APPROPRIATE EMPIRICAL ANTIBACTERIAL THERAPY FOR SEVERE INFECTIONS WITH NON-FERMENTERS
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ABSTRACT
The outcome of patient with severe infection depends on time of initiation of right antimicrobial therapy. Aim: The emergence of antimicrobial resistance and the severity of nosocomial infections determined by non-fermenters prompted our interest to study the overall antimicrobial resistance profile of Gram negative non-fermenters in order to determine the appropriate empirical antibacterial therapy. Material and methods: In our study were included 85 Pseudomonas aeruginosa strains and 39 Acinetobacter baumannii strains isolated from patients admitted in the Clinic of Infectious Diseases of Iasi from 2006 to 2011. These strains were tested to beta-lactams, fluoroquinolones, aminoglycosides and colistin. Results: Pseudomonas aeruginosa strains showed resistance to ceftazidime (55.6%), cefoperazone (64.6%), ciprofloxacin (87.5%), amikacin (55.6%), imipenem (53%) and to colistin in 2% of cases. Acinetobacter baumannii strains were resistant to ceftazidime (80%), cefoperazone (87.5%), ciprofloxacin (76.9%), amikacin (45.5%), piperacillin - tazobactam (64.7%), imipenem (44.1%) and colistin in 38.9% of cases. Conclusions: If an infection with Pseudomonas aeruginosa is suspected, the choice of imipenem in starting treatment may be conducive to treatment failure in almost half of cases. Colistin is an effective alternative, but encumbered by renal toxicity. For infections with Acinetobacter baumannii remains the same risk of treatment failure if imipenem therapy is the first intention. More than one third of the cases treated with colistin may result also in failure.

Keywords: first-line therapy, pseudomonas aeruginosa, acinetobacter baumannii.

INTRODUCTION
Severe bacterial infections can be acquired in the community, hospital or long – term chronic facilities and are associated with high mortality rate if not treated promptly and effectively. In most cases initiating an efficient antimicrobial therapy is required before establishing etiology. One of the primary goals of the clinician is to minimize development of antimicrobial resistance, to optimize first-choice therapy and to determine the duration of effective treatment. They may be met when knowing the general profile of antimicrobial resistance and patient’s risk factors for colonization or infection with resistant bacteria.

MATERIAL AND METHODS
In order to choose correctly the first line antimicrobial therapy we studied the profile of resistance for 85 strains of Pseudomonas aeruginosa and Acinetobacter baumannii isolated from 39 of 2006-2010, isolated from urine (UC), blood cultures (BC), cerebrospinal fluid (CSF), wounds, bronchoalveolar fluid (BAF) (tab. I).
TABLE I. Strains isolated in biological products

<table>
<thead>
<tr>
<th>STRAINS</th>
<th>UC</th>
<th>BC</th>
<th>CSF</th>
<th>WOUNDS</th>
<th>BAF</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>74</td>
<td>87.1</td>
<td>8</td>
<td>9.4</td>
<td>1</td>
<td>1.2</td>
</tr>
<tr>
<td><em>A. baumannii</em></td>
<td>11</td>
<td>28.2</td>
<td>15</td>
<td>38.5</td>
<td>4</td>
<td>10.3</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSIONS

The *P. aeruginosa* and *A. baumannii* strains were tested at aminopenicillins, beta-lactams, beta-lactamases inhibitors, cephalosporins, fluoroquinolones, aminoglycosides and carbapenems (tab. II).

TABLE II. Profile of overall antimicrobial resistance

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th><em>Pseudomonas aeruginosa</em></th>
<th><em>Acinetobacter baumannii</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strains</td>
<td>Resistance</td>
</tr>
<tr>
<td>Ceftazidim</td>
<td>63</td>
<td>35 (55.6%)</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>48</td>
<td>31 (64.6%)</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>16</td>
<td>9 (56.3%)</td>
</tr>
<tr>
<td>Ticarcilin/clavulanic acid</td>
<td>10</td>
<td>7 (70%)</td>
</tr>
<tr>
<td>Piperacillin-Tazobactam</td>
<td>32</td>
<td>19 (59.4%)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>76</td>
<td>35 (53%)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>66</td>
<td>40 (71.4%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>48</td>
<td>42 (87.5%)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>26</td>
<td>15 (57.7%)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>45</td>
<td>25 (55.6%)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>44</td>
<td>36 (42.4%)</td>
</tr>
<tr>
<td>Colistin</td>
<td>50</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

*P. aeruginosa* is known as a major cause of nosocomial infections associated with invasive devices, ventilation, both in immunocompromised and immunocompetent patients [7].

*P. aeruginosa* has intrinsic resistance to many classes of antibiotics, a special ability to acquire resistance mutations and high virulence potential. Resistance is the result of extensive association of multiple mechanisms [9]. Intrinsic resistance (due to decreased outer membrane permeability, production of beta-lactamase type cAMP and the presence of numerous genes encoding multidrug efflux pumps) and acquisition of genes encoding enzymes that modify aminoglycosides and beta-lactamase, compromise any antibiotic, less polymixins. Resistance to carbapenems has been attributed to production of metallo-beta-lactamase (MBL) which hydrolyzes most beta-lactams except Aztreonam [9].

**Cephalosporin Resistance**

63 strains of *P. aeruginosa* strains were tested to ceftazidime (one of anti-pseudomonas cephalosporins widely used in clinical practice) and 35 were resistant (representing a rate of 55.6%). Although the number of isolates is quite small (most of the *P. aeruginosa* nosocomial infections, patients are commonly treated with antibiotics before their transfer to our clinic so that in most cases in which *P. aeruginosa* is suspected the
bacteriological results are negative) and we can not draw definite conclusions with statistical value, resistance to ceftazidime is seen in more than half of them. According to EARSS 2009 [16], 28 countries have reported 7937 strains of P.aeruginosa and 1171 were resistant to ceftazidime (14.75%). Romania reported only 10 cases of ceftazidime-resistant strains of P.aeruginosa while in countries like the Czech Republic and Greece the resistance rate to ceftazidim ranged from 25 to 50%. According to the study MYSTIC 2006, 1012 P.aeruginosa strains were isolated in 40 European centers and 25% were ceftazidim-resistant (over 40% in Italy and Greece) [15].

Ureido-penicillin Resistance

Of 85 strains of P.aeruginosa isolated in our clinic, only 16 strains were tested to piperacillin and 32 strains were tested at piperacillin-tazobactam. Piperacillin-tazobactam resistance was demonstrated in 19 strains (59.4% of those tested), and to piperacillin in 56.3% of cases.

Resistance to piperacillin-tazobactam for P.aeruginosa was the lowest in 2006 according to the MYSTIC study (15%) [15].

According to EARSS 2009 (2006-2009), a higher resistance rate was noted in some countries like Hungary and France, of 21% and 19% in 2009. [17] MAR-T study indicates a rate of 50.3% piperacillin-tazobactam – resistant strains of P.aeruginosa isolated in ,, Matei Bals ,, Institute (INBS).[10]. The results are consistent with those obtained in Iasi Infectious Disease Clinic. Development of locally adapted antimicrobial therapy recommendations should take into account the possibility that piperacillin-tazobactam empiric therapy to be ineffective in at least half cases of infections determinated by P.aeruginosa.

Ten P.aeruginosa strains were tested to ticarcillin / clavulanic acid and resistance was demonstrated in 7 cases (70%).

Aminoglycosides

At European level, the report brings together EARSS 2009 data on resistance to aminoglycosides for 8253 strains of P.aeruginosa, of which 1884 were resistant to aminoglycosides, resistance rate reaching 33.3% in Bulgaria, 41.3% in Greece and 45.5% in Romania [17].

MAR-T Study results indicated in Romania a percentage of 53.1% sensitivity for amikacin in the INBS and 78.8% for strains from Iasi, Constanta and Timisoara [10]. In the study conducted in our clinic, 35 P.aeruginosa strains were tested for amikacin, the stamina of 55.6%. Gentamicin resistance was observed in the rate of 81.8%, being used for a longer time than amikacin.

Fluoroquinolone Resistance

Antipseudomonas fluoroquinolones have been widely used to treat infections with P.aeruginosa, usually in combination with an anti-pseudomonas beta-lactam, according to many clinical guidelines. To get an idea of the effectiveness of these antibiotics as first-line treatment in severe infections, 48 strains of P.aeruginosa ciprofloxacin were tested to ciprofloxacin and the resistance rate was 87.5%. 26 strains were tested to ofloxacin and showed a resistance rate of 57.7%. EARSS report 2009 showed a lower rate of resistance, ranging from 1-5% in Denmark and Norway. Romania was registered with a resistance rate of 20% (but in 2008 were reported only three strains of P.aeruginosa). In Eastern Europe, the percentage of strains of P.aeruginosa fluoroquinolones - resistant ranged between 36.36% and 33.36% for Bulgaria in 2007 and 2008, over 40% in the Czech Republic, 37.27% in Poland (2007), 26.71% in Hungary (2009). In Greece was noted the highest resistance rate, 49.75% in 2008 [17].

MYSTIC Study 2006, conducted in 40 European centers, showed a resistance rate of 33% for P.aeruginosa strains to ciprofloxacin.
Nationally, the MAR-T study shows a sensitivity of 39.7% for 292 *P. aeruginosa* strains tested at ciprofloxacin, isolated from INBS and 82.6% for 275 strains isolated in the other three participating centers [10]. The number of strains isolated in our hospital is relatively small, we followed in particular the effectiveness of ciprofloxacin (the fluoroquinolone most commonly used), but a resistance rate higher than the one communicated by INBS or by countries with major antibiotic resistance (Greece and Italy) issues was observed. This could be explained by the frequent use of ciprofloxacin in treating many types of infections, while other antipseudomonal fluoroquinolones were more rarely used.

**Carbapenem Resistance**

Long time recommended as first-line therapy in nosocomial infections with non-fermenters, the use of carbapenems (imipenem and meropenem) was limited by the emergence of resistance. Of 85 *P. aeruginosa* strains studied, 66 were tested to imipenem and the resistance rate was 53% (35 resistant strains), and 56 strains were tested to meropenem and the resistance rate was 71.4%.

In the 2009 EARSS report, a statistical analysis was performed after the study of resistance profile for 8129 strains of *P. aeruginosa* of which 1541 were resistant to carbapenems. They surveyed 28 European countries. Percentage of resistance to carbapenems is under 1% in Iceland, ranged between 20-25% in Bulgaria and Poland, 44% in Greece, 30% in Italy (188 strains) and 54 5% for Romania, the number of strains studied being 11 [17].

MAR-T study indicates in INMB for *P. aeruginosa* a rate of sensitivity of 50.5% to imipenem (293 strains) and 50% for meropenem (208 strains tested) so that, for the other centers participating in the study (data were been aggregated and presented in comparison with results from INMB), the percentage of susceptibility to carbapenems is over 75%. The study coordinators explained this difference by the existence of intensive care unit intensively operating in the INMB. This may be an explanation for our clinical study, in the intensive care unit patients being hospitalized with serious infections, many of them being transferred from other hospitals from Moldova [10].

The existence of a high resistance rate to carbapenems questions their effectiveness as first-line choice therapy in severe infections with non-fermenters. Inefficiency of meropenem conduces in many cases to the impossibility of treating nosocomial meningitis where the area of possibilities is already limited by the penetrability of the hemato meningeal membrane.

**Colistin Resistance**

50 strains of *P. aeruginosa* were tested to colistin and the resistance rate was low (2%). Colistin is an antibiotic that preserve in vitro activity for a significant percentage of strains and is recommended as first-choice therapy in cases where this etiology is suspected. For colistin, MAR-T study indicates a rate of sensitivity of 90.2% for strains isolated in [10].

Unfortunately, colistin is the only option available for treating infections with *P. aeruginosa* XDR (Extended Drug Resistance). According to the SENTRY study conducted in Europe between 2001 and 2004 *P. aeruginosa* showed a low resistance rate to polymyxin B (1.1%) [6]. There was noted an increased frequency of resistant strains of *P. aeruginosa* to polymyxins according their more extensive use. In a previous SENTRY study (1998), polymyxin resistance was not observed in *P. aeruginosa* [6].

**Acinetobacter baumannii Resistance**

*Acinetobacter baumannii* is a non-fermentor frequently encountered in the surrounding environment. Although it was initially considered avirulent, *A. calcoaceticus-baumannii* complex emerged
as a nosocomial pathogen with the ability to create serious issues in intensive care units, becoming in evolution MDR, XDR, and then RDP [7].

The incidence of severe infections with MDR and even XDR A. baumannii increased worldwide as a result of its ability to survive in the environment, to acquire mechanisms of resistance (plasmids, transposomi, and integrons containing various gene that encodes resistance), by intrinsic resistance to various antibiotics as a result of decreased outer membrane permeability, efflux pumps [8] and the production of intrinsic beta-lactamase Amp-C type and variants of OXA type 51/69 with carbapenemases properties [8].

Cephalosporins Resistance

39 A. baumannii strains were tested at ceftazidim and 24 were resistant (80%). Alarming resistance rate in our study is in agreement with results from European countries with serious issues of antibiotic resistance, so that we can assume that the initiation of treatment with ceftazidime in a severe infection in which we suspect this etiology is a mistake.

Acinetobacter baumannii resistance to ceftazidime was observed also in MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) in which were studied 490 isolates from 37 centers in 11 European countries during 1997-2000. The resistance rate to ceftazidime was estimated at 58%. More recent data from 2006, reported by 40 centers in 12 countries participated at MYSTIC program, indicate a rate of 68.8% resistance to ceftazidime. [4]

National studies indicate lower resistance rate of Acinetobacter baumannii to ceftazidime: 41.3% (Spain 2000-2003), 58.2% in Italy (2004-2008), 84% (Turkey 2000-2003) and 96, 9% in Greece (February 2006) [13].

A study conducted in Iasi at the Institute of Public Health in 2004, performed on 34 strains of A. baumannii and A. lwaffii isolated from clinical specimens and environmental samples from the hospitals were investigated according the biochemical behavior and antimicrobial susceptibility showed a susceptibility rate of 16.6% to ampicillin and 25% to ceftriaxone, ceftazidime, gentamicin and kanamycin [2].

Piperacillin-Tazobactam Resistance

17 strains of Acinetobacter baumannii were tested to piperacillin-tazobactam with a resistance rate of 64.7%. Although it is a small number of cases, the result is similar to those published in MYSTIC 2002 and 2006 studies (65-66%). Greek National studies indicate a resistance rate higher than 95% [14].

This resistance rate to Piperacillin-tazobactam restricts their use as first-choice antimicrobial treatment in severe nosocomial infection.

Aminoglycosides Resistance

22 strains of Acinetobacter baumannii were tested to Amikacin, with a resistance rate of 45.5%.

The MYSTIC report for 2006 showed a rate of Acinetobacter baumannii resistance to amikacin of 45% in Europe, and national studies indicated a resistance rate of 87.3% in Greece [14].

Gentamicin resistance was documented in 28 of the 30 strains tested (93.3%), probably by improper administration of this antibiotic in many types of infections. The combination of aminoglycosides to imipenem showed a synergistic effect for strains of Acinetobacter baumannii imipenem-susceptible, so that their use may be beneficial as first choice therapy in severe infections [2].

Fluoroquinolones Resistance

26 strains of Acinetobacter baumannii were tested to Ciprofloxacin and the resistance was observed in 20 cases (76.9%). The result is similar to those achieved in surveillance studies: MYSTIC 2002 ( 60% ), MYSTIC 2006 (67.9%), Spain (87%, 2000-
Carbapenems (imipenem and meropenem) were indications of choice in severe nosocomial infections with non-fermenters. The emergence of carbapenem resistance is a reality worldwide. For the strains of *Acinetobacter baumannii* isolated in SENTRY study in 30 European countries between 2001 and 2004, the resistance rate to imipenem and meropenem was 26.3% and 29.6% [1].

The MYSTIC Program (Meropenem Yearly Susceptibility Test Information Collection) reported antimicrobial susceptibility profile for 490 strains of *Acinetobacter baumannii* collected in 37 centers in 11 European countries between 1997 and 2000. Imipenem and meropenem were the most effective antibiotics in *Acinetobacter baumannii* infections, with resistance rates ranging from 16% to 18%. Higher differences were observed between participating countries, Turkey at that time reported the highest resistance rate to all antibiotics, including carbapenems, followed by Italy and England [15]. More recent data, MYSTIC 2006, indicated an increase in resistance rate for meropenem (43.4%) and imipenem (42.5%) [14]. In Greece, the percentage of imipenem-resistant strains of *Acinetobacter baumannii* isolated from patients hospitalized from 1996 to 2007 ranged from 0 to 85.1% (in intensive care units), 60.4% (internal medicine services) and 59% (departments of surgery) [15]. Many small-range studies documented the emergence of carbapenem-resistant strains of *Acinetobacter spp*. A study conducted in Turkey in intensive care units indicated a resistance rate of 80.3% and 71.2% to meropenem, imipenem respectively [5]. In Bulgaria, a study developed in one center in intensive care units in 2006, indicates a resistance rate to carbapenems of 75% [12] and in England, a retrospective study on 399 strains indicates an increase in resistance rate from 0% in 1998 to 55% in 2006 [16].

In our study, 28 strains of *Acinetobacter baumannii* were tested to meropenem, resistance proven in 22 cases (78.6%) and the imipenem resistance was observed in 44.1% cases. So if the number of cases is small, the high percentage of resistance to carbapenems reached the upper limits encountered in Europe, especially for meropenem.

In the MAR-T study conducted in Romania, the differences between the participating centers are very important. If all other centers reported a rate of of susceptibility to meropenem of 75%, the percentage of sensitive strains isolated in INMB was 27.4% [10].

**Colistin Resistance**

*A. Baumannii* is an opportunistic pathogen that is frequently involved in outbreaks of infection, occurring mostly in intensive care units, so that resistance to carbapenems is a big warning [7].

Unfortunately, there is emergence of resistance of colistin-resistant *A. baumannii* strains in Europe. Results of survey European SENTRY study indicates a rate of 2.7% colistin *A. baumannii* resistant strains (2001-2004) [74].

In a study conducted in Greece on 100 strains of *A. baumannii* isolated in intensive care units a rate of 3% resistance to colistin was observed [14].

In our clinic 18 strains of *A. baumannii* were tested to colistin, the resistance rate being 38.9% (7 strains resistant). High percentage of resistance can be explained by the small number of isolates and by the fact that all patients were at risk for infection with multidrug-resistant *A. baumannii*, admitted from other medical services (surgery, intensive care units). Colistin-resistant isolates should be tested to multiple antibiotics. Resistance to colistin can induce phenotypic changes in the outer membrane penetrability favoring other antimicrobial agents (ampicillin-sulbactam, aztreonam,
cefoxitin, cefepime, ceftriaxone, and cefuroxime), but not carbapenems or tobramycin. Minocycline and tigecycline are valuable reserve for the treatment of infections with multidrug-resistant A. baumannii, regardless of susceptibility to colistin [11].

CONCLUSIONS
1. The resistance rate of strains of P. aeruginosa to ceftazidime is 55.6%, to ciprofloxacin 87.5% and 55.6% to imipenem. Initiation of treatment with one of these antibiotics in a severe infection may lead to treatment failure in at least half of the cases.
2. A. baumannii strains showed a resistance rate of 80% to ceftazidime, 76.9% to ciprofloxacin, and to amikacin 45.5%. Acinetobacter baumannii resistance to meropenem was observed in 78.6% of strains, and the resistance rate to imipenem was 44.1%.
3. A high rate of strains resistant to carbapenems is consistent with previous published national data (MAR-T study) from hospital with important intensive care units.
4. Because of the increased resistance rate of non-fermenters to carbapenems, colistin remains the correct first choice for severe infections when this etiology is suspected.

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