key words: Microbial Sensitivity Tests; Acinetobacter; Anti-Infective Agents; Cross Infection

Introduc"
to three or more groups of antimicrobial drugs. Cross-resistant bacteria are those that have developed resistance to a variety of drugs that have a similar mechanism of action [2]. The main cause for development of antibiotic resistance is irrational use of antibiotics [3].

Representatives of the genus Acinetobacter are strictly aerobic, gram-negative, immobile (Greek “akinetos” - immobile), catalase positive, indole negative, non-fermentable coccobacilli. Within this genus there are over 20 different species, the most important among them - Acinetobacter calcoaceticus-baumannii complex which is responsible for most human infections [4]. Most of the clinical isolates of Acinetobacter baumannii, which cause infections of hospitalized patients, using genotyping methods, are divided into three European (international) clones, of which the European clone I (EU I) and European clone II (EU II) are most dominant. The permeability of the outer membrane layer of Acinetobacter baumannii (less than 5% in comparison to other gram-negative microorganisms), congenital decreased susceptibility to antibiotics, in combination with rapid acquisition of resistance mechanisms using mutation or genetic elements (plasmids, transposons and insertion sequences) are responsible for rapid emergence of multi-resistant isolates. Another important feature of this microorganism is the ability to survive in wet and dry conditions up to 4 months, with the ability to form a biofilm which plays a significant role. Survival of isolates that have such capability is twice as long (36 vs. 15 days) in comparison to isolates which are not capable to form a biofilm. Isolates that are capable to form biofilms are multiresistant isolates, because a biofilm is a community of well-structured microcolonies which has the ability for long-term survival and causes changes to the genetic material. Colonization by Acinetobacter baumannii precedes the biofilm formation, often in intensive care units [5].

Acinetobacter may be part of normal bacterial flora of the skin and mucous membranes or various secretions of healthy people. Also, Acinetobacter colonizes the oral cavity, the respiratory and gastrointestinal tract. As an opportunistic bacteria it can be a significant cause of serious hospital infections, mainly contaminating catheters and the equipment for mechanical ventilation. Because of this, it commonly causes bacteremia and nosocomial pneumonia in patients on mechanical ventilation, urinary tract infections, infections of the skin and soft tissues, as well as meningitis and endocarditis [4].

These infections are most common in immunocompromised patients, patients with severe underlying disease, and those who have undergone invasive diagnostic procedures. Risk factors for colonization and infection include recent surgery, central venous catheters, tracheostomy, parenteral nutrition and uncontrolled use of broad-spectrum antibiotics (third generation cephalosporins, fluoroquinolones, carbapenems) [4].

Acinetobacter has the ability to survive on various surfaces for long periods of time. These bacteria have a number of mechanisms of resistance, from enzymes that break down the beta-lactam ring, through the modification of the enzyme against aminoglycosides and changing binding site of quinolones, to changing different mechanisms of drug elimination and outer membrane proteins, so that the outcome of treatment of infections caused by Acinetobacter is uncertain. These mechanisms, either individually or in cooperation, define the resistance of Acinetobacter spp to antibiotics. Infection with MDR strains in hospitals further complicates the patients’ conditions, especially in intensive care units, prolonging hospitalization and increasing mortality. Hospital mortality of patients with Acinetobacter spp infections accounts for 8 – 23%, and in intensive care units even for 10 – 43% [6].

Acinetobacter is resistant to various antibiotics from different groups. Its multiresistant isolates are increasingly common around the world and the infections they cause represent a serious therapeutic problem. Carbapenems (imipenem, meropenem) are β-lactam antibiotics with the broadest spectrum of activity. When they emerged in the 1980s, for a long time they were the first line drugs in the treatment of infections caused by gram-negative nonfermentable pathogens, and they represented a new treatment option for serious infections. However, despite the early efficiency of carbapenem in the treatment of infections caused by Acinetobacter, nowadays increasing carbapenem-resistant Acinetobacter isolates are reported worldwide [4]. Multidrug resistant isolates are resistant to three or more groups of antibiotics that can be applied in the treatment of infections caused by these microorganisms (aminoglycosides, carbapenems and quinolones). Although rare, pandrug resistant isolates have also been described, which show resistance to sulbactam, minocycline, tigecycline and colistin.

Material and Methods

The study was conducted as a retrospective study, analyzing medical records of the patients treated at the Clinic of Infectious Diseases of the Clinical Center of Vojvodina (CCV) in the period from January 1, 2012 to December 31, 2015. It included 1,673 patients in cooperation, define the resistance of Acinetobacter spp to antibiotics. Infection with MDR strains in hospitals further complicates the patients’ conditions, especially in intensive care units, prolonging hospitalization and increasing mortality. Hospital mortality of patients with Acinetobacter spp infections accounts for 8 – 23%, and in intensive care units even for 10 – 43% [6].

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and bronchial aspirates) routinely sampled at the Clinic of Infectious Diseases of the CCV in the aforementioned period, was performed to establish the incidence of infections caused by Acinetobacter spp and antimicrobial resistance.

The inclusion criteria were: clinical picture of bacterial infection, as demonstrated by laboratory findings and X-ray findings of pneumonia.

The results of bacterial isolation and identification and antimicrobial susceptibility test results were obtained from standard reports on bacteriological examination. Isolation and identification was done in the laboratories of the Microbiology Center of the Institute of Public Health of Vojvodina in Novi Sad using standard bacteriological techniques. The causes are shown as the absolute number of isolates, as well as the percentage of their representation in the calendar year when they were isolated. The bacterial susceptibility to antimicrobials was expressed as percentage of resistant and susceptible strains and only primoisolates were analyzed. Susceptibility to antimicrobial drugs was analyzed only if there were more than two isolates in a single year. Strains showing an intermediate degree of susceptibility were classified as resistant, whereas MDR strains showed resistance to three or more groups of antimicrobial drugs.

Results

During the four-year research, 4,460 samples were examined, of which 825 were positive, and 101 Acinetobacter spp were isolated.

During this period, 1,682 blood samples were analyzed and a total of 260/1,682 (15.5%) positive isolates were found. In 2012, there were 64/353 (18.1%); in 2013, 46/474 (9.7%); in 2014, 62/512 (12.1%); and in 2015, 88/343 (25.6%).

The most common identified pathogens from blood cultures were coagulase-negative Staphylococcus spp in 121/260 (46.5%), followed by Staphylococcus aureus in 94/260 (36.2%), Klebsiella pneumoniae in 18/260 (6.9%), and Escherichia coli in 26/260 (10.0%).

During the same period, bacteriological tests of 770 throat swabs were examined and 124/770 (16.1%) positive isolates were found. In 2012, there were 30/35 (84.6%); in 2013, 34/47 (72.3%); in 2014, 35/63 (55.6%); and in 2015 there were 29/37 (75.7%) positive isolates. The most commonly isolated microorganisms were Acinetobacter spp in 33/128 (25.8%), followed by Staphylococcus aureus in 28/128 (22.2%), Pseudomonas aeruginosa in 19/128 (15.0%), Proteus mirabilis in 18/128 (14.3%), Enterococcus spp in 14/128 (11.0%), and Klebsiella pneumoniae in 10/128 (8.0%).

During the research period, 1,344 urine cultures were analyzed, and 198/1345 (14.7%) positive isolates were found. In 2012, there were 34/320 (10.6%); in 2013, 47/274 (17.1%); in 2014, 38/372 (10.2%); and in 2015, 79/348 (22.7%) isolates. The most commonly isolated were Escherichia coli in 69/198 (34.8%), Enterococcus spp in 36/198 (18.2%), Klebsiella pneumoniae in 28/198 (14.1%), Proteus mirabilis in 26/198 (13.1%), Pseudomonas aeruginosa in 25/198 (12.6%) and Acinetobacter spp in 7/198 (3.5%).

During the four-year research period, 770 throat swabs were examined and 124/770 (16.1%) positive isolates were found. In 2012, there were 30/35 (84.6%); in 2013, 34/47 (72.3%); in 2014, 35/63 (55.6%); and in 2015 there were 29/37 (75.7%) positive isolates. The most commonly isolated microorganisms were Acinetobacter spp in 33/128 (25.8%), followed by Staphylococcus aureus in 28/128 (22.2%), Pseudomonas aeruginosa in 19/128 (15.0%), Proteus mirabilis in 18/128 (14.3%), Enterococcus spp in 14/128 (11.0%), and Klebsiella pneumoniae in 10/128 (8.0%).

The test results of 325 samples of cerebrospinal fluid showed that 43 (13.2%) were positive. In 2012, there were 12/70 (17.1%) positive isolates; in 2013, 10/69 (14.5%); in 2014, 13/104 (12.5%); and in 2015 there were 8/82 (9.8%). During the four-year research, the most common microorganism isolated from the cerebrospinal fluid was Streptococcus pneumoniae in 14/43 (32.6%), followed by coagulase-negative Staphylococcus spp in 12/43 (27.9%); Listeria monocytogenes and Neisseria meningitidis each in 3/43 (6.9%); Acinetobacter spp and Strepococcus viridans each in 2/43 (4.7%). Other causes were found in less than 3%. One isolate of Acinetobacter spp was found in the cerebrospinal fluid during 2012 and 2014, while in 2013 and 2015 not a single positive isolate was found.

The bacteriological examination of 183 wound/decubitus swabs was performed during the research period. Positive isolates were found in 128/183 (69.9%). In 2012, there were 30/35 (84.6%); in 2013, 34/47 (72.3%); in 2014, 35/63 (55.6%); and in 2015 there were 29/37 (75.7%) positive isolates. The most commonly isolated microorganisms were Acinetobacter spp in 33/128 (25.8%), followed by Staphylococcus aureus in 28/128 (22.2%), Pseudomonas aeruginosa in 26/128 (20.3%), Proteus mirabilis in 14/128 (10.9%), Enterobacter spp in 10/128 (7.8%), Klebsiella pneumoniae in 9/128 (7.0%), and Enterococcus spp in 8/128 (6.5%).

Other pathogens were found in less than 6%. The incidence of Acinetobacter spp isolates in wound/decubitus swabs per year was: in 2012, 6/30 (20.0%); in 2013, 10/34 (29.4%); in 2014, 8/35 (22.9%); and in 2015, 9/29 (31.0%).

During the four-year research period, 770 throat swabs were examined and 124/770 (16.1%) positive isolates were found. In 2012, there were 30/35 (84.6%); in 2013, 34/47 (72.3%); in 2014, 35/63 (55.6%); and in 2015 there were 29/37 (75.7%) positive isolates. The most commonly isolated microorganism was Staphylococcus aureus found in 61/124 (49.2%). The incidence of Staphylococcus aureus isolates in 2012 was 62.8% (22/35); in 2013, 37.8% (14/37); in 2014, 45.7% (16/35); and in 2015 it was 52.9% (9/17). The incidence of Acinetobacter spp was 14/124 (11.3%), Klebsiella pneumoniae in 13/124 (10.5%), Streptococcus pyogenes in 9/124 (7.3%), Enterobacter spp in 8/124 (6.5%), and Pseudomonas aeruginosa in 5/124 (4.0%). Other pathogens were found in less than 4%. The incidence of Acinetobacter spp isolates in wound/decubitus swabs was as follows: in 2012, 4/35 (11.4%); in 2013, 5/37 (13.5%); in 2014, 3/35 (8.6%); and in 2015, 2/17 (11.8%).

During the same period, bacteriological tests of 153 swabs taken from cannula tubes and bronchial aspirate were performed, and positive isolates were found in 72/153 (47.1%). In 2012, there were 18/37 (48.6%) positive isolates, in 2013, 21/40 (52.5%); in 2014, 21/59 (35.6%); and in 2015, 12/17 (70.6%) isolates. The most common pathogens were Acinetobacter spp in 32/72 (44.4%), Pseudomonas aeruginosa in 18/72 (25.0%), Klebsiella pneumoniae in...
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10/72 (13.9%), Enterobacter spp and coagulase negative Staphylococcus spp in 7/72 (9.7%) each, and Stenotrophomonas maltophilia in 6/72 (8.3%). The incidence of Acinetobacter spp per year was: in 2012, 6/37 (16.2%); in 2013, 9/21 (42.9%); in 2014, 7/21 (33.3%); and in 2015, 10/12 (83.3%).

The study showed that the incidence of Acinetobacter spp was highest in swabs taken from cannula/tubes and bronchial aspirate - 32/72 (44.4%), then in wound/decubitus swabs in 33/128 (25.8%) of isolates, in throat swabs in 14/124 (11.3%), in blood cultures in 14/260 (5.4%), in cerebrospinal fluid cultures in 2/43 (4.7%), and the lowest incidence of Acinetobacter spp was found in urine cultures, in 6/198 (3.0%) isolates.

Acinetobacter spp isolates from blood cultures were resistant to all tested antibiotics, except colistin and tobramycin. All Acinetobacter spp isolates from blood tested during 2013, 2014 and 2015, were 100% susceptible to colistin, while in 2012 susceptibility to this antibiotic had not been studied. In the reported period, the susceptibility to tobramycin was 88.9% (Table 1).

### Table 1. Antimicrobial susceptibility of Acinetobacter spp isolated from blood

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>2012</th>
<th>2014</th>
<th>2015</th>
<th>Total/Ukupno</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0 (0.0)</td>
<td>2 (100.0)</td>
<td>0 (0.0)</td>
<td>6 (100.0)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0 (0.0)</td>
<td>2 (100.0)</td>
<td>0 (0.0)</td>
<td>6 (100.0)</td>
</tr>
<tr>
<td>Piperacillin/Tazobactam</td>
<td>0 (0.0)</td>
<td>2 (100.0)</td>
<td>0 (0.0)</td>
<td>6 (100.0)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0 (0.0)</td>
<td>2 (100.0)</td>
<td>0 (0.0)</td>
<td>6 (100.0)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>0 (0.0)</td>
<td>2 (100.0)</td>
<td>0 (0.0)</td>
<td>6 (100.0)</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>2 (100.0)</td>
<td>0 (0.0)</td>
<td>5 (83.3)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0 (0.0)</td>
<td>2 (100.0)</td>
<td>0 (0.0)</td>
<td>6 (100.0)</td>
</tr>
<tr>
<td>LevoTobramycin</td>
<td>0 (0.0)</td>
<td>2 (100.0)</td>
<td>0 (0.0)</td>
<td>6 (100.0)</td>
</tr>
<tr>
<td>Colistin</td>
<td>–</td>
<td>–</td>
<td>6 (100.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Total/Ukupno</strong></td>
<td>2</td>
<td>6</td>
<td>5</td>
<td>13</td>
</tr>
</tbody>
</table>

S - susceptible/osetljivo, R - resistant/rezistentno

10/72 (13.9%), Enterobacter spp and coagulase negative Staphylococcus spp in 7/72 (9.7%) each, and Stenotrophomonas maltophilia in 6/72 (8.3%). The incidence of Acinetobacter spp per year was: in 2012, 6/37 (16.2%); in 2013, 9/21 (42.9%); in 2014, 7/21 (33.3%); and in 2015, 10/12 (83.3%).

The study showed that the incidence of Acinetobacter spp was highest in swabs taken from cannula/tubes and bronchial aspirate - 32/72 (44.4%), then in wound/decubitus swabs in 33/128 (25.8%) of isolates, in throat swabs in 14/124 (11.3%), in blood cultures in 14/260 (5.4%), in cerebrospinal fluid cultures in 2/43 (4.7%), and the lowest incidence of Acinetobacter spp was found in urine cultures, in 6/198 (3.0%) isolates.

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Antimicrobial resistance of Acinetobacter spp taken from wound/decubitus swabs during the study period was almost 100% to all tested antibiotics.

### Table 2. Antimicrobial susceptibility of Acinetobacter spp isolated from wound/decubitus swabs

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>Total/Ukupno</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Ampicillin/ Sulbactam</td>
<td>0 (0.0)</td>
<td>6 (100.0)</td>
<td>1 (10.0)</td>
<td>9 (90.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Piperacillin/Tazobactam</td>
<td>0 (0.0)</td>
<td>6 (100.0)</td>
<td>0 (0.0)</td>
<td>1 (100.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>1 (16.7)</td>
<td>5 (83.3)</td>
<td>0 (0.0)</td>
<td>10 (100.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>1 (16.7)</td>
<td>5 (83.3)</td>
<td>0 (0.0)</td>
<td>10 (100.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>4 (66.7)</td>
<td>2 (33.3)</td>
<td>8 (80.0)</td>
<td>2 (20.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>0 (0.0)</td>
<td>6 (100.0)</td>
<td>0 (0.0)</td>
<td>10 (100.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>2 (33.4)</td>
<td>4 (66.6)</td>
<td>4 (40.0)</td>
<td>6 (60.0)</td>
<td>2 (25.0)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0 (0.0)</td>
<td>6 (100.0)</td>
<td>0 (0.0)</td>
<td>10 (100.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>LevoTobramycin</td>
<td>3 (50.0)</td>
<td>3 (50.0)</td>
<td>0 (0.0)</td>
<td>10 (100.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Colistin</td>
<td>–</td>
<td>–</td>
<td>10 (100.0)</td>
<td>0 (0.0)</td>
<td>8 (100.0)</td>
</tr>
<tr>
<td><strong>Total/Ukupno</strong></td>
<td>6</td>
<td>10</td>
<td>8</td>
<td>9</td>
<td>33</td>
</tr>
</tbody>
</table>

S - susceptible/osetljivo, R - resistant/rezistentno
except colistin and tobramycin. In the examined period, the susceptibility to colistin was 100% and to tobramycin 30.8%. The isolates were susceptible to gentamicin and levofloxacin during 2012, while during 2014 and 2015, resistance to these antibiotics was 100% (Table 2).

Resistance to carbapenem of isolates from the throat swabs was 100%, in all isolates in 2012, 2014 and 2015, while in 2013, it was recorded in three isolates (60%). Susceptibility to ampicillin/sulbactam was not recorded during the first three years of testing, while in 2015 there were no information about susceptibility to this antibiotic. Susceptibility to colistin was 100% in 2013, the only year that focused on examining the susceptibility to this antibiotic. Susceptibility to tobramycin was tested in 2013 and 2014, and it was 100% (Table 3).

Antimicrobial susceptibility of Acinetobacter spp isolated from cannula/tubes swabs and bronchial aspirate during the period of research was recorded only to colistin (100%). The antimicrobial susceptibility to this antibiotic was carried out only in 2015, in 2 isolates. A lower susceptibility (20%) was recorded to ampicillin/sulbactam and 17.4% to tobramycin. Resistance of Acinetobacter spp to other antibiotics was over 82.6% (Table 4).

**Discussion**

During the past decades, Acinetobacter baumannii has become one of the leading causes of nosocomial infections worldwide. A study that examined the prevalence of infections in intensive care units in 75 countries on five continents, confirmed that infections caused by Acinetobacter baumannii are among the five most frequent infections [5]. In the European countries, it approximately causes from 2% to 10% of all infections caused by gram-negative microorganisms, and in the United States (US) about 2.5% [7]. Our study shows that Acinetobacter spp causes blood infections in 2.2% up to 9.7% of inpatients.

In regard to data from our country, the study of Šuljagić and Mirović in 2006, showed that Acinetobacter spp caused nosocomial blood infections with an incidence of 7.1% [8], which corresponds with our data on blood infections in 2015, with an incidence of 5.7%. In our study, the incidence of Acinetobacter spp in blood cultures was 5.4%, taking the fourth place, behind the cannula/tubes swabs and bronchial aspirate, the wound/decubitus swabs, and throat swabs, whereas according to the results of American authors it was in the second place, after isolates from the respiratory tract, with an incidence of 23.9% [9]. Our results from 2014 are identical with the data from Saudi Arabia, where the incidence was also 9.7% [10].

The lowest incidence of Acinetobacter spp was found in cerebrospinal fluid and urine. Acinetobacter spp was not isolated from urine in 2013, while the highest incidence was recorded in 2012, 5.9%.

During 2013 and 2015, not one strain of Acinetobacter was isolated from the cerebrospinal fluid, and in 2012 and 2015, one isolate was found each year.

During the four-year research, Acinetobacter spp was isolated from wound/decubitus swabs in 25.8% of hospitalized patients. According to literature data, Acinetobacter spp was found in skin swabs of patients who were not hospitalized, from 0.5 to 3%, and in hospitalized patients in up to 75% [11–13]. The results of our research on the dominance of Acinetobacter spp from wound swabs are similar to the findings of other authors who examined the the
dominance of gram negative bacteria from swabs of infected decubital wounds, whose localization is usually near urogenital and the end of gastrointestinal tract [14, 15].

The results of our research on the incidence of Acinetobacter spp in patient material routinely sampled at the Clinic of Infectious Diseases of the CCV, in the period from January 1, 2012, to December 31, 2015, is somewhat similar to the findings from US [9] and Great Britain [16], where the highest percentage of Acinetobacter spp was also isolated from cannula/tubes swabs and bronchial aspirate. In our study, the prevalence ranged from 16.2% in 2012, up to 83.3% in 2015, which shows that Acinetobacter spp in our environment.

Table 4. Antimicrobial susceptibility of Acinetobacter spp isolated from the cannula/tubes swabs and bronchial aspirate

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>Total/Ukupno</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin/ clavulinate</td>
<td></td>
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<tr>
<td>Piperacillin/ tazobactam</td>
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<tr>
<td>Imipenem</td>
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<tr>
<td>Meropenem</td>
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<tr>
<td>Gentamicin</td>
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<tr>
<td>Amikacin</td>
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<td>Tobramycin</td>
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<td>Ciprofloxacin</td>
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<td>Levofloxacin</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Colistin</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Total/Ukupno 6 9 7 10 32

S - susceptible/ osetljivo, R - resistant/rezistentno

while in our study, ten years later, the susceptibility to these antibiotics was not observed, as shown by our results from 2014 and 2015, where resistance was recorded to all antibiotics except colistin and tobramycin. The study of Šuljagić and Mirović, conducted in 2006, examined the resistance of Acinetobacter spp isolated from blood of patients hospitalized in the intensive care units, showed that all isolates were resistant to gentamicin, and 71% to ciprofloxacin, but did not register resistance to imipenem [8]. Rapid development of resistance to carbapenems in the last decade [17] explains existence of differences in the susceptibility of isolates of Acinetobacter spp to imipenem in the aforementioned and our study. All strains of Acinetobacter spp were susceptible to colistin, as shown by studies carried out in Bulgaria from 1999 to 2006, where only colistin proved effective [18]. These strains that are susceptible only to colistin were also described in Korea in a study published in 2008 [19]. In Taiwan, there were isolates that were resistant to all available antibiotics, including colistin [20].

Due to the small number of isolates of Acinetobacter spp from cerebrospinal fluid and urine, data on their susceptibility were not analyzed.

Isolates of Acinetobacter spp from wound swabs in our study indicate that it is a MDR bacteria. The study conducted at the Institute of Public Health of Vojvodina at the Center for Microbiology, included strains of Acinetobacter spp isolated from wound swabs of patients hospitalized at institutes and clinics in CCV in Novi Sad, show that it is a MDR bacteria [4]. The above-mentioned study, recorded a lower rate of resistance to carbapenems of 61.8%, and in our study it was 83.3% in 2012, 100% in 2013 and 2014, and 88.9% in 2015. The emergence of an
increasing number of strains resistant to carbapenems caused the empirical use of these antibiotics [21]. Acinetobacter spp in our study did not show resistance to colistin, as found in literature data [22–24]. Susceptibility testing to colistin in our study was conducted in 2013, 2014, and 2015, but not for all isolates.

Susceptibility of isolates from throat swabs to colistin was done only in 2013, when all isolates were susceptible to this antibiotic. Resistance to carbapenems observed in the four-year period was 85.7%. Resistance was recorded in all isolates in 2012, 2014 and 2015, while in 2013 it was recorded in 60% of isolates. A disturbing percentage of resistant isolates of Acinetobacter baumanii to carbapenems in recent years has been observed in Croatia, according to the Committee for Antibiotic Resistance in the Croatian Academy of Medical Sciences, amounting to 90% in major Croatian hospitals [5]. In our study, susceptibility to tobramycin during 2013 and 2014 was 100%, while in 2012 and 2015, susceptibility to this antibiotic was not examined. Almost all isolates were resistant to piperacillin/tazobactam, ampicillin/sulbactam, gentamicin, amikacin, ciprofloxacin and levofloxacin. A combined application of ampicillin/sulbactam, may be effective, especially in hospitals where this agent is rarely used [25]. In our study, the resistance to this antibiotic in the surveyed period was 100%.

High resistance of Acinetobacter spp, isolated from cannula/tubes swabs and bronchial aspirate, to almost all tested antimicrobials except colistin were observed, but susceptibility to this antibiotic was carried out only in the course of 2015, only in 2 isolates. Also, in 2015, susceptibility to ampicillin/sulbactam and piperacillin/tazobactam was not tested, because since 2015, susceptibility testing to antibiotics is performed according to the EUCAST standard, which does not include testing to these antibiotics. Some authors recommend the use of a combination of ampicillin/sulbactam [4]. In our study, the resistance to this antibiotic was 80%.

Some of the epidemiological studies may have included isolates that were not responsible for infections, but simply colonized ill patients. In terms of resistance, 34% of Acinetobacter isolates in the National Healthcare Safety Network of the United States were resistant to cephalosporins, carbapenems, fluoroquinolones and aminoglycosides [26], as show the findings of antibiotic resistance of Acinetobacter spp isolated from cannula/tubes swabs and bronchial aspirates. In another national surveillance study conducted in the US in 2010, 44.7% and 49.0% were resistant to imipenem and meropenem, respectively, and 5.3% were resistant to colistin in vitro, whereas in our study resistance to colistin was not recorded [27].

Current data indicate that colistin is the main and only therapeutic option, and its unique pharmacokinetic properties have led many to suggest the use a combination of antibiotics. To maintain the susceptibility of colistin, carbapenems, sulbactam, rifampicin and tigecycline have been the most studied, in order to find a combination that would provide the best clinical efficacy and reduce toxicity [27].

**Conclusion**

Representatives of the genus Acinetobacter are a common cause of infections in hospitalized patients with bacterial infections. During the four-year research, the incidence of Acinetobacter spp isolated from blood cultures was 5.4% (14/260), from urine cultures 3% (6/198), from cerebrospinal fluid cultures 4.7% (2/43), from wounds/decubitus swabs 25.8% (33/128), from throat swabs 11.3% (14/124) and from cannula/tubes swabs and bronchial aspirate 44.4% (32/72). All strains of Acinetobacter spp isolated from patient material during the investigation period were susceptible to colistin (100%). In the examined period, resistance of Acinetobacter spp to β-lactams, carbapenems, aminoglycosides and fluoroquinolones was over 63.6%, except to tobramycin, but only from blood cultures, with resistance of 11.1%. All strains of Acinetobacter spp were multiresistant.

**References**


