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ORIGINAL PAPER

ANTIMICROBIAL RESISTANCE OF ACINETOBACTER BAUMANNII STRAINS ISOLATED IN „MATEI BALŞ" NATIONAL INSTITUTE OF INFECTIOUS DISEASES

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Abstract. Acinetobacter baumannii is a gram-negative bacillus with an emerging multiresistance to antibiotics, making more and more difficult the treatment of hospital-acquired infections in which it is involved. **Objectives:** To analyse the antimicrobial resistance for Acinetobacter baumannii strains, in order to develop the first line treatment alternatives for the syndromes with a frequent involvement of this germ. **Methods:** The antimicrobial resistance tests of the non-duplicate Acinetobacter baumannii strains were analysed. These strains were isolated from patients hospitalized at the INBI „Prof.Dr. Matei Balş" between the 1st of July 2008 and June 30, 2010. The evolution of the resistance was studied for the four semesters of the analysed period. **Results:** 147 strains of Acinetobacter baumannii were analysed. The main specimen which provided the Acinetobacter baumannii isolates was tracheobronchial aspirate (68 strains, 46.25%). More than half of the patients from which the bacteria were isolated had been recently hospitalized in other medical units (55.10%). The proportion of carbapenem resistant strains recorded a slight decrease between the first period (91.42%) and the final period of the study (80.64%), p=0.146. The associations containing sulbactam proved to be efficient in less than 14% of the cases, in each of the studied time periods and the difference in the susceptibility rate was insignificant between the first and the final studied time periods (p=0.78). The level of resistance to amikacin remains high, between 73.07% and 91.3%, and the progressive improvement of susceptibility rates between the analysed periods was insignificant (p=0.10). The efficiency of tobramycin is higher than that of amikacin, but has only reached the level of statistical value in two of the analysed periods. The in vitro resistance of ciprofloxacin was high, rating over 80% in all four intervals. The activity of trimethoprim-sulfamethoxazole was also low and oscillatory, without a significant change between the two time periods (p=0.16). A single strain proved resistance to colistin. **Conclusions:** Most antibiotics have a less than moderate activity against Acinetobacter baumannii. The only antibiotic that proved a high activity against these strains was colistin; no data were collected for tigecycline.
Keywords: acinetobacter baumannii, carbapenems, colistin, nosocomial infections

Introduction

Although the infections caused by Acinetobacter baumannii are not so frequent, nevertheless, their clinical and epidemiological importance is high, because of their antimicrobial multiresistance and the fact that these bacteria affect mostly hospitalized patients, especially in surgery and intensive care units, with horizontal transmission.

Recent data highlight the globally increasing rate of antibiotic resistance of Acinetobacter baumannii.

For this reason, the present study aims to analyse the antimicrobial susceptibility for Acinetobacter isolates in the National Institute of Infectious Diseases "Prof. Dr. Matei Balş" (INBI). Furthermore, since 2007, our institute includes an intensive care unit, and the management of multiresistant Acinetobacter baumannii infections often represents a concern for clinicians.

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Objectives

The purpose of this paper is to analyse the antimicrobial resistance of Acinetobacter spp strains, in order to establish the optimal treatment alternatives for the antimicrobial management of these infections.

Material and methods

We conducted a non-interventional, cross-sectional study with the purpose of analysing Acinetobacter baumannii susceptibility to antimicrobials, using real-life data provided by the microbiology laboratory. The data were used as they were communicated to clinicians, without making any additional considerations about resistance profiles. After excluding bacterial duplicates (the same strain isolated from a patient in less than 4 weeks from the same specimens or isolated simultaneously from different specimens) the antibiograms of all remaining 147 Acinetobacter baumannii isolates were studied. The antimicrobial susceptibility tests were performed for the majority of the strains by semi-automated methods (API, Vitek, Microscan): 139 strains (94.56%). For 8 strains (5.44%) the susceptibility tests were performed by disk diffusion method (for 7 strains in T1 and for 1 strain in T2). The investigated Acinetobacter baumannii strains were isolated from patients hospitalized at INBI "Prof. Dr. Matei Balş" between the 1st of July 2008 and June 30, 2010. A comparative study of Acinetobacter baumannii antimicrobial resistance was done for four six-month intervals: T1 (July - December 2008), T2 (January - June 2009), T3 (July - December 2009), T4 (January - June 2010). The study is part of a more complex project conducted in this period; during this larger study, 4858 strains from several bacterial species were analysed.

The statistical analysis of the collected data was performed with EpiInfo software 3.4.3. The statistical significance was assessed using the statistical significance threshold (p value), through the Fisher test. P was considered significant for values less than 0.05.

Results

The distribution of Acinetobacter baumannii isolates

The 147 strains of Acinetobacter baumannii corresponded to each time period as follows: T1 - 35 strains, T2 - 35 strains, T3 - 45 strains, T4 - 32 strains. Compared to other bacterial agents involved in infectious pathologies, the proportion of Acinetobacter baumannii isolates was low, representing only 3.02% of the germs isolated throughout the study (figure 1).

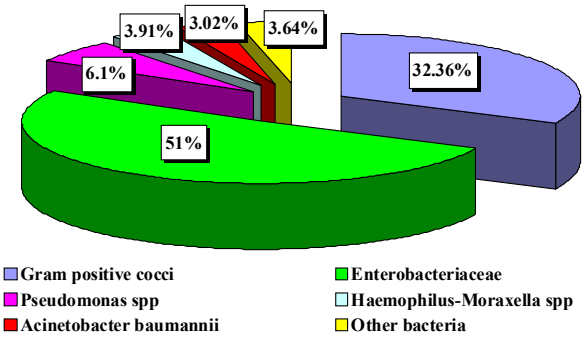

Figure 1. The proportion of Acinetobacter baumannii isolates, compared to other germs

Legend: Acinetobacter baumannii represented the smallest proportion (3.02%) of all the isolated bacteria throughout the 2-years study period

The specimens that provided the Acinetobacter baumannii strains

The main specimen that provided the Acinetobacter baumannii strains was the tracheobronchial aspirate (68 strains, 46.25%). Less often, other positive samples were: wound secretions (24 strains, 16.32%), sputum (18 strains, 12.24%), urine (9 strains, 6.12%), blood cultures (8 strains, 5.44%), removed foreign material (7 strains, 4.76%), deep collections (4 strains, 2.72%), cerebrospinal fluid (4 strains, 2.72%), other pathologic products (5 strains, 3.4%) (figure 2).

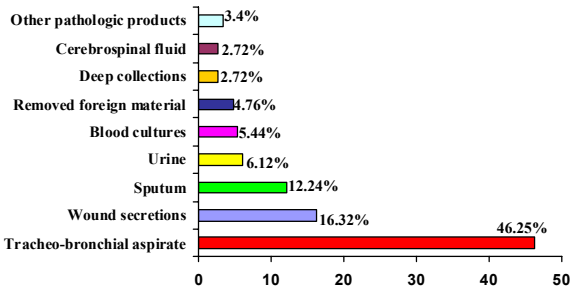

Figure 2. The distribution of Acinetobacter baumannii isolates from various specimens

Legend: Most Acinetobacter baumannii strains were isolated from tracheobronchial aspirate (46.25%). Other specimens included: wound secretions (16.32%), sputum (12.24%), urine (6.12%), blood cultures (5.44%), removed foreign material (strains, 4.76%), deep collections (2.72%), cerebrospinal fluid (2.72%), other pathologic products (3.4%)

Regarding the background of the patients who provided these isolates, 66 of the cases (44.90%) were patients who did not provide data about other recent hospitalizations. In 81 cases (55.10%), the patients were transferred to our institute from other

medical units. For these cases there weren't data available regarding the length of stay and the type of unit (intensive care unit, surgery, or regular unit) of the prior hospitalization, nor the antibiotic treatment administered in the previous medical center.

Antimicrobial susceptibility

Among the tested antibiotics included in testing panels for Gram negative rods, the following were considered clinically and epidemiologically relevant: ampicillin/sulbactam, aminoglycosides (amikacin, tobramycin), carbapenems (imipenem, meropenem), fluoroquinolones (ciprofloxacin, levofloxacin), trimethoprim/sulfamethoxazole, colistin.

The analysis of Acinetobacter baumannii strains' susceptibility for the entire study period revealed a very low susceptibility rate, under 50% for most of the investigated antibiotics (tobramycin: 44.45%, carbapenems: 19.18%, amikacin: 18.86%, trimethoprim-sulfamethoxazole: 13.77%, fluoroquinolones: 10.89%, ampicillin-sulbactam: 9.58%). Colistin was the only one to prove a high activity (99.06%) against these strains (figure 3). No data were collected for tigecycline.

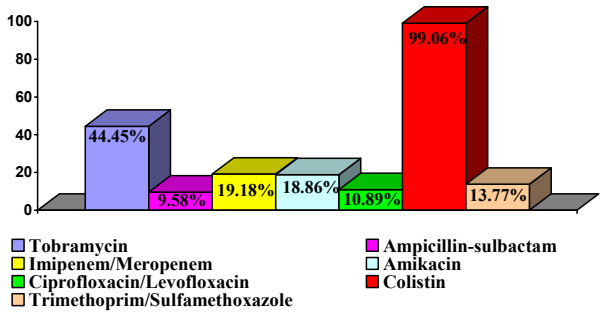

Figure 3. The antimicrobial susceptibility of Acinetobacter baumannii (over the entire study duration)

Legend: The antimicrobial susceptibility rates of Acinetobacter baumannii isolates were low for most of the investigated antibiotics: ampicillin-sulactam-9.58%, fluoroquinolones-10.89%, trimethoprim-sulfamethoxazole-13.77%, amikacin-18.66%, tobramycin-44.45%. imipenem/meropenem-19.18%, Only for colistin the susceptibility rate was very good: 99.06%

When we compared the strains isolated from patients with previous hospitalization versus no recognized hospitalization we found no statistically significant differences in resistance rates for any of the analyzed antimicrobials: ampicillin/sulbactam (92.73% vs. 87.18%, p=0.36), amikacin (87.67% vs. 75%, p=0.06), tobramycin (61.84% vs. 48.53%, p=0.12), carbapenems (81.08% vs. 80.59%, p=0.94), fluoroquinolones (92.5% vs. 85.07%, p=0.87), trimethoprim/sulfamethoxazole (86.66% vs. 85.71%, p=0.87), colistin (1.49% vs. 0%, p=0.44).

In order to verify the evolution of the antimicrobial susceptibility of Acinetobacter baumannii during the study period, the resistance was monitored throughout the four semesters of the study (figure 4).

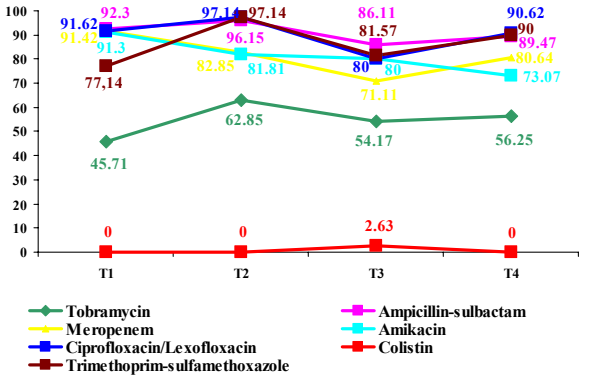

Figure 4. Evolution of Acinetobacter baumannii resistance for six-months time intervals

Legend: The analysis of the evolution of the resistance rate did not reveal any statistically significant differences between the initial and the final stage of the study

This analysis allowed us to draw the following conclusions:

- the rate of carbapenem resistant strains remained above 70% in all of the studied time spans. Although this resistance rate recorded a slight decrease from 91.42% (T1), to 82.85% (T2) and 71.11% (T3) respectively, this decrease was followed by an increase in T4 (80.64%), making the difference between T1 and T4 statistically insignificant (p=0.146). The only time period in which the evolution had a statistical significance was between T1 and T3 (p=0.02).
- more than 86% of the isolates proved resistant to sulbactam associations for each time period. No statistically significant differences were recorded between T1 and T4 (p=0.78) or the other analyzed time intervals (T1-T2: p=0.60, T2-T3: p=0.18, T3-T4: p=0.72).
- the level of amikacin resistance remains high (91.3% - T1, 81.81% - T2, 80% - T3 and 73.07% - T4). The progressive decrease in the resistance rate between the analyzed intervals did not reach statistical value (p=0.10).
- the efficiency of tobramycin is superior to that of amikacin, with a proportion of resistant strains of 45.71% in T1, 62.85% in T2, 54.17% in T3, 56.25% in T4. This superiority is statistically significant for two of the analyzed intervals: T1 (p=0.0004) and T3 (p=0.02), but not for the other two: T2 (p=0.08) and T4 (p=0.18).
- the in vitro resistance to ciprofloxacin was high: 91.62% (T1), 97.14% (T2), 80% (T3) and 90.62% (T3). The difference had no statistical

value between T1 and T4 (p=0.9). Only the decrease in the resistance rates between T2 and T3 had statistical significance, p=0.02.

- the efficiency of trimethoprim-sulfamethoxazole was also low and oscillatory, without a statistically significant difference between T1 and T4 (p=0.16). For two of the analyzed time periods, the modifications were significant: a decrease in the susceptibility rate between T1 and T2 (p=0.01) and an increase between T2 and T3 (p=0.03).
- Colistin seems to be the current "rescue" therapy: no resistant strains were identified in the T1, T3 and T4 periods, and only one out of the 38 tested in T3 proved to be resistant.

An important feature of the infections caused by Acinetobacter baumannii is their high severity due to the clinical status of the affected patients (intensive care units patient, or patients that underwent major surgery) and their treatment difficulty, because most of these strains are resistant to almost all of the currently used antibiotics. This is a significant problem all over the world.

In our study we found high resistance rates for almost all the analyzed antimicrobials, even higher that the levels reported in other regions. For example, the MYSTIC program, that assessed the problem of Acinetobacter baumannii antimicrobial resistance in Europe, found an average rate of resistance of 66% to fluoroquinolones (compared with 89.11% found in our study) and 52.4% to aminoglycosides (compared with resistance rates of 80.82% to amikacin and 55.55% to tobramycin found in our study) [1]. However, the resistance levels for these antimicrobials are similar with those found in another Romanian recently published study, which was performed between 2001-2003 and which identified a rate of resistance to ciprofloxacin of 93.1% and to amikacin of 83.8% [2]. Although sulbactam is known to have a good activity against Acinetobacter, only 9.58% of the strains proved to be susceptible to the association that included it, this level of susceptibility being much lower than the one found in other regions, e.g. 60% in Asia [3]. The emergence, spread and increasing rate of carbapenem-resistant strains of Acinetobacter baumannii is a concerning issue. In our study, we found a resistance rate of 80.82% to this antibiotic class, much higher than the one observed by Radu-Popescu et al. 5 years earlier, in their study the carbapenem-resistance rate being 21.8% [2]. The level of carbapenem-resistance found by us is also higher that the ones noted for other geographic regions. For example, according to the MYSTIC program the level of resistance to carbapenems in Europe is 26.9% to meropenem, 30.2% to imipenem [1], for Asia, carbapenem-resistance exceeds 25% [3] and for South Africa the level of resistance to carbapenems is about 30% [4]. In the U.S., if in 1986 there were no Acinetobacter baumannii resistant strains to carbapenems described, in 2003 the resistance reached 20% [5], and in 2005 in certain regions, it reached 60.2% [6]. For Latin America, the mentioned carbapenem-resistance rates are higher than the one found in our study (71%) [1].

Unfortunately, the evolution of Acinetobacter baumannii antimicrobial resistance seems to be towards pan-resistance, as colistin and tigecycline (the backline antimicrobials) resistant isolates are now described [5,7,8]. In our study we found only one colistin-resistant strain; no susceptibility tests to tigecycline were performed for these isolates.

The particular resistance pattern displayed by Acinetobacter baumannii strains is a result of the combination between its intrinsic resistance and acquisition of resistance genetic determinants over time, most probably due to extensive use of broad-spectrum antimicrobials. The lack of uniformity in the use of antibiotics in different geographical areas is likely to result in different resistance rates noted all over the world. Because we did not have data regarding antibiotic consumption in our institute in order to make a correlation between them and the resistance rate, and also between them and the evolution that we have noticed during the study period in the resistance rates, we can only speculate that the high resistance levels and their evolution were influenced by the amount and the fluctuations of antibiotic consumption.

Concerning the lack of the differences in resistance rates between the strains isolated from patients with prior hospitalizations and the patients without prior hospitalizations, we consider that this was due to the heterogeneity of the patients admitted in our institute from other hospital units. We did not have any data to allow us to say which patients with prior hospitalizations were already colonized with Acinetobacter baumannii, so we could not determine the exact moment of the acquisition of these strains (in our institute, or in the other hospital).

The limitations of this study are related to the lack of information about the patients with a prior hospitalization, such as: the type of the unit in which the patients were admitted, the duration of the previous hospitalization, the antibiotic treatment that the patients had received, the presence or the absence of Acinetobacter baumannii colonization while the patients were still hospitalized in the other medical institution. Another limitation was linked to the small number of isolates for each temporal interval and the limited periods of time between which the resistance evolution was analyzed; this is the reason why we did not find more important

changes in the dynamics of the susceptibility patterns of Acinetobacter baumannii. Therefore, in order to be able to draw more significant conclusions regarding the temporal evolution of the resistance patterns of A. baumannii, further monitoring over more extended periods of time would be helpful.

Conclusions

Acinetobacter baumannii is a concerning germ, especially for hospitalized patients from intensive care units. In our study, 55.10% of the patients who had infections due to A. baumannii came from other medical units. The high proportion of isolates provided by tracheobronchial aspirate (46.25%) certifies the high prevalence of this bacteria in intensive care units.

Acinetobacter baumannii represented only 3.02% of the bacteria isolated in our hospital during a 24-months period.

Although the frequency of these isolates was low, this germ impresses through its extremely low antimicrobial susceptibility. It even proved resistant to antibiotics considered as second-line therapy, the main concern being the resistance levels to carbapenems. The only antibiotic that remains active in these cases is colistin, and probably tigecycline, but no susceptibility results were collected for this antimicrobial.

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