PREVALENCE OF ACINETOBACTER BAUMANNII 
ISOLATED FROM CLINICAL SPECIMENS 
IN ADAM MALIK HOSPITAL

Evita Mayasari, Cherry Siregar

Abstract

Acinetobacter baumannii is the most frequent species of Acinetobacter spp. isolated from humans and more common in nosocomial infection than it is in community acquired infection. A.baumannii existence in environment is associated with the diversity of its reservoirs, its capacity to accumulate genes of antimicrobial resistance, and its resistance to desiccation. Infection of Multidrug resistant (MDR) strain of A.baumannii is not easy to manage and it has become a problem in many countries. The aim of this retrospective study was to investigate the prevalence of A.baumannii from routine clinical specimens sent to clinical microbiology laboratory RSUP HAM Medan and its susceptibility pattern to various antibiotics. Identification and susceptibility testing of A. baumannii was performed by Vitek 2 with Advanced Expert System (AES). A total of 644/3693 (17.44%) A.baumannii isolates were identified from various clinical specimens. From those isolates, there were 147 (23%) isolates of carbapenem-resistant A.baumannii (imipenem and meropenem). A.baumannii mainly isolated from sputum specimens, and most isolates were highly sensitive to colistin, amikacin and tigecycline. Low prevalence of A.baumannii was found in this study. However, the isolates showed high resistance level to antibiotics, particularly penicillin, cefalosporin and fluoroquinolones.

Keywords: A.baumannii, clinical specimens, susceptibility pattern, carbapenem-resistant A.baumannii

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MKA, Volume 37, Nomor 1, April 2014
INTRODUCTION

Acinetobacter are opportunistic Gram-negative bacilli that occasionally appear round on smears (so termed cocobacilli). These bacteria are widely distributed in soil and water. They can occasionally be cultured from skin, mucous membranes, secretions, and hospital environment. A. baumannii is the species most commonly isolated. A. baumannii has been isolated from blood, sputum, skin, pleural fluid, and urine, usually in device associated infections. Acinetobacter encountered in nosocomial pneumonia often originates in the water of room humidifiers or vaporizers. In patients with A. baumannii bacteremia, intravenous catheters are the main source of infection. In patients with burns or with immune deficiencies, acinetobacter acts as an opportunistic pathogen and can produce sepsis.

Acinetobacter strains are often resistant to antimicrobial agents, and therapy of infection can be difficult. They respond most commonly to gentamicin, amikacin, or tobramycin and to newer penicillins or cephalosporins. To treat serious A. baumannii infections, carbapenems has been considered for many years as the drug of choice. Until recently, the majority of clinical A. baumannii isolates had been sensitive to the carbapenems. Unfortunately, the rapidly escalating prevalence of carbapenem-resistant A. baumannii (CRAB) in many parts of the world in the past few years has undermined the reliability of carbapenems. A. baumannii resistance to carbapenems mediated by the lack of drug penetration (i.e., porin mutations and efflux pumps) and/or carbapenem-hydrolyzing beta-lactamase enzymes such as OXA carbapenamases and metallo-beta-lactamases (MBLs).

Recently, a large number of class D OXA-type enzymes which against carbapenems were characterized in locations that include Scotland, Spain, France, Japan, Singapore, China, Brazil, Cuba, and Kuwait. Some Acinetobacter strains express class B MBLs, which hydrolyze a broad array of antimicrobial agents, including carbapenems. MBLs pose a significant threat because they are often located on mobile genetic elements easily transferred among bacteria. Carbapenem-resistant isolates of A. baumannii are usually resistant to all classes of antimicrobials, and show intermediate resistance to rifampin, while usually retaining susceptibility to tigecycline and colistin. Susceptibility testing should be done to help select the best antibiotics for therapy.

Carbapenem resistance in A. baumannii is now an emerging issue worldwide. Surveillance studies indicate that the percentage of CRAB isolates gradually increased over the last ten years in Europe, North and Latin America. Numerous outbreaks of CRAB were reported from hospitals in Northern Europe, Southern Europe and the Middle East, North and Latin America, Tunisia, South Africa, China, Taiwan, Singapore, Hong Kong, Japan, South Korea and Australia. Outbreaks caused by CRAB have also been observed in developing countries such as Morocco, Thailand, India and Indonesia. At present, the article of CRAB prevalence in Indonesia is limited. A study in Sanglah General Hospital Bali reported that of all A. baumannii isolates, almost 40% was multidrug-resistant and three of them produced carbapenemase. A study in internal medicine ward of dr. Soetomo Hospital Surabaya reported that 9.8% A. baumannii isolated from 467 urine specimens and antibiotics were potent towards these isolates. In an effort to provide information regarding prevalence and antibiotic susceptibility pattern of A. baumannii isolated from clinical specimens of a hospital in Medan, we conducted this study.

METHODS

We conducted a retrospective study on various clinical specimens sent to clinical microbiology laboratory RSUP HAM, January until December 2012. Identification and susceptibility testing of A. baumannii were performed by Vitek2 system with Advanced Expert System (AES). Vitek2 AES is able to predict the presence of carbapenemases (metallo- or OXA) in A. baumannii. The entire beta lactam minimum inhibitory concentration (MIC) profile was taken into account and analysed by the AES software, providing the capability to flag isolates as potential carbapenemase producers, even those producing a low-level of enzyme.

Except for sterile fluids, the clinical specimens were cultured in blood agar and MacConkey agar, incubated overnight at 37°C. The sterile fluids were put in Fan
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Aerob Culture Bottles (BacT/ALERT), and incubated at 37°C for five days. The culture bottles with bacterial growth were proceeded for Gram stain and primary isolation in blood agar and MacConkey agar, incubated overnight at 37°C. The pure colonies from cultured isolates were picked and mixed with three milliliters of sterile saline (0.45% NaCl), prepared for inoculum suspension. Two tubes were prepared for each isolate. The inoculum turbidity was adapted with the turbidity of 0.5 McFarland standard using Den-sichek. First tube was placed in cassette for identification and second tube was placed for sensitivity test. A suitable Vitek2 card (GN/AST-N089/100) was placed according to instruction from bioMérieux.

The antibiotics used for sensitivity test in this study included ampicillin, amoxicillin/clavulanic-acid, ampicillin-sulbactam, meropenem, ciprofloxacain, cefazidime, cefepime, ceftriaxone, cefotaxime, gentamicin, imipenem, trimethoprim/sulfametoxazol, tobramycin, levofloxacain, tigecycline, amikacin, dan piperacillin-tazobactam.

RESULTS AND DISCUSSION

There are three most clinically relevant species of *Acinetobacter* that have been implicated in the vast majority of both community-acquired and nosocomial infections, i.e., *A. baumannii*, *Acinetobacter* genomic species 3 and *Acinetobacter* genomic species 13TU. According to the taxonomy, these organisms are classified as the *A. calcoaceticus-A. baumannii* complex, together with *A. calcoaceticus* that have frequently been recovered from soil and water but has never been implicated in serious clinical disease. The three clinically relevant members of the *A. calcoaceticus-A. baumannii* complex cannot be separated by currently available commercial identification systems. In fact, *A. baumannii*, *Acinetobacter* genomic species 3, and *Acinetobacter* genomic species 13TU are uniformly identified as *A. baumannii* by the most widely used identification systems (including Vitek2). The term *A. baumannii* group for these species instead of *A. calcoaceticus-A. baumannii* complex reflects the fact that *A. baumannii*, *Acinetobacter* genomic species 3, and *Acinetobacter* genomic species 13TU share important clinical and epidemiological characteristics and also eliminates the confusion resulting from inclusion of an environmental species, *A. calcoaceticus*. Consistent with Vitek2 results, we simply use the species name *A. baumannii*.

A study revealed that Vitek2 cards were promising for rapid identification and reliable antibiotic susceptibility test (AST) for most species of non-glucosefermenting Gram-negative bacilli (including *Acinetobacter* spp.). A study stated that Vitek2 method was the only automated susceptibility method that satisfied Food and Drug Administration (FDA) criteria for approval when used for imipenem and meropenem. The latter result is consistent with the former that observed a very major errors (VME) rate of 0.7% for the Vitek2 instrument. Therefore we supposed that our data are reliable enough to describe *A. baumannii* prevalence. We successfully identified a total of 644/3693 (17.44%) isolates of *A. baumannii* from various clinical specimens over the period January-December 2012 (Figure 1).

Figure 1. *A. baumannii* Prevalence in Total Bacterial Isolates

Vitek2 is able to determine the MIC and the production of carbapenemase during one test cycle. It detects metabolic changes by fluorescence based methods and identifies bacteria by monitoring the kinetics of bacterial growth. The MIC phenotype detected for the test isolate is interpolated with all the patterns of the database and the best is identified. We found 147/644 (23%) CRAB (imipenem and meropenem). Because the problems of
false resistance and false susceptibility with imipenem and meropenem may occur, the detection of CRAB strains by Vitek2 should be confirmed by an additional test\textsuperscript{14,18} (e.g. modified Hodge test, PCR), which we did not perform in this study. Therefore, our result regarding CRAB prevalence in \textit{A. baumannii} isolat remain as a prediction.

In most institutions, the majority of \textit{A. baumannii} isolates are from the respiratory tracts of hospitalized patients.\textsuperscript{4} A study from South Africa revealed that from 232 CRAB complex isolates, 149 (64.2\%) of the specimens were from endotracheal aspirates.\textsuperscript{19} Recent study from Turkey revealed that from 74 strains isolated and identified as \textit{A. baumannii}, most of the strains (46\%) were isolated from tracheal aspirate specimens, and the study results is correspond to three other similar studies from Turkey.\textsuperscript{20} We found \textit{A. baumannii} isolates in sputum 59.94\%, pus 12.35\%, urine 6.02\%, blood 4.07\%, cerebrospinal fluid (CSF) 2.11\%, pleural fluid 0.9\%, bronchoalveolar lavage (BAL) 0.3\% and other specimens 14.31\%, as shown in Figure 2. Most of \textit{A. baumannii} were isolated from respiratory tract (sputum), therefore our result is consistent with previous studies.

Antibiotic susceptibility pattern of \textit{A. baumannii} isolates to some tested antibiotics is describe in table 1. Those isolates were sensitive to colistin (95.86\%), amikacin (76.23\%), tigecycline (75\%), imipenem (63.39\%), meropenem (62.43\%), ampicillin-sulbactam (57.35\%), tobramycin (53.54\%) and trimethoprim/sulfamethoxazole (51.55\%).

![Figure 2. Distribution of \textit{A. Baumannii} Isolates According to Clinical Specimens.](image)

**Table 1. Antibiotic Susceptibility Pattern of \textit{A. Baumannii} isolates**

<table>
<thead>
<tr>
<th>Antibiotic Tested</th>
<th>No. tested</th>
<th>No. sensitive</th>
<th>% sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin (AN)</td>
<td>551</td>
<td>420</td>
<td>76.23</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid (AMC)</td>
<td>508</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Ampicillin (AM)</td>
<td>644</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ampicillin-sulbactam (SAM)</td>
<td>136</td>
<td>78</td>
<td>57.35</td>
</tr>
<tr>
<td>Cefepime (FEP)</td>
<td>644</td>
<td>242</td>
<td>37.58</td>
</tr>
<tr>
<td>Cefotaxime (CTX)</td>
<td>508</td>
<td>85</td>
<td>16.73</td>
</tr>
<tr>
<td>Ceftazidime (CAZ)</td>
<td>644</td>
<td>227</td>
<td>35.25</td>
</tr>
<tr>
<td>Ceftriaxone (CRO)</td>
<td>136</td>
<td>13</td>
<td>9.56</td>
</tr>
<tr>
<td>Ciprofloxacin (CIP)</td>
<td>644</td>
<td>240</td>
<td>37.26</td>
</tr>
<tr>
<td>Colistin (CS)</td>
<td>508</td>
<td>487</td>
<td>95.86</td>
</tr>
<tr>
<td>Gentamicin (GM)</td>
<td>644</td>
<td>263</td>
<td>40.84</td>
</tr>
<tr>
<td>Imipenem (IPM)</td>
<td>508</td>
<td>322</td>
<td>63.39</td>
</tr>
<tr>
<td>Levofloxacin (LEV)</td>
<td>644</td>
<td>250</td>
<td>38.82</td>
</tr>
<tr>
<td>Meropenem (MEM)</td>
<td>551</td>
<td>344</td>
<td>62.43</td>
</tr>
<tr>
<td>Piperacillin/tazobactam (TZP)</td>
<td>642</td>
<td>248</td>
<td>38.63</td>
</tr>
<tr>
<td>Tigecycline (TGC)</td>
<td>644</td>
<td>483</td>
<td>75</td>
</tr>
<tr>
<td>Tobramycin (TM)</td>
<td>508</td>
<td>272</td>
<td>53.54</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole (SXT)</td>
<td>644</td>
<td>332</td>
<td>51.55</td>
</tr>
</tbody>
</table>
In this study, the sensitivity test results of *A. baumannii* towards ampicillin-sulbactam, imipenem, tobramycin meropenem, trimethoprim sulfamethoxazole were tolerable. Ampicillin-sulbactam or a carbapenem (imipenem or meropenem) is adequate for the treatment of infections caused by antibiotic-susceptible strains of *Acinetobacter* spp. A study of *A. baumannii* bacteremia in patients who presented with critical illness found that patients with empirical combination therapy (e.g., carbapenem plus sulbactam or an aminoglycoside) had a lower mortality rate than patients with monotherapy. We found high (76.23%) amikacin-sensitive *A. baumannii* isolates, so this recommendation may be important for critical patients at risk of *A. baumannii* infections or in the clinical settings of the presence of MDR *A. baumannii*. Among the antibiotics that are considered as agents against MDR *A. baumannii*, tigecycline has received significant attention. Where the dissemination of MBLs accounts for the increasing prevalence of CRAB, the combination of colistin and rifampin with or without tigecycline should be considered. But there have been reports of *A. baumannii* resistance to tigecycline. In this study, we found 25% tigecycline-resistant *A. baumannii* isolates. We found those isolates were most sensitive to colistin (95.87%), and our result is correspond to the previous studies from India and Morocco. When CRAB is suspected, intravenous colistin combined with rifampin and imipenem is a recommended therapy. However, the optimal empirical combination regimens directed against *A. baumannii* infections should be based on local antimicrobial susceptibility patterns.

*A. baumannii* rate of carriage by hospitalized patients is high. It causes a variety of nosocomial infections, comprising of bacteremia, urinary tract infection, surgical site infection and ventilator-associated pneumonia, mainly in intensive care unit (ICU) patients. It has emerged as a major cause of hospital acquired infections and unfortunately, some of them are MDR strains which probably caused by extensive use of antimicrobial agents. *A. baumannii* has a broad range of resistance mechanisms to all existing antibiotic classes and a capacity to acquire new determinants of resistance. Genome sequence analysis of six *A. baumannii* clinical strains has shown the presence of a resistance island with a variable composition of resistance genes interspersed with transposons, integrons, and other mobile genetic elements in three of them. Plasmids carrying resistance genes involved in horizontal gene transfer have been described in *A. baumannii* strains. A study showed that infections with MDR *Acinetobacter* are associated with length of hospitalization and its mortality rate higher than patients with infection by susceptible *Acinetobacter*.

Within the last five years, the increasing number of CRAB is of major importance in the context of resistance to beta lactams. Between 1998 and 2004, this rate of resistance in Europe, North America, South America, and Asia ranged between 0% and 40%. But in most studies after 2005, the rate of resistance was greater than 50%. High rates of resistance were found in India (90%), Morocco (74%), Iran (63%), Italy (62.5%), China (55.6%), Turkey (53.7%), Korea (51%), and India (51%). A study in ICU Cipto Mangunkusumo Hospital Jakarta found high rate of resistance (50.5%). Sanglah Hospital Bali showed a moderate rate of resistance (36.9%). To compare CRAB prevalence with previous studies, we still need confirmation of Vitek2 CRAB results by additional test (e.g., modified Hodge test).

**CONCLUSION**

We found low prevalence of *A. baumannii*, and most of them are from sputum. Those isolates showed high resistance level to antibiotics, particularly penicillin, cephalosporin and fluoroquinolones. We detected CRAB prevalence from the isolates, but we need to perform additional tests in further studies to confirm the result. Antibiotic susceptibility pattern presented in this study may help clinicians to select appropriate empirical antimicrobial therapy for *A. baumannii* infections.
REFERENCES


