The Role of *Acinetobacter* as a Cause of Nosocomial Bacteremia

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**INTRODUCTION**

Nosocomial bacteremia is major cause of morbidity and mortality elevation in the hospital. It is also causes longer patient hospitalization so that it is finally increasing the hospitalization cost that should be paid by the patient. Nosocomial bacteremia that caused by *Acinetobacter* occurs because this microorganism is resistant to many kinds of antibiotics. This microorganism frequently infected patient with severe condition and frequently related to the usage of parenteral nutrition, central vein catheter, and ventilator equipment that have been used by the patient in ICU.  

Since 1970, The National Nosocomial Infections Surveillance System (NNIS) as quoted by Horan TC et al has been collecting and analyzing data about the amount of nosocomial infection United State Hospital. The data that has been achieved by NNIS suggested nosocomial infection during the year of 1984. It was occurred on 3-4 occurrence per 100 patient. That data equal to the data that has been reported by Study on the Efficacy of Nosocomial Infection Control (SENIC) in United State hospital in the year of 1984, that implemented nosocomial infection surveillance as being quoted by Horan TC et al that from 26,965 infected patient there was 84 % with infection caused by pathogen microorganism, that was 86 % caused by aerobic bacteria, 2 % by anaerob bacteria, 8 % by fungi, and 4 % caused by virus, parasite or protozoa. It was reported that 5% - 25% hospitalized patient was experiencing infection by pathogenic microorganism of negative-Gram non-fermentative basil.  

*Acinetobacter* is a kind of microorganism, isolated from soil, water and plants. It is also found on human skin and medical equipment used in hospitals. Various report and study said that nosocomial bacteremia in fact has strong correlation with the usage of medical utility.

Various human infection that caused by *Acinetobacter* species has been reported by Lyons as being quoted by Hamory BH. That infection related to endotracheal tube usage or tracheostomy that could cause pneumonia. *Acinetobacter* is known as major cause of pneumonia nosocomial infection related to ventilator installation on the patient in ICU. *Acinetobacter* is also found in endocarditis, meningitis, skin and tissue infection, and urinary tract infection. There is also report about sporadic case of conjunctivitis, osteomyelitis and sinovitis.

A. baumannii is the most found species *Acinetobacter* genus and it is the most often cause of nosocomial infection.

**NOSOCOMIAL BACTERIEMIA**

The microorganism in blood circulation, if it is in there in certain time and small amount, it will not cause clinical symptoms immediately. That microorganism is immediately destructed by antibody system, complement or macrophage in liver cells, spleen and bone marrow.

Nosocomial bacteremia is defined as clinically significant after the patient being hospitalized for 48 hours and positive blood culture. There is two kinds of nosocomial bacteremia i.e. primary bacteremia and secondary bacteremia.

Primary bacteremia is positive finding of microorganism in blood, without any focal infection in other site such as urinary tract, lungs or tissue injury that caused by microorganism that the same as being found in blood. Generally primary bacteremia is related to invasive utility usage as intra venous fluid administration, surgery, gynecologic, parenteral drugs administration in children and primary skin lesion of newborn management.
Secondary bacteremia is positive finding of microorganism in the blood as the result of infection of other site that caused by the same microorganism as founded in the blood. If there is purulent phlebitis in intravenous line administration then bacteremia is assumed as secondary of that infection. Secondary bacteremia is frequently related to urinary tract infection, lower respiratory tract infection, and skin infection.1,2,7

### Table 1. The Secondary Bacteremia Microorganism Based on NNIS

<table>
<thead>
<tr>
<th>Year</th>
<th>E.coli</th>
<th>S. aureus</th>
<th>Klebsiella sp</th>
<th>P.aeruginosa</th>
<th>Proteus-providencia</th>
<th>Coagulase negative staphylococci</th>
<th>Serratia</th>
<th>Acinetobacter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1975</td>
<td>20.2%</td>
<td>36.7%</td>
<td>11.7%</td>
<td>8.1%</td>
<td>6.2%</td>
<td>6.4%</td>
<td>3.6%</td>
<td></td>
</tr>
<tr>
<td>1983</td>
<td>19.8%</td>
<td>36.7%</td>
<td>11.1%</td>
<td>8.1%</td>
<td>6.2%</td>
<td>6.4%</td>
<td>3.6%</td>
<td></td>
</tr>
</tbody>
</table>

Bacteremia is assumed as secondary of that infection. Secondary bacteremia is frequently related to urinary tract infection, lower respiratory tract infection, and skin infection.1,2,7

### Table 2. The Primary Bacteremia Microorganism Based on NNIS and Bryan

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>14.3%</td>
<td>14.2%</td>
<td>15.2%</td>
</tr>
<tr>
<td>E.coli</td>
<td>14.0%</td>
<td>12.8%</td>
<td>13.6%</td>
</tr>
<tr>
<td>Klebsiella sp</td>
<td>9.1%</td>
<td>9.1%</td>
<td>7.9%</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>6.5%</td>
<td>7.3%</td>
<td>7.3%</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>6.3%</td>
<td>6.9%</td>
<td>6.6%</td>
</tr>
<tr>
<td>Group D streptococci</td>
<td>6.0%</td>
<td>6.1%</td>
<td>5.5%</td>
</tr>
<tr>
<td>Enterobacter sp</td>
<td>5.7%</td>
<td>5.6%</td>
<td>5.0%</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>4.5%</td>
<td>3.4%</td>
<td>4.7%</td>
</tr>
<tr>
<td>Proteus-Providencia</td>
<td>3.9%</td>
<td>2.8%</td>
<td>4.5%</td>
</tr>
<tr>
<td>Serratia</td>
<td>3.8%</td>
<td>2.8%</td>
<td>4.1%</td>
</tr>
</tbody>
</table>

The microorganism that frequently cause nosocomial bacteremia based on NNIS is categorized as follow (Table 1,2).

At division of Clinical Microbiology, Department of Clinical Pathology, School of Medicine, Cipto Mangunkusumo Hospital in January 2001 to November 2003, there were 443 cases that caused by Acinetobacter, that is in the year of 2001 were 45 cases, in the year of 2002 were 131 cases, January up to November 2003 were 267 cases. The specimen usually show small diplo-basillus form, size 0.7x1.0 um, we could find it as couple or chain-like, and it does not form any spora. Even it has fimbriae at one of its end, this microorganism is dismotile but it shows twitching movement. This microorganism gives positive catalase, oxygenase, nitrate, urease reaction and positive indol reaction, and it does not grow on SS agar.3,10,11

### Characteristics of Acinetobacter

#### Classification

Genus Acinetobacter first discovered by Morax in France and Axenfeld in German on 1896 so that it is recognized as Morax-Axenfeld bacillus. Baumann, Dou dorof and Stainer on 1968, which is quoted by Rubininstein E et al named it as genus Acinetobacter because it has cocobasillus form, negative gram, immotile, and negative oxidase. At first, we assumed that Acinetobacter only consist of one species that is A. calcoaceticus with 2 sub species that is A. calcoaceticus var. anitratus and A. calcoaceticus var. Iwoffii. Then 2 other variant were found i.e. A. calcoaceticus var. haemolyticus and A. calcoaceticus var. alcaligenes. 6,10

#### Morphology and Identification

Genus Acinetobacter is an obligatory aerob microorganism, basillus type or coco-basillus, negative gram, with diameter of 1.5-2.5 um. Gram staining of direct specimen usually show small diplo-basillus form, size 0.7x1.0 um, we could find it as couple or chain-like, and it does not form any spora. Even it has fimbriae at one of its end, this microorganism is dismotile but it shows twitching movement. This microorganism gives positive catalase, oxygenase, nitrate, urease reaction and negative indol reaction, and it does not grow on SS agar.3,10,11
All of its strain could grow at temperature 20° C – 30° C but the optimal growing temperature are 33° C – 35° C, except A. junii that could grow on temperature 42° C and A. baumannii on temperature 42° C - 44° C. This microorganism grows well on every media that containing carbon and it uses ammonium and nitrate salt as its nitrogen source. Some of strain could use glucose, lactose, xylose, or maltose as their carbon sources. 5,9,11

The growth on blood agar after 24 hours shows colony form with diameter of 0,5-2 mm, translucent-opact, no pigmentation, convex, fine edge, no blood hemolysis except A. haemolyticus and genom-species 6. This microorganism grows well on Mac Conkey agar, pale red in color with diameter of 0,5-2 mm, convex, fine edge, and some of them do not make any lactose fermentation. 5,10,14

Identification of species acinetobacter is also based on the presence of cytochrome oxidase activity, immotile, positive citrate test unless genom species 12, and resistant to penicillin. 5,10,12

A. johnsonii and A. lwoffii have non-sacharolytic nature. A. johnsonii can be differed from other acinetobacter spesies because it could not grow on temperature 37° C but it is well developed on temperature 20°-30° C. 5,6,10,13

EPIDEMIOLOGY OF ACINETOBACTER BACTEREMIA

Nosocomial infection has been known for years, since the development of health clinic that provided facility of hospitalization. This infection case has been much reported in countries over the world. The occurrence of nosocomial infection is a global problem that covering at least 9% (3%-12%) of 1,4 million hospitalized patient in all hospitals around the world. This amount was reported by WHO from survey result of 14 countries, which includes 47 hospitals on 1996.1,14

The nosocomial infection that caused by A. calcoaceticus is fluctuating according to the season; averagely it doubles on summer compared to early winter. A. calcoaceticus is opportunistic pathogen in nature, and it is widely known as the cause of recurrent infection. Nosocomial infection that has been caused by A. calcoaceticus generally high in hospital that is 3,11 per 10,000 patients and that number remain constant every year.1,4,15

The result of NNIS surey as quoted by Horan TC found that morbidity because of nosocomial bacteremia on the hospitalized patient is over than 50%. It is difficult to prevent this because the host factor has a very important role, i.e. very young or very old age, immunity status defect, bad nutrition, obesity, anatomical defect or malignancy. The nosocomial bacteremia morbidity that caused by Acinetobacter in hospital vary between 0,3 –2,0 per 1000 hospitalized patient. From 70 % hospitalized patient in ICU room, we found Acinetobacter microorganism on first week and 10% had bacteremia on the 18th - 20th day care. The proportion of microorganism culture that has been reported from nosocomial infection survey result in Cipto Mangunkusumo Hospital year 1998, we found Pseudomonas (20,1%), E. aerogenes (11,7%), E. coli (8%), S. epidermidis (3%), S. anhemolitik (1,8%), Bacillus sp (0,6%) dan Acinetobacter (0,2%).16

MECHANISM OF ACINETOBACTER CAUSING NOSOCOMIAL BACTEREMIA

Important stage of Acinetobacter infection is started by colonization at injury site, respiratory tract, gastrointestinal tract, pharynx and skin. That colonization is caused by acinetobacter that stick on cell and the fragile wound surface. This microorganism nature makes a biofilm so that it is free from phagocytosis mechanism and it is also could inhibit some antibiotics mechanism of work. 6,17

First stage mechanism of bacteremia is the fibrin formation around the insertion of canule into blood vessel. Microorganism penetrate into blood suplly through 2 mechanism. That microorganism could switch through the outer surface of catheter, which is assumed caused by capillary power from the border between catheter passage and skin or through inner wall of lumen through catheter tip. This condition could cause colonization in the catheter tip. The microorganism then grow on fibrin surface and then get into blood flow. Skin colonization is strong predictor of infection through catheter. The other intra vascular infection could be caused by contamination of intravenous fluid, or hematogenically on secondary bacteremia. 2,18

Bacteremia that caused by A. baumannii frequently cause septic shock. Cisneros JM et al and other examiner found that 25%-30% patient had septic shock. Bacteremia A. baumannii could be fulminant that it could cause fast death in 48 hours after bacteremia. Clinical manifestation of bacteremia A. baumannii is not spesific, except DIC usually occur. 4,10

The source of nosocomial bacteremia that is caused by Acinetobacter varies based of risk factor. The intravascular catheter infection because of inadequate sterilization is the cause of biggest nosocomial bacteremia epidemics that has been reported ever. Nosocomial bacteremia because of A. baumannii is
frequently caused by respiratory tract infection that is 55%-71 % on the patient with mechanical ventilator. Clinical isolation of acinetobacter mostly fund on the respiratory tract specimen, then if there is any colonization of \textit{Acinetobacter} on sputum. It has important role of bacteremia.  

Even though, the source of \textit{Acinetobacter} bacteremia frequently unknown. In some cases, bacteremia source is suspected came from the intestine. This hypothesis is more obvious after we know it clearly that colonization of gastrointestinal tract occurs often by \textit{A. baumannii}. This colonization occurs faster than colonization of respiratory tract. Colonization in intestine occurs after the patient being hospitalized for 7 days in ICU.  

\textbf{LABORATORY EXAMINATION}  

The precise diagnosis is established by positive finding of \textit{Acinetobacter} from patient’s blood. In order to determine whether there is any bacteremia or not we need to perform blood culture. The specimen place must be selected and clean. Blood sampling from arterial or vein canule must be avoided because it has contamination risk. Low blood volume culture (3,5 ml or less) correlates significantly to the low level of microorganism detection in blood compared to standard volume (>7 ml). Volume of blood sample is the most important factor that could affect blood culture sensitivity. For adult, we suggest 10-20 ml blood volume for every blood sampling. This suggestion based on studies that reveals about microorganism concentration in 50 % infected adults is \( \leq 1 \) CFU (colony forming unit)/ml. Base on some studies, blood culture positive result in adults will increase as 3% for every ml blood sample.  

\textit{Acinetobacter} identification by Gram staining gives a negative Gram basillus microorganism, or double coccobasillus or chain-like coccosbacillus, diameter 0,9-1,6 um, and 1,5-2,5 um of length.  

Selective media that usualy used for \textit{Acinetobacter} are made of blood ingredients such as manitol and phenil alanine selective media, which contain agar (jelly), soy peptone, pancreatic digest of casein, natrium chloride, lactose, maltose or sucrose, salt bile or ox bile, violet bromresol, antibiotic ampicillin, cefsulodine, and vancomycine.  

On the blood agar, the colony of microorganism is white-gray in colour, translucent or opact, convex surface, find border, diameter 0,5-2 mm, negative hemolysis except for species acinetobacter hemolitycus and genomsesies. That microorganism grows well in Mac Conkey agar media, the colony has pale reddish colour and has negative lactose fermentation.  

On Triple Sugar Iron (TSI) agar, \textit{Acinetobacter} gives base result at the agar slope, but agar base is not changing. The biochemistry test result reveals positive catalase, and citrate, negative oxydase, indol, ornitine, nitrate and urease. OF oxydative glucose and immotile microorganism. The complete identification could been seen on table 4.  

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
Characteristic & \textit{A. calcoaceticus} & \textit{A. baumannii} & \textit{A. haemolyticus} & \textit{A. junii} & \textit{A. johnsonii} & \textit{A. lwoffii} & \textit{Anomim species} \\
\hline
Growth & & & & & & & 3 6 10 11 12 \\
\hline
44\degree C & - & + & - & - & - & - & - \\
\hline
42\degree C & - & + & + & - & - & - & - \\
\hline
37\degree C & + & + & + & + & + & + & + \\
\hline
Haemolysis & - & - & + & + & + & + & + \\
\hline
Citrate & + & + & + & + & + & + & + \\
\hline
Gelatine hydrolisis & - & - & - & - & - & - & - \\
\hline
Phenylalanine & + & + & - & - & - & - & - \\
\hline
Omitine & + & + & + & - & - & - & - \\
\hline
OF : Glucose & + & + & + & - & - & - & - \\
\hline
Xylose & - & - & + & + & + & + & + \\
\hline
Lactose & - & + & + & + & + & + & + \\
\hline
Arginine & - & + & + & + & + & + & + \\
\hline
\end{tabular}
\caption{Differentiation Species Characteristic of Genus \textit{Acinetobacter}}
\end{table}  

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textit{Acinetobacter} Spp & \textit{Herellea Agar} & \textit{Holton Agar} & \textit{Leeds Agar} \\
\hline
Pale violet colony and yellow base colour & Pink colour colony and light violet base & Pink colour colony and light violet base \\
\hline
\end{tabular}
\caption{The Difference of \textit{Acinetobacter} Colony on Herellea Agar, Holton Agar, and Leeds Agar.}
\end{table}
Acinetobacter resistency to antibiotics has been known since 1975 especially for isolation result from ICU room, so that it becomes problem in therapy. Species Acinetobacter tends to resistant against some antibiotics even A. lwoffii I is more sensitive than other species. 19,20

Imipenem is an effective antibiotics, but there is some reports about A. baumanii strain from France, United States and Singapore that have been resistant to imipenem. The excessive therapeutic use of imipenem for klebsiella pneumonia infection that resistant to cephalosporin, reveals elevation of A. baumanii resistance to imipenem. 9

Sulbactam is a beta lactamase inhibitor that use in combination with ampicilline for infection therapy by species Acinetobacter that has been resistant to imipenem. On the study that has been carried out by Cisneros JM et al, ampicilline sulbactam is the most effective antibiotic for acinetobacter in vitro. In the group of other beta lactam, there is only tircacilline/ clavulanat and ceftazidime that show any eff19,21,22,23 Resistance of Acinetobacter species to cephalosporine is caused by the ability to produce cephalosporinase. Effectiveness reduction of the third generation cephalosporine is very progressive a long with the frequent use of this antibiotic. The same condition occurs in elevation of Acinetobacter resistance to aminoglycocide. On Cisneros study, only netilmicine that still effective.(61%). 9,21,23

There are variations of fluoroquinolone activity to acinetobacter. In the Vila et all study that being quoted by Cisneros JM, about 30% of Acinetobacter baumanii strain resistant to ciprofloxacin and in Cisneros study itself, there is 97% resistant to that antibiotic. Sparfloxacine is more effective (45% strain sensitive), followed by ciprofloxacin (30%), and pefloxacin (25%). Resistance occurs because of chromosomal mutation that alternate DNA gyrase or topoisomerase II which is the main target of fluoroquinolone in negative gram microorganism. Other resistance mechanism to quinolone is about the alteration of outside cell envelope as the result of outer membrane missing and alteration of lipopolisaccharide that has a role in the process of intra cell antibiotic binding. Mutation cause reduction of intracellular drugs concentration through that mechanism. The multi-resistant locus expression which is induced on microorganism cell against various antibiotics confirm that resistance is occured including against quinolone through pump system out of active cell membrane. 9,24

Doxycycline clearly active against Acinetobacter baumanii. 54% strain has been reported sensitive to it in Cisneros study and 98% in the Vila et al study as being quoted by Cisneros. 9

Recently, most of Acinetobacter strain resistant to aminopenicillin, ureidopenicillin, first, second, and third generation of cephalosporin, cefamycine and aminoglicocide. There is also much strain that resistant to tetracycline, chloramphnicole, and fluoroquinolone. The basic of this high resistance is because of low permeability of the microorganism’s outer membrane, which then accompanied by secondary mechanism such as induction of cephalosporinase induction or pump out antibiotic system that could cause high acinetobacter resistance, which occur almost for all antibiotic. For infection case and high resistance to all kinds of antibiotics, there are no other choices except polymyxine B or sulbactam or combination of trimetroprim sulfonamide 9,20,22,23

CONCLUSION

Nosocomial bacteremia is the main cause of morbidity and mortality elevation in hospital. Besides, it is also the cause of longer period of hospitalization. Acinetobacter is a kind of microorganism, isolated from soil, water and plants. It is also found on human skin and medical equipment used in hospitals. There is correlation between Acinetobacter microorganism as the cause of nosocomial infection in hospitalized patient with bacteremia, urinary tract infection, and secondary meningitis.

Acinetobacter is a kind of negative gram microorganism with basil or coccobasillus form, obligatory aerobe, has spore, fimbrae at one of it ends, grows on temperature 20⁰ -30⁰ C, but optimally on temperature 33⁰ -35⁰ C. After 24 hours growth on blood agar reveals colony with translucent-opact form, no pigmentation, not blood hemolysis except for A. hemolyticus. On Mac Conkey agar it has pale red color, convex, fine border, some of them fermentating lactose. Identification of Acinetobacter species is based on negative activity of cytochrome oxydase, immotile, positive catalase and citrate, negative nitrate, urease, and indol, OF oxidative, no growth on agar SS.

Nosocomial bacteremia is defined as positive blood culture and clinically significant after the patient being hospitalized for 48 hours. Acinetobacter is one of microorganism that causes nosocomial bacteremia.
Despite of respiratory tract, surgery wound as the most frequent bacteremia source, then intravenous catheter usage, burn injury and urinary tract infection respectively. 70% hospitalized patient on first week care is reported experienced nosocomial bacteremia that occurs because of acinetobacter.

The mechanism of Acinetobacter causing nosocomial bacteremia is started by the presence of colonization at wound site, respiratory tract, gastrointestinal tract, pharynx, and skin surface.

In order to determine whether there is bacteremia, we performed blood culture. For adults, the blood volume that needed for blood culture examination is suggested 10-20 ml. The precise diagnosis is established based on positive findings of Acinetobacter that came from the patient blood on culture media.

Acinetobacter species tends to develop resistance against some antibiotics such as amino penicillin, ureidopenicillin, second and third generation of cephalosporin, cefamycine, aminoglycoside, tetracycline, chloramphenicol, and fluoroquinolone so that there is some difficulty in nosocomial bacteremia management. Recently the management is using polymixine B, sulbactam or combination of antibiotic of trimetroprim sulfonamide.

REFERENCES