

Isolation of environmental microorganisms from clinical specimens: A report of the occurrence of *Acinetobacter anitratus* in blood of hospitalized patients in Jakarta in a 7 year period

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Abstrak

Tujuan Menunjukkan adanya *A. anitratus* pada isolat klinik (darah) yang berasal dari pasien rawat di rumah sakit di Jakarta selama periode 2002-2008 dan pola sensitivitas mikroorganisme ini terhadap antibiotika.

Metode Penelitian ini merupakan penelitian retrospektif dari semua spesimen darah yang masuk ke Laboratorium Mikrobiologi Klinik Fakultas Kedokteran Universitas Indonesia (LMK-FKUI) dari tahun 2002-2008. Kultur dan pemeriksaan kepekaan terhadap antibiotik dilakukan berdasarkan praktek standar di LMK-FKUI dan Clinical Laboratory Standard Institute pada tahun yang bersangkutan. Data dikumpulkan dengan menggunakan program WHO-NET 5.4. Semua mikroorganisme Gram negative yang diisolasi dari spesimen darah ditabulasi juga termasuk dengan uji kepekaan *A. anitratus* terhadap antibiotik. Selain itu juga dilakukan analisis terhadap asal spesimen atau dari institusi mana spesimen tersebut berasal.

Hasil *A. anitratus* merupakan bakteri Gram negatif yang paling banyak diisolasi selama tujuh tahun sejak 2002 sampai 2008 dari spesimen darah, dan selalu ditemukan setiap tahunnya. Hampir 50% bakteri yang diisolasi terdiri dari bakteri tersebut dan *Pseudomonas aeruginosa*, dan keduanya adalah bakteri lingkungan. Pemeriksaan kepekaan bakteri *A. anitratus* terhadap antibiotik menunjukkan adanya resistensi terhadap beberapa antibiotik yang diuji. Evaluasi asal spesimen darah menunjukan sebagai berikut: 88 spesimen (74%) berasal dari Rumah Sakit pemerintah, 18 spesimen (15%) dari Rumah Sakit swasta, 3 spesimen (3%) dari pasien praktek dokter dan 10 spesimen (8%) tidak diketahui asalnya.

Kesimpulan Ditemukan *A. anitratus* setiap tahun sejak 2002 sampai 2008 dari spesimen darah dari pasien rawat inap di beberapa Rumah Sakit di Jakarta. Diperlukan penelitian lebih lanjut untuk mencari faktor resiko bakteremia *A. anitratus* agar dapat mengurangi kemungkinan terjadinya infeksi rumah sakit. Selain itu sangat dianjurkan untuk melanjutkan sampai tahap genotyping untuk menentukan hubungan antara strain yang ada di Rumah Sakit dengan strain yang diisolasi dari pasien. (*Med J Indones* 2009; 18:227-32)

Abstract

Aim To report the presence of environmental microorganisms, *A. anitratus*, in blood of hospitalized patients in Jakarta from 2002 to 2008 and their susceptibility to antibiotics.

Methods A Retrospective study was performed on all blood specimens that were received in Clinical Microbiology Laboratory (CML) Faculty of Medicine University of Indonesia during 2002-2008. Culture and antimicrobial susceptibility examination were carried out according to up to date standard practice in CML and Clinical Laboratory Standard Institute, respectively. Data was collected by WHONET 5.4 program. All Gram-negative microorganisms that were isolated from blood specimens were tabulated, and so the antibiotics susceptibility of *A. anitratus*. The origin of the specimens in term of institutions where the specimens came from was also analyzed.

Results In a 7 year period up to 2008, *A. anitratus* was found in blood specimens, and these environmental bacteria were in fact the most predominant isolated Gram negative microorganisms. Together with another environmental microorganism, *Pseudomonas aeruginosa*, it composed nearly 50%. Antimicrobial susceptibility test of this microorganism showed some degree of resistance to all tested antibiotics. The origin of those blood specimens which yielded *A. anitratus* were mainly from government-owned hospitals, that was 88 specimens (74%), followed by private hospitals (18 specimens, 15%), individuals (3 specimens, 3%), and unknown source (10 specimens, 8%).

Conclusion Persistent occurrence of *A. anitratus* in blood specimens of hospitalized patients in hospitals in Jakarta was observed. In the near future, a study to find risk factors for the acquisition of *A. anitratus* bacteremia is needed to reduce potential hospital associated infection. Moreover, genotyping is advised in order to determine the relationship of hospital and patient derived strains. (*Med J Indones* 2009; 18:227-32)

Key words: gram - negative microorganisms, antimicrobial susceptibility

The prevalence of *Acinetobacter* infection has increased worldwide in the past two decades. Centers for Disease Control and Prevention (CDC) and National Nosocomial Infection Surveillance (NNIS) in the United States indicated that *Acinetobacter* was the cause of 1% of all nosocomial bloodstream infections, and 3% of nosocomial pneumonia in US hospitals compared with 5% to 10% for Latin America hospitals.^{1,2} In 2008 CDC defined a new terminology 'Health care-associated infection' instead of nosocomial infection.³ *Acinetobacter species* are commensals, pleomorphic aerobic Gram negative bacilli, and usually coccobacillary or coccal in appearance. This microorganism is able to survive on both moist and dry surfaces and may be part of the normal skin flora of humans. *Acinetobacter species* are often misinterpreted to be other Gram negative organisms that are more commonly associated with clinical syndromes, e.g. *Neisseria meningitidis* in cerebrospinal fluid, and *Haemophilus influenza* in sputum.^{4,5} DNA-DNA hybridization investigations have shown the presence of 25 DNA homology groups that were called genomospecies among *Acinetobacter* strains. For clinical purposes, 2 phenotypic groups were determined i.e. non hemolytic and hemolytic groups.⁶

Acinetobacter usually colonizes patients in the intensive care setting. The bacteria have low virulence but are capable of causing suppurative infection in almost every organ. In patients with *Acinetobacter* bacteremia, intravenous catheters are almost always the source of infection. In patients with burns or with immune deficiencies, *Acinetobacter* acts as an opportunistic pathogen and can produce sepsis. Signs and symptoms that occur depend on the involved organ system.⁷ Mortality and morbidity rates are increased in patients who are very ill with multi-system disease, due to their underlying illness rather than the superimposed infection with *Acinetobacter*. When associated with polymicrobial bacteremia, mortality rate from *Acinetobacter* bacteremia had been reported 17% to 46%, especially for those associated with *A. baumannii*.^{8,9}

The ability of *Acinetobacter* to use a variety of carbon sources via diverse metabolic pathways expands its habitat. The organisms are widely distributed in soil, sewage, water and in the hospital environment. *Acinetobacter* has been isolated from a variety of foods, hospital air, vaporizer mist, tap water faucets, peritoneal dialysate bath, bedside urinals, wash cloths, angiography catheters, ventilators, laryngoscope, contaminated gloves, duodenoscopes, reused needles, multi dose medications, plasma protein fraction, hospital pillows, and soap dispensers. Some strains recovered from sink basins have been found to be tolerant

of soap.¹⁰ *Acinetobacter* has been grown from numerous human sources, including skin, sputum, urine, feces and vaginal secretions. Up to 25% of healthy adults exhibit cutaneous colonization and 7% of adults and infants have transient pharyngeal colonization.⁴ Residency in an Intensive Care Unit (ICU), particularly in the presence of other patients who are colonized with *Acinetobacter*, predisposes to colonization.⁷ Several studies concerning *Acinetobacter sp.* in critical clinical settings had been conducted in Indonesia. Investigation on Ventilator Associated Pneumonia (VAP) patients hospitalized in National General Hospital Cipto Mangunkusumo in Jakarta in 2006-2007 demonstrated that *A. anitratus* (32.4%) was the most Gram negative bacteria that was isolated from lower respiratory secretion, followed by *Pseudomonas aeruginosa* (*P. aeruginosa*) (24.7%), *Klebsiella pneumonia* (10.4%), and Methicillin Resistant *Staphylococcus aureus* (2.6%).¹¹ In 2005, a study was carried out in Burn Unit of the above mentioned hospital and antibiogram similarity suggested an association between *Acinetobacter sp.* isolated from skin of patients in Burn Unit and those from environment.¹²

This study aimed to report the presence of environmental microorganism, *A. anitratus*, in blood specimens of hospitalized patients in Jakarta and its proximity during 2002-2008 and their susceptibility to antibiotics.

METHODS

A retrospective study was performed on all blood specimens received in Clinical Microbiology Laboratory (CML) of Department of Microbiology, Faculty of Medicine University of Indonesia (FMUI) from 2002 to 2008. Culture and identification procedures were carried out according to standard operating procedure in CML FMUI.¹³ Susceptibility to antimicrobials was determined as in Clinical and Laboratory Standards Institute (CLSI).¹⁵ Data was collected from WHONET,^{5,4} a computer program that was designed for recording and analyzing microbiology data. All isolated Gram negative organisms from blood specimens, from 1 January 2002 up to 31 December 2008 and susceptibility of *A. anitratus* to antibiotics were tabulated by years. Origin of specimens in term of institutions where the specimens came from was also analyzed.

RESULTS

Among ten most frequent Gram negative microorganisms that were found in blood specimens during 2002-2008 in Jakarta, *A. anitratus* was the most predominant bacteria with a total of 119 isolates (28%) out of 432 total isolates.

Figure 1 shows the presence of *A. anitratus* every year, where the result of the year 2005 seemed to be the lowest (14%), and 2003 was the highest (34%). The ten Gram negative bacteria isolated during this 7 year period were as follow: *A. anitratus* (28%), *P. aeruginosa* (21%), *Klebsiella pneumonia* (16%), *Enterobacter aerogenes* (9%), *Salmonella typhi* (8%), *Escherichia coli* (7%), *Alkaligenes faecalis* (6%), *Klebsiella oxytoca* (3%), and the last two were *Enterobacter gergoviae* and *Klebsiella*

ozanae (each was 2%) (see Figure 2). Interestingly, environmental microorganisms such *A. anitratus* and *Pseudomonas aeruginosa* composed nearly 50% of the clinical isolates during this period, despite these two bacteria were vastly related to nosocomial infections.

Close examination of the origin of the specimens showed that the specimens came from hospitals and individuals. Of those yielded *A. anitratus* derived

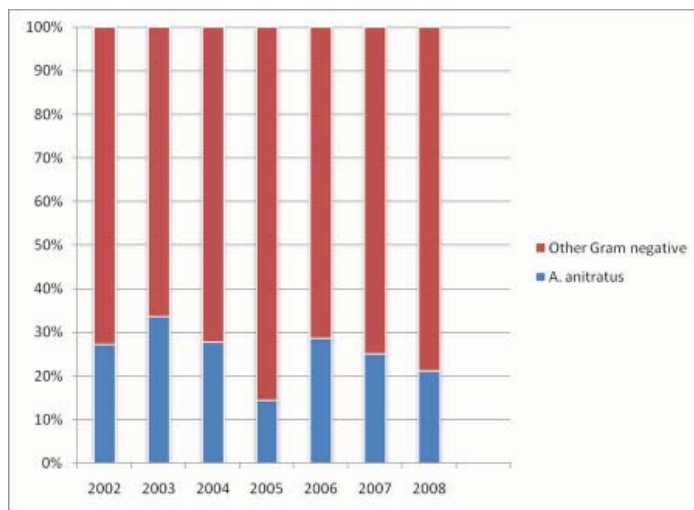


Figure 1. Percentage of isolated *Acinetobacter anitratus* compared to other Gram negative bacteria

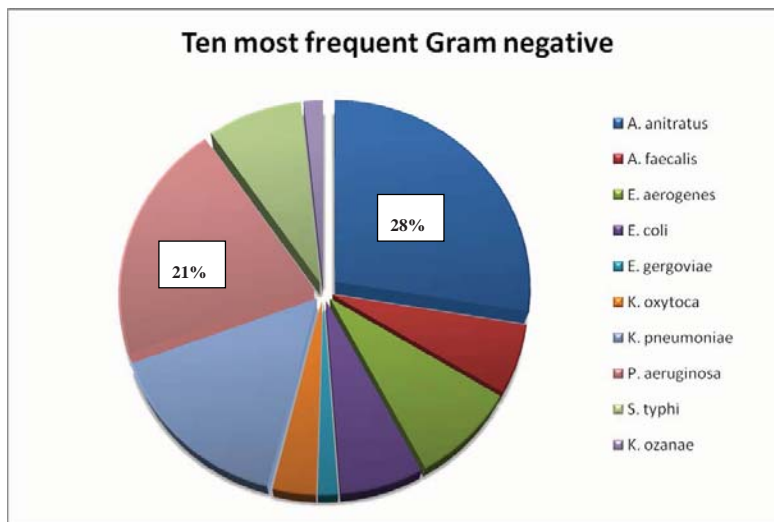


Figure 2. Ten most frequent Gram negative bacteria from blood specimens (2002-2008)

Most environmental microorganisms isolated from blood specimens: *A. anitratus* (28%) and *P. aeruginosa* (21%). Both composed nearly 50% of blood specimen isolates.

mainly from government-owned hospitals, that was 88 specimens (74%), followed by private hospitals (18 specimens, 15%), individuals (3 specimens, 3%), and unknown source (10 specimens, 8%). The specimens from government-owned hospitals mostly derived from adults in-patient (58%), followed by neonate and children in-patients (25%), while ICU patients, adults and neonates/children in emergency room (ER) contributed 10%, 2%, and 5% respectively (Table 1).

Susceptibility of *A. anitratus* to antimicrobials recommended by CLSI¹⁵ was shown in Figure 3. To most antimicrobial tested, *A. anitratus* was showing some degree of resistance. However, the bacteria showed 80% or greater susceptibility to antimicrobials as follows: Tazobactam-Piperacillin (TZP) 100%, Cefepime (FEP) 91%, Meropenem (MEM) 89%, Levofloxacin (LVX) 83%, Gatifloxacin (GAT) 100%, and Sulfamethoxazole (SXT) 89% (Figure 3).

Table 1. Origin of *A. anitratus* isolates

Origin	Total Specimens	ICU	In Adults	ER Adults	In Neo-Child	ER Neo-Child
Government Hospitals	88 (74%)	9 (10%)	51 (58%)	2 (2%)	22 (25%)	4 (5%)
Private Hospitals	18 (15%)	Nk	Nk	Nk	Nk	Nk
Individuals	3 (3%)	Nk	Nk	Nk	Nk	Nk
Unknown	10 (8%)	Nk	Nk	Nk	Nk	Nk

In: In-patients; ICU: Intensive care unit; ER: Emergency Room; Nk: Not known

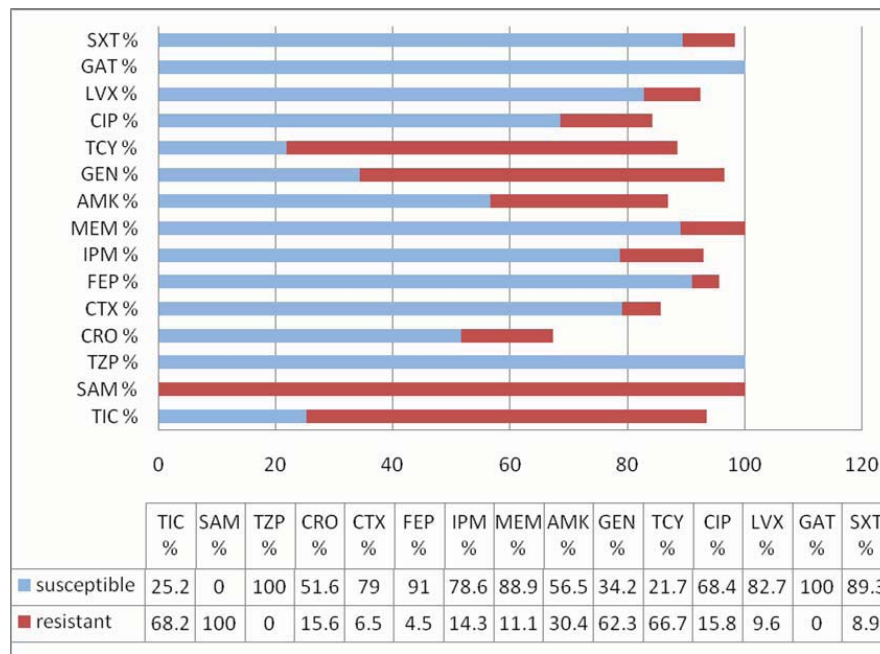


Figure 3. Resistance Patterns of *Acinetobacter anitratus* to antibiotics (2002-2008)

TIC = Ticarcilin; SAM= Ampicilin-Sulbactam; TZP= Piperacilin-Tazobactam; CRO= Ceftriaxone; CTX= Cefotaxime; FEP = Cefepime; IPM= Imipenem; MEM= Meropenem; AMK= Amikacin; GEN= Gentamycin; TCY= Tetracycline; CIP = Ciprofloxacin; LVX= Levofloxacin; GAT= Gatifloxacin; SXT= Trimethoprim-Sulfamethoxazole.

DISCUSSIONS

Persistent discovery of *A. anitratus* in blood specimens during a 7 year period from 2002-2008 from particular hospitals in Jakarta was a strong indication of the existence of the sources of the microorganisms in the hospitals. Moreover, this bacteria and *P. aeruginosa* composed approximately 50% of positive clinical isolates during that period. Our finding was in agreement with earlier study which examined VAP patients in hospitals in Jakarta which showed *A. anitratus* as predominant environmental microorganism in lower respiratory tract.¹¹

In *Acinetobacter* cases, diagnostic is the most problem. The difficulty in diagnosis is mainly in distinguishing between colonization of these bacteria from infection. Generally, colonization means the presence of microorganisms on skin, on mucous membranes, in open wounds, or in excretions or secretions but are not causing adverse clinical signs or symptoms, while inflammation is a tissue response to injury or stimulation that is caused by non infectious agents, such as chemicals and mechanicals, although it can also occur in infection.³

Other aerobic Gram negative bacilli such as *Enterobacter species*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia*, *P. aeruginosa*, *Flavobacterium meningosepticum* and *Serratia marcescens* should be considered as differential diagnosis especially in patients with pulmonary infiltrates in ICU, continuous ambulatory peritoneal dialysis (CAPD) associated peritonitis, meningitis, wound infection, or catheter-associated bacteremia.⁷ Pseudo bacteremia resulting from improper specimen collection and blood culture technique should be distinguished from true *Acinetobacter* bacteremia. Therefore, specimen handling is a very critical step in microbiology examination.

Outbreaks of health care-associated infections due to multi-resistant strains of *A. anitratus* have been reported, and mainly from ICU and surgical intensive care unit.^{16,17} A centre in Netherland performed a case control study, in which risk factors for the acquisition of *A. anitratus* were investigated by comparing epidemiological characteristics of patients who became colonized or infected with those of control patients without colonization. The study showed that ventilators in use were the reservoirs of *A. anitratus* and caused frequent nosocomial respiratory tract infections.¹⁸ A study in Jakarta in 2006-2007 showed superimposed infection due to *A. anitratus* in patients with mechanical ventilation.¹¹ Molecular epidemiology have been carried out in many studies to identify the strain of *Acinetobacter sp.*, in which pulsed-field gel

electrophoresis apparently was more discriminative compared to other methods.¹⁹⁻²¹

Acinetobacter strains are often resistant to antimicrobial agents, and therapy of infection can be difficult. In general, first, second, and third-generation of cephalosporins, macrolides, and penicillins have little or no anti-*Acinetobacter* activity, and their use may predispose to *Acinetobacter* colonization.^{7,22} Overall, there is a trend of increasing resistance of *Acinetobacter*. However, there are significant differences in *Acinetobacter* antimicrobial resistance patterns according to species, country of isolation and region.²³ The current approach to treat a serious infection involving *Acinetobacter* is based on sensitivity of the specific isolate and the use of combination therapy. In the event of hospital outbreak that involve multidrug-resistant *Acinetobacter* strains with similar antibiogram, a review of infection control procedures including hand washing, patient isolation, ventilator care, and housekeeping should be carried out.

In conclusion, the occurrence of *A. anitratus* in blood specimens of patients in hospitals in Jakarta was observed persistently from 2002 to 2008. This microorganism showed resistance in some degree to most antibiotic tested except for Piperacilin-Tazobactam (TZP) and Gatifloxacin (GAT). Further study to find risk factors for *A. anitratus* bacteremia is needed to reduce potential hospital associated infection. In addition, molecular approaches are advised to be performed, so that the strains (genotypes) of *A. anitratus* can be determined, and the relationship of hospital and patient derived strains can be elucidated.

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