การพิสูจน์เอกลักษณ์ของเชื้อ *Acinetobacter* species ที่แยกได้จากสิ่งส่งตรวจของผู้ป่วยด้วยเทคนิค ดีเอ็นเอ - ดีเอ็นเอ ไฮบริไดเซชั่น โดยติดฉลากด้วยสารโฟโตไบโอติน

นางสาว ชุติมา เสรีกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาจุลชีววิทยาทางการแพทย์ สหสาขาวิชาจุลชีววิทยาทางการแพทย์
บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2544

ISBN 974-03-1658-1

IDENTIFICATION OF *ACINETOBACTER* SPECIES ISOLATED FROM CLINICAL SPECIMEN BY PHOTOBIOTIN LABELLING DNA - DNA HYBRIDIZATION

Miss Chutima Sereekul

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Medical Microbiology
Inter-Departmental of Medical Microbiology
Graduate School
Chulalongkorn University
Academic Year 2001

ISBN 974-03-1658-1

Thesis Title	Identification of *Acinetobacter* species Isolated from Clinical Specimen by Photobiotin Labelling DNA - DNA Hybridization
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Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master 's Degree

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ชุติมา เสรีกุล: การพิสูจน์เอกลักษณ์ของเชื้อ *Acinetobacter* species ที่แยกได้จากสิ่งส่งตรวจของผู้ป่วยด้วยเทคนิคดีเอ็นเอ-ดีเอ็นเอไฮบริไดเซชั่นโดยติดฉลากด้วยสารโฟโตไบโอติน. (IDENTIFICATION OF *ACINETOBACTER* SPECIES ISOLATED FROM CLINICAL SPECIMEN BY PHOTOBIOTIN LABELLING DNA-DNA HYBRIDIZATION) อ. ที่ปรึกษา : รศ. ดร. พิณทิพย์ พงษ์เพ็ชร, อ.ที่ปรึกษาร่วม : รศ. ดร. สมบูรณ์ ธนาศุภวัฒน์ จำนวน 126 หน้า. ISBN 974-03-1658-1.

การศึกษาเพื่อพิสูจน์เอกลักษณ์ของแบคทีเรียแกรมลบรูปแท่งทั้งหมด 350 isolates ซึ่งได้จากโรงพยาบาลจุฬาลงกรณ์ 75 isolates และได้จากโรงพยาบาลศิริราช 275 isolates สามารถจัดกลุ่มได้เป็น *Acinetobacter* species 150 isolates โดยอาศัยลักษณะทางฟีโนไทป์จำเพาะเบื้องต้น จากการทดสอบลักษณะทางฟีโนไทป์ ผลจาก Vitek 32 system และผลจากการทดสอบความคล้ายคลึงทางดีเอ็นเอ รวมถึงการศึกษาองค์ประกอบ ubiquinone ของเชื้อ สามารถแยกเชื้อ *Acinetobacter* ได้ออกเป็น 7 กลุ่ม โดยใช้ความคล้ายคลึงทางดีเอ็นเอเป็นหลัก กลุ่มที่ 1 จำนวน 105 isolates มีระดับความคล้ายคลึงทางดีเอ็นเอมากกว่า 70.11% เมื่อเทียบกับ *A. baumannii* JCM 6841^T ได้จัดเชื้อเหล่านี้เป็น *A. baumannii* เชื้อในกลุ่มนี้สามารถใช้น้ำตาล glucose และเจริญที่อุณหภูมิ 44 ^{0}C เชื้อทดสอบมี ubiquinone ชนิด Q-9 เป็นองค์ประกอบหลัก กลุ่มที่ 2 จำนวน 5 isolates มีระดับความคล้ายคลึงทางดีเอ็นเอมากกว่า 71.22% เมื่อเทียบกับ *A. calcoaceticus* DMST 2270^T ได้จัดเชื้อเหล่านี้เป็น *A. calcoaceticus* เชื้อกลุ่มนี้ไม่สามารถเจริญที่อุณหภูมิ 41 ^{0}C และเชื้อทดสอบมี ubiquinone ชนิด Q-8 เป็นองค์ประกอบหลัก กลุ่มที่ 3 จำนวน 2 isolates มีระดับความคล้ายคลึงทางดีเอ็นเอมากกว่า 70.33% เมื่อเทียบกับ *A. lwoffii* JCM 6840^T ได้จัดเชื้อเหล่านี้เป็น *A. lwoffii* เชื้อในกลุ่มนี้ ไม่สามารถใช้น้ำตาล glucose ไม่ย่อยสลาย L-arginine และ citrate และไม่สามารถเจริญที่อุณหภูมิ 41 ^{0}C เชื้อทดสอบมี ubiquinone ชนิด Q-9 เป็นองค์ประกอบหลัก กลุ่มที่ 4 มีจำนวน 10 isolates มีระดับความคล้ายคลึงทางดีเอ็นเอมากกว่า 72.08% เมื่อเทียบกับ *A. junii* DMST 2274^T ได้จัดเชื้อเหล่านี้เป็น *A. junii* เชื้อในกลุ่มนี้ไม่สามารถใช้น้ำตาล glucose แต่สามารถย่อยสลาย L-arginine และ citrate และบางสายพันธุ์สามารถเจริญที่อุณหภูมิ 41 ^{0}C และเชื้อทดสอบมี ubiquinone ชนิด Q-9 เป็นองค์ประกอบหลัก เชื้อกลุ่มที่ 5 จำนวน 4 isolates มีระดับความคล้ายคลึงทางดีเอ็นเอมากกว่า 82.45% เมื่อเทียบกับ *A. haemolyticus* DMST 2273^T ได้จัดเชื้อเหล่านี้เป็น *A. haemolyticus* กลุ่มที่ 6 จำนวน 3 isolates มีระดับความคล้ายคลึงทางดีเอ็นเอมากกว่า 81.22% เมื่อเทียบกับ *Acinetobacter* genospecies 3 DMST 2272^T ได้จัดเชื้อเหล่านี้เป็น *Acinetobacter* genospecies 3 เชื้อทดสอบมีubiquinone ชนิด Q-9 เป็นองค์ประกอบหลัก เชื้อกลุ่มที่ 7 จำนวน 21 isolates มีระดับความคล้ายคลึงทางดีเอ็นเอ ระหว่าง 10.00 - 69.09% เมื่อเทียบกับ *Acinetobacter* species สายพันธุ์มาตรฐาน (Type strains) ที่ใช้ทดสอบทั้ง 9 สายพันธุ์คือ *A. buamannii* JCM 6841^T, *A. calcoaceticus* DMST 2270^T,*A.haemolyticus* DMST 2273^T, *A. lwoffii* JCM 6840^T, *A. junii* DMST 2274^T, *A. johnsonii* DMST 2276^T, *A. radioresistance* JCM 9326^T, *Acinetobacter* gerospecies 3 DMST 2272^T, *Acinetobacter* gerospecies 6 DMST 2275^T จึงไม่สามารถพิสูจน์เอกลักษณ์ได้ว่าเป็น *Acinetobacter* ในสปีชีส์ใด เชื้อทดสอบมี ubiquinoneชนิด Q-8 หรือ Q-9 เป็นองค์ประกอบหลัก จากการพิสูจน์เอกลักษณ์ของเชื้อทั้งหมด 52 isolatesโดยใช้เครื่องมือ Vitek 32 system พบว่าเชื้อจำนวน 23 isolates พิสูจน์เอกลักษณ์ได้เป็น *A. baumannii* จำนวนเชื้อ18 isolates เป็น *A. calcoaceticus- A. baumannii* complex และอีก 11 isolates เป็น *A. lwoffii* / *A. junii* การพิสูจน์เอกลักษณ์ของเชื้อ *Acinetobacter* species ครั้งนี้พบว่าความคล้ายคลึงทางดีเอ็นเอเป็นวิธีการที่เหมาะสมมากกว่าการใช้วิธีทางฟีโนไทป์ และการใช้ Vitek 32 system

ภาควิชาสหสาขาวิชาจุลชีววิทยาทางการแพทย์ ลายมือชื่อนิสิต.....

สาขาวิชาจุลชีววิทยาทางการแพทย์ ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา 2544 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4175211730 : MAJOR MEDICAL MICROBIOLOGY

KEY WORD: *ACINETOBACTER* SPECIES / DNA HOMOLOGIES / UBIQUINONE

CHUTIMA SEREEKUL: THESIS TITLE. IDENTIFICATION OF *ACINETOBACTER* SPECIES ISOLATED FROM CLINICAL SPECIMEN BY PHOTOBIOTIN LABELLING DNA-DNA HYBRIDIZATION THESIS ADVISOR : ASSOC. PROF. PINTIP PONGPECH, Ph.D, THESIS COADVISOR : ASSOC. PROF. SOMBOON TANASUPAWAT, Ph.D, 126 pp. ISBN 974-03-1658-1.

Identification of a total 350 rod-shaped gram-negative bacterial isolates, obtained from King Chulalongkorn Memorial Hospital (75 isolates) and Siriraj Hospital (275 isolates) was performed. One hundred-and fifty isolates were grouped as *Acinetobacter* by the presumptive phenotypic characteristics. Their phenotypic characteristics, Vitek 32 system, DNA relatedness and ubiquinone components of the isolates were carried out. On the basis of the DNA relatedness, the organisms were separated into 7 groups. One-hundred and five isolates (Group I) showed DNA homologies more than 70.11% with *A. baumannii* JMC 6841^{T}. They were identified as *A. baumannii*. These isolates oxidized glucose, grew at 44 ^{0}C and produced acid from glucose. The strains tested contained the major ubiquinone with 9 isoprene units (Q-9). Five isolates (Group II) showed DNA homologies more than 71.22% with *A. calcoaceticus* DMST 2270^{T}. They were identified as *A. calcoaceticus*. They could not grow at 41 ^{0}C. The strain tested had Q-8 as a major ubiquinone component. Two isolates (Group II) showed DNA homologies more than 70.33% compared to *A. lwoffii* JCM 6840^{T}. They were identified *as A .lwoffii*. They could not oxidize glucose, not utilize L-arginine, and citrate and no growth at 41 ^{0}C. The strain tested contained Q-9 as a major ubiquinone component. Ten isolates (Group IV) showed DNA homologies more than 72.08% with *A. junii* DMST 2274^{T}. They were identified as *A. junii*. These isolates could not oxidize glucose but could utilize L-arginine and citrate and some tested strains grew at 41 ^{0}C. The strain tested had Q-9 as a major ubiquinone component. Four isolates (Group V) showed DNA homologies more than 82.45% with *A. haemolyticus* DMST 2273^{T} and they were identified as *A. haemolyticus*. The isolated (Group VI) showed DNA homologies more than 81.22% with *Acinetobacter* genospecies 3 DMST 2272^{T}. They were identified as *Acinetobacter* genospecies 3. They colud oxidize glucose and grow at 41 ^{0}C. The strains tested contained Q-9 as a major ubiquinone component. Twenty-one isolates (Group VII) showed DNA homologies of less than 10.0-69.67% with all the 9 type strains of *Acinetobacter* species including *A. baumannii* JMC 6841^{T}, *A. calcoaceticus* DMST 2270^{T}, *A. lwoffii* JCM 6840^{T} *A. junii* DMST 2274^{T}, *Acinetobacter* genospecies 3 DMST 2272^{T}, *A. haemolyticus* DMST 2273^{T}, *A. johnsonii* DMST 2276^{T}, *Acinetobacter* genospecies 6 DMST 2275^{T}, and *A. radioresistens* JCM 9326^{T}. The strains tested contained Q-8 or Q-9 as a major ubiquinone component. The identification of 52 isolates by Vitek 32 system showed that 23 isolates were *A. baumannii*, 18 isolates were *A. calcoaceticus-A. baumannii* complex (A*bc* complex) and 11 isolates were identified as *A. lwoffii* / *A. junnii*. The identification of *Acinetobacter* species based on DNA relatedness method revealed to be more appropriate than the phenotypic method and Vitek 32 system.

Department Medical Microbiology Student's signature.....

Field of study Medical Microbilogy Advisor's signature.....

Academic year 2001 Co-advisor's signature.....

ACKNOWLEDGEMENTS

I wish to express my deep gratitude to Associate Professor Dr. Pintip Pongpech, Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, my advisor, and Associate Professor Dr. Somboon Tanasupawat, Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, my co-advisor, for their valuable advices, indispensable help, encouraging guidance, initiating ideas and construction criticisms through out my study.

My sincere gratitude are also given to the members of my advisor committee, Associate Professor Pongpun Nunthapisud, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, and especially to Associate Professor Dr. Chertsak Dhiraputra, Department of Microbiology, Faculty of Medicine, Siriraj Hospital, Mahidol University, for their kindness, valuable comments helpful suggestion for the completeness of this thesis.

I also wish to thanks all the staff members of Siriraj Hospital and Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for their co-operation, encouragement and kindness in every way.

Finally, I wish to express all my deepest gratitude to my beloved parents for their eternal love, encouragement, kindness and adoration will always be remembered.

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ABBREVIATIONS

^{0}C	degree celsius
DNA	deoxyribonucleic acid
et al	et alii
g	gram
mg	milligram
min	minute
ml	millilitre
No., no	number
rpm	round per minute
μg	microgram
μl	microlitre
%	percent
ATCC	American Type Culture Collection
DMST	Department of Medical Scienes, National Insitiue of Health
JCM	Japan Collection of Microorganisms

CHAPTER I

INTRODUCTION

The control of hospital-acquired infection caused by multiply resistant gram-negative bacilli has proved to be a particular problem over the last 20 years in developed countries. An increasing incidence during the 1970s of the resistant members of the family Enterobacteriaceae involved in nosocomial infections was followed by the therapeutic introduction of newer broad-spectrum antibiotics in hospitals and subsequent increase in the improtance of strictly areobic gram-negative bacilli, including *Pseudomonas aeruginosa*, *Stenotrophomonas (Xanthomonas) malthophilia*, and *Acinetobacter* species. (1)

Of these pathogens, it is now recognized that *Acinetobacter* species play a significant role in the colonization and infection of the patients admitted to hospitals. They have been recognized as opportunistic nosocomial pathogens responsible for severe nosocomial infections including bacteremia, urinary tract infection, and secondary meningitis, but their predominant role is as agents of nosocomial pneumonia, particularly ventilator-associated pneumonia in patients confined to hospital intensive care units (ICUs) (1, 2). The majority of infection are of epidemic origin, and treatment has become difficult because many strains are resistant to a wide range of antibiotics, including broad-spectrum β-lactams, aminoglycosides, and fluoroquinolones (3, 4, 5, 6) . Most hospital outbreaks are attributed to *Acinetobacter baumannii* (1, 7) however, other *Acinetobacter* species such as genomic species 3, *A. johnsonii* (genomic species 7), *A. lwoffii* (genomic species 8), and *A. radioresistens* (genomic species 12) may also be found among clinical *Acinetobacter* isolates. These

species are usually considered to represent contamination or colonization rather than infection when they are isolated from clinical specimens (4), particularly since these species are also present on the skin and mucous membranes of human as part of their normal bacteria flora (4). The increasing pathogenic importance of *Acinetobacter* species has stimulated the development of reliable identification methods for these strains. Unfortunately, there is still lack of simple methods for the rapid indentification of *Acinetobacter* strains in clinical laboratories (1, 2)

The widely adopted Biochemical Scheme by Bouvet and Grimont (8) showed promising results for identification of *Acinetobacter* species, but further studies have shown that this schemes does not cover the phenotypic variability of all genomic species (9, 10, 11). Commercial phenotypic identification system also been shown to have a limited capacity for differentiation of all genomic species (12, 13). However, the efficiency of these phenotypic identification methods would be considerably higher if strains belonging to *A. baumannii* and genomic species 1, 3 and 13 TU are identified as members of the *A. calcoaceticus-A. baumannii* complex (A*cb* complex) rather than as genomic species (11, 13). Apart from phenotypic methods, a variety of genotypic methods have been explored for species identification (14, 15, 16, 17), but most are too expensive, require too much experience or standardization, or have simply proven to be unsatisfactorily discriminative. Thus, is still need the simple, reliable and inexpensive identification methods for *Acinetobacter* strains. DNA-DNA hybridization is one of these methods proved to have more reliability and resolution than other methods. Its efficacy for identification of several other bacterial species has been proven (17, 18, 19).

Eventhough, the genus *Acinetobacter* has recently been shown to comprise at least 19 genomic species included; *A. baumannii*, *A. calcoaceticus*, *Acinetobacter* genospecies 3, *A. haemolyticus*, *A. junii*, *Acinetobacter* genospecies 6, *A. johnsonii*, *A. lwoffii*, *Acinetobacter* genospecies 9, *Acinetobacter* genospecies 10, 11, *A. radioreistens*, *Acinetobacter* genospecies 13TU, 14TU, 14BJ, 15BJ, 15TU, 16, and 17 defined by grouping in DNA-DNA hybridization technique in the previous study (20), there are only seven named species. Many strains described so far have remained unclassified (8, 18, 21). At presence, there is still a limitation in the identification of *Acinetobacter* species isolated from clinical isolate in our country. Traditional, method used in the clinical laboratory for the identification of *Acinetobacter* species is based on the limited phenotypic characteristics, because many of the other characterization such as the assimilation of the carbon source is difflicult to perform and still could not identify most of these organisms to the species level. In the presence study, DNA-DNA hybridization technique would be used along with the conventional biochemical tests, Vitek 32 system and ubiquinone analysis in order to identify *Acinetobacter* isolates from clinical specimens to determine the possibility to adopt such techniques for the clinical laboratory.

The main objectives of this presence study are as followed:

1. Identification of *Acinetobacter* species isolates from clinical specimen by photobiotin labelling DNA-DNA hybridization including the phenotypic characteristics and ubiquinone system.
2. Comparison of the identification based on the DNA relatedness results with Vitek 32 system.

CHAPTER II

LITERATURE REVIEWS

1. Taxonomy of *Acinetobacter*

Bacteria now classified as a member of the genus *Acinetobacter* have suffered a long history of taxonomy change. The original concept of the genus *Acinetobacter* included a herterogeneous collection of nonmotile, gram-negative, catalase-positive and oxidase-negative saprophytes that could be distinguished from other bacteria by their lack of pigmentation (22, 23). Extensive nutritional studies (24) showed clearly that the oxidase-negative strains differed from the oxidase-positive strains. In 1971, the subcommittee on the taxonomy of *Moraxella* and allied bacteria recommended that the genus *Acinetobacter* comprised only oxidase-negative strains (25). This division has been supported by the use of the tranformation tests, which have new been used for over two decades as the basis for inclusion of individual isolates within the genus (26).

Gram-negative, nonfermentative bacteria currently recognized as genus *Acinetobacter* have been classified previously under at least 15 different "gerneric"names, the best known of which are "*Bacterium anitratum*" (26); "*Herellea vaginicola*" and "*Mima polymorpha*" (28), *Achromobacter*, *Alcaligenes*, *Micrococcus calcoaceticus* and B5w (29) and "*Moraxella glucidolytica*" and "*Moraxella lwoffii*" (1). It is only recently that rational taxonomic proposals for these organisms have emerged and the delineation of species within the genus is still the subject of research.

The genus *Acinetobacter* is now definded as gram-negative coccobacilli, with a

DNA G+C content of 39 to 47 mol%, that are strictly aerobic, nonmotile, catalase positive and oxidase negative. Good growth occur on complex media between 20^0C and 30^0C without growth factor requirments, while nitrate is rarely reduced. Most *Acinetobacter* strains can grow in a simple mineral medium containing ammonium or nitrate salts and a single carbon and energy source such as acetate, lactate or pyruvate.

Bergey's Manual of Systematic Bacteriology classified the genus *Acinetobacter* in the family *Neisseriaceae* (30), with one species , *Acinetobacter calcoaceticus*. This species was once subdivided in the literature into two subspecies, subsp. *anitratus*(formerly *Herellea vaginicola*) and subsp. *lwoffii* (formerly *Mima polymorpha*) (29), but this arrangement has never been formally approved by taxonomists. More recent taxonomic of the genus classified these organisms in the new family *Moraxellaceae,* which includes *Moraxella*, *Acinetobacter*, *Psychrobacter* and related organism (31). Recently, the taxonomy of the genus *Acinetobacter* has changed considerably. In 1986, Bouvet and Grimont distinguished 12 different groups (genospecies) within the genus by the use of DNA-DNA hybridization (8). According to this identification method , three DNA groups, numbered 2, 5, and 7 were given new species names as *A. baumannii*, *A. junii*, and *A. johnsonii*, while he previously used names, *A. calcoaceticus*, *A. haemolyticus*, and *A. lwoffii* were allocated *in sensu stricto* to group 1, 4, and 8, respectively. Another species *A. radioresistens*, was described in 1988 (32) and appeared later to correspond to DNA group 12 (18). The seven species names published since 1986 (8, 18) are included in the Index of Bacterial and Yeast Nomenclatural Changes (33), which comprises nomenclatural changes since 1980. In 1989, Tjernberg and Ursing presented five additional DNA groups, code 13

through 15 concurrently, Bouvet and Jeanjean (21) described five DNA groups of proteolytic *Acinetobacter* isolates, numbered 13 through 18. The group 13 described by Bouvet and Jeanjean appears to correspond to the group 14 described by Tjernberg and Ursing, while for the other groups no correlation was apparent. Thus, different DNA groups have the some numbers, which may add to the confusion regarding the present subdivision of the genus. For identification of *Acinetobacter* isolates belonging to DNA groups 1 through 12, a scheme of phenotypic tests has also been described (8). However, a recent study has shown that phenotypic identification could be problematic (34), and this was especially the cases for DNA groups 1 (*A. calcoaceticus*), DNA groups 2 (*A. baumannii*), group 3, and group 13 (18), which were genetically and phenotypically closely related and were referred to by some research groups as the *A. calcoaceticus* - *A. baumannii* complex (A*cb* complex). There were still a need for a rapid and reliable method of assigning new isolates to individual genospecies (8, 11, 18).

Thus, today, at least 19 different groups (seven of which have species names) can be distinguished within the genus by DNA-DNA hybridization.

1.1. General characteristics of *Acinetobacter* species

As already described, *Acinetobacter* species are aerobic gram-negative, nonmotile, oxidase-negative, coccobacilli, typically 0.9-1.6 μm by 1.5 to 2.5 μm. Pairing or clustering of cells often occurs. Gram stain variability, as well as variations in cell size and arrangement can often be observed within a single pure culture (35). All strains grow between 20 ^{0}C and 30 ^{0}C, with most strains having temperature optima of 30 ^{0}C-35 ^{0}C. They grow well on all common complex media. After 24 hours of

Table 1 Differentiation of the species of the genus *Acinetobacter*.

Characteristics	*A. baumannii*	*A. calcoaceticus*	*A. haemolyticus*	*A. johnsonii*	*A. junii*	*A. lwoffii*	genomic species				
							"3"	"6"	"10"	"11"	"12"
Growth at :											
44 °C	+	-	-	-	-	-	-	-	-	-	-
41 °C	+	-	-	-	90	-	+	-	-	-	-
37 °C	+	+	+	+	+	+	+	+	+	+	+
Gelatin hydrolysis	-	-	96	-	-	-	-	+	-	-	-
Hemolysis	-	-	+	-	-	-	-	+	-	-	-
Glutamyltransterase	99	+	4	-	-	-	+	66	-	-	-
Citrates (Simmons)	+[c]	+	91	+	82	-	+	+[c]	+	+	-
Acid from glucose	95	+	52	-	-	6	+	66	+	-	33
β-Xylosidase	95	-	52	-	-	6	+	66	-	-	-
Utilization of :											
DL-Lactate	+	+	-	+	+	+	+	-	+	+	+
Glutarate	+	+	-	-	-	-	+	-	+	+	+
L-Phenylalanine	87	+	-	-	-	-	+	-	-	-	+
Phenylacetate	88	+	-	-	-	94	66	-	25	50	+
Malonate	98	+	-	13	-	-	87	-	-	-	+
L-Histidine	98	+	96	-	+	-	94	+	+	+	-

Table 1 (Cont.) Differentiation of the species of the genus *Acinetobacter*

Characteristics	*A. baumannii*	*A. calcoaceticus*	*A. haemolyticus*	*A. johnsonii*	*A. junii*	*A. lwoffii*	genomic species				
							"3"	"6"	"10"	"11"	"12"
Azelate	90	+	-	-	-	+	+	-	50	25	+
D-Malate	98	-	96	22	+	76	+	66	+	+	-
L-Aspartate	+	+	64	61	40	-	+	66	+	75	-
L-Leucine	97	38	96	-	11	-	94	+	-	-	+
Histamine	-	-	-	-	-	-	-	-	75	+	-
L-Tyrosine	+	+	5	70	60	3	+	66	+	75	+
β-Alanine	95	+	-	-	-	-	94	-	+	+	-
Ethanol	+[c]	+	96	+	+	97	+	+	+	+	+
2,3-Butanediol	+	+	-	35	-	-	+	-	+	+	+
trans-Aconitate	99	+	52	-	-	-	+	-	-	-	-
L-Arginine	98	+	96	35	95	-	+	+	-	-	+
L-Ornithine	93	+	-	4	-	2	+	-	-	-	
DL-4- Aminobutyrate	+	+	+	35	88	40	+	-	+	+	+

[a] Data from Bouvet and Grimont, Int. J. Syst. Bacteriol. 36: 228-240, 1986.

[b] Symbols: +, all strains positive; -, all strains negative. The numbers are percentages of positive strains.

[c] All strains except one or two auxotrophic strains.

growth on blood agar, the colonies are between 0.5 and 2 mm in diameter, convex, entire, gray to white and variably hemolytic, on MacConkey agar colonies are smooth, opague colorless and slightly pinkish (1, 36). Presumptive identification of *Acinetobacter* species can be made on the basis of the lack of cytochrome oxidase activity, lack of motility and resistance to penicillin, which made them different from other nonfermentative bacteria (35). Most strains are unable to reduce nitrate to nitrite. Some clinical isolates, particularly those belonging to genomic species 4 (*A. haemolyticus*), may show hemolysis on sheep blood agar plates. Although rare *Acinetobacter* strains showing growth factor requirments have been isolated (35), most strains can grow in a simple mineral medium containing a single carbon and energy source. Few strains can use glucose. But the pentoses such as D-ribose, D-xylose and L-arabinose can also be utilized as carbon sources by some strains (1, 35). *Acinetobacter* can not utilize lysine and ornithine in Moeller's decarboxylase medium (36). Differentiation of the species of the genus *Acinetobacter* were show in Table 2.

. **1. 2. Characterization methods of *Acinetobacter***

There are variety of methods for identification of these organisms, for example, phenotypic methods that detect characteristics expressed by the microorganism, chemical methods and genotypic methods. The following section described the different characterization methods that are currently being applied in the identification of *Acinetobacter* species.

1. 2. 1 Phenotypic characterization

Identification methods that assess phenotypic differences are inherently limited by the capacity of microorganisms to alter the expression of the underlying genes (37).

Several factors can influence the accuracy of identification, usually, a bacterial species consists of different strains and / or biotype displaying variation in phenotypic which can also be affected by absence or presence of plasmid encoding for certain metabolic function. Variation in the inoculum size and duration of incubation may affect interpretation of the result. Sometime strictly control of temperature, incubation time, suspension liquid and inoculum size are necessary in these identification methods (38).

The phenotypic characteristics of bacteria comprised the morphological, cultural, physiological, and biochemical features. These methods are laborious, requiring a vareity of media, reagents, and tested conditions, and are time consuming and susceptible to interpretation error (39, 40). However, it must be emphasized that traditional tests continue to be valuable as these phenotypic characters are important for identification of bacteria. A variety of commercial test systems and automated systems based on phenotypic characteristics such as API 20NE (bioMerieux, Marey / Etoile, France), Crystal Enteric / NonFermenter ID Kit (Crystal E/NE) (Becton Dickinson, Cockeysville, Md.), autoSCAN-W/A (Dade Berhring MicroScan Inc., West Sacramento, Calif.) and the Vitek AutoMicrobic System (AMS) (bioMerieux Vitek Systems Inc., Hazelwood, Mo.) have been developed for the identification of *Acinetobacter* species (41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52), but all these methods sometime has problems with sensitivity and reproducibility.

Kitch et al. (44) evaluated the ability of the RapID NF Plus system (Innovative Diagnostic System, Inc., Atlanta, Ga.) in the identification of clinically isolated gram-negative nonfermentative rods (NFB). The result showed that 311 of 345 strains (90.1%) were correctly identified, with 21 strains (6.1%) requiring additional tests for accurate

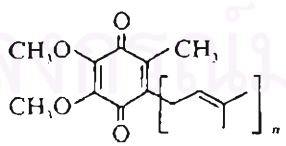
identification and 13 strains (3.8%) misidentified. The system does not differentiate between all *Acinetobacter* species, because the clinical relevance of this expanded classification remains to be established, no definitive data are available on how far commercial kits should go in the genomic species identification of these organisms. In 1995, Robinson et al. (42) performed the comparison study using Crystal/Nonferment system (CE/NF), API system and Vitek automicrobic system for identification of gram negative. The result showed that API 20NE and CE/NF correctly identified 90.3 and 91.6%, respectively, of the Enterobacteriaceae, while Vitek identified 92.4% but all these systems could not identified all species of nonfermentative gram-negative bacteria (NFB). There was no significant differences between the three systems in their ability to identify gram negative nonfermentative bacilli isolates. Wauters et al. (45), reported that only two *Acinetobacter* species are recognized by Crytal E/NF as *A. buamannii* and *A. lwoffii*, while *A. junii, A. johnsonii* and *A. haemolyticus* and non-glucose oxidizer other than *A. lwoffii* were misidentified. In 1996, Bernards et al. (13), evaluated the ability of the API 20 NE system in identification of *Acinetobacter* genomic species. This system did not discriminate between the genomic species 1, 2, 3, and 13TU. Therefore, the discriminative power of the test in the API system are insufficient for correct identification of all *Acinetobacter* genomic species.

The Vitek AutoMicrobic System(AMS) (bioMerieux Vitek Systems Inc., Hazelwood, Mo.) has been used to correctly identified 216 isolates of the total 299 isolates (71.8%) (50), but this system misidentified some species and the error rate were 7.6% (23 isolates). The most frequently misidentified species were *A. lwoffii, Cryseobacterium indologenes* and *Comamonas acidovorans*. Various investigators

included O'Hara et al., Bourbeau and Heiter., Funk et al., Lung et al., Sung et al., and Pfaller et al., pointed out the weaknesses of the Vitek GNI card and Vitek GNI + card in the identification of *A. lwoffii* and other non-glucose oxidizer. For the GNI system, O'Hara et al., reported that one of the *A. lwoffii* isolate was misidentified as "*Acinetobacter anitratus*" and Pfaller et al. (53) reported that three out of five isolates of *A. lwoffii* were interpreted as unidentified. As for the GNI + system, O'Hara et al. (47) reported one isolate out of and Bourbeau and Heiter (48), reported one out of three isolates were unable to be identified but were interpreted as no growth or unidentification by using the GNI + card. In 2000, Sung et al. (50) showed that two out of the 10 isolates of *A. lwoffii* failed to grow in the GNI + card, with an unidentified result, and two were misidentified due to false-positive citrate and /or urea reaction. The remaining 6 isolates were correctly identified after the additionnal testing.

1. 2. 2 Chemotaxonomic characterization

The chemical methods based on the use of chemical analysis of microorganisms components included the analysis of ubiquinone systems (Figure 1) and fatty acid profiles. These method have been recommended for the characterization of bacterial genera and species.



Ubiquinone

Figure 1 Ubiquinone (n, isoprene unit).

In 1988, Moss et al. (54) analyzed the cellular fatty acids and ubiquinone contents in nonfermentative gram negative bacteria. *Acinetobacter* species contained the major

fatty acids of C18:1ω9c, C16:1 ω7c, and C16:0 followed by the small amounts of C12:0, C12:1, 3-OH-C12:0, C17:1, C:17:0,C18:2, C18:1 ω7c, and C18:0. For ubiquinone system of *Acinetobacter* species was shown in Table 2 (55). No differences in fatty acid composition was observed among these *Acinetobacter* species. This method could not identify *Acinetobacter* at the species level, but could be distinguished *Acinetobacter* species from all other organisms only at the genus level.

Table 2 Ubiquinone system of *Acinetobacter* species.

Species	Isoprenolog (s)	
	Major	Minor
A .baumannii ATCC 9955 , ATCC 17961	Q-8	Q-7, Q-6
A. calcoaceticus IAM 12087	Q-9	
A. lwoffii GIFU 1951	Q-9	
A. radioresistens IAM 13186	Q-9	
A. junii ATCC 17908^{T}	Q-8	Q-7, Q-6
A. jonnsonii ATCC 17923	Q-8	Q-7, Q-6
A. haemolyticus ATCC 19002, ATCC 19194	Q-8	Q-7, Q-6
Acinetobacter genospecies 3 ATCC 19004^{T}	Q-8	Q-7, Q-6
Acinetobacter genospecies 6 ATCC 17979^{T}	Q-8	Q-7, Q-6

*Data from Yokota. A., et al. Bulletin of the Japan Federation for Culture Collections. 8: 136-171, 1992 (55).

In 1989, Veys et al. (56) applied the used of gas-liquid chromatography in the identification of nonfermenting-gram-negative bacteria from clinical specimens. They reported that qualitative interpretation of the fatty acid composition provides insufficient

criteria to differentiate between the distinct *Acinetobacter* species. In agreement with the observations made by Moss et al. (54), the fatty acid analysis appears to be only genus specific.

Both cell envelope and whole-cell protien patterns have been used in a series to the identification of *Acinetobacter* species. Analysis of cell envelope protien patterns by sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) has shown herterogeneity in unrelated strains, while multiple isolates from patients or outbreaks were indistinguishable (34). This method has been used sucessfully to trace specific strains during endemic episodes and outbreaks in hospitals. Electrophoretic analysis of whole-cell protien fractions has the advantage that simple preparation is most simple than preparation of cell envelopes. Similarities between *Acinetobacter* isolates from outbreaks and discrimilarities in unrelated control strains have been reported in several studies (57), but as with other phenotypically based methods, apparent differences between isolates from a common origin should be interpreted with caution.

In 1991, Osterhout et al. (58) identified the clinical isolates of gram-negative nonfermentative bacteria using the automated cellular fatty acid identification system. The result showed that of the 42 strains of *Acinetobacter* evaluated, 7 strains were incorrectly identified. Because of its heterogeneity and taxonomic complexity, this identification method was on the basis of genus level rather than the species level. However, this method was an accurate, efficient, and relatively rapid method for the identification of gram-negative nonfermentative bacteria, but sufficiently or ability to differentiate chemotaxonomically closely related species as well as the complicated technique.

1. 2. 3 Genotypic characterization

Genotypic methods have now provided a much more reliable basis than phenotypic similarity for measuring relatedness in bacteria as well. The underlying principle is that the progressive divergence of organism in the evolution which is directly reflected in the DNA (59). Several genetic methods have been used for identification of *Acinetobacter* species.

Genotypic identification is emerging as an alterative or complement to established phenotypic methods. Typically, genotypic identification of bacteria involves the use of conserved sequences within phylogenetically informative genetic targets, such as analysis of plasmid profiles, ribotyping, analysis of chromosomal DNA by pulsed-field gel electrophoresis (PFGE) and fingerprinting by arbitrarily primed PCR (60). All these methods have been introduced in order to improved the technique in identification of *Acinetobacter* species.

Plasmid profile have now been used in many studies as a rapid and simple method for identifying *Acinetobacter* strains (61, 62). Seifert et al. (62) reported that the analysis of plasmid profiles has been useful in delineating several out break of *Acinetobacter* infections. Analysis by pulsed-field gel electrophoresis of restriction fragment length polymorphisms generated from intact chromosomal DNA has also been used to compare fingerprints obtained from *Acinetobacter* strains following restriction with *Apa*I (60, 63), *Sma*I , *Apa*I and *Sma*I , and *Nhe*I and *Sma*I. These studies have indicated considerable DNA polymorphism in the clinically important genomic species 2 (*A. baumannii*), even within biotypes, and good correlation between strains from within defined outbreaks or multiple isolates from single patients. Equipment for pulsed-field

gel electrophoresis is costly, while the preparation of intact chromosomal DNA and subsequent digestion and elecrophoresis required several days. Nevertheless, pulsed-field gel electrophoresis seems to provide highly discriminatory results and extremely useful epidemiological information.

In 1995, Dolzani et al. (64) demonstrated a polymorphism in the spacer sequences between the genus for the 16S and 23S rRNAs among bacteria in the genus *Acinetobacter* and has exploited it for their identification. The analysis in this studies showed that the restriction genospecies profiles obtained are highly reproducible and characteristic for each genospecies and allows identification of *Acinetobacter* genospecies in the *A. calcoaceticus-A. baumannii* complex (A*cb* complex). However, the detection of restriction polymorphism of 16S-23S rRNA intergenic spacer sequences is therefore a less discrimminatory identification method compared with DNA-DNA hybridization. Thus, the resolution of 16S rRNA sequences analysis is insufficient to distinguish closely related genomic species because of extremely slow rate of base substitution in 16S rRNA (65), this method is still not suitable for routine analysis in diagnostic laboratories.

In 1999, Berlau et al. (20) used the random amplified polymorphic DNA (RAPD) fingerprint profiles in the identification of *Acinetobacter* species. They concluded that it was possible to identify 88% of the 112 isolates to the genospecies level. Problems were encountered with the used of *Bfa*I, the only enzyme so far to distinguish between genospecies 4 and 7, and 5 and 17. The enzyme did not digest the crude PCR product, although it produced novel patterns with purified DNA.

The genetic identification method is DNA-DNA hybridization. This method could

provide more resolution than other genotypic methods, and the 70% criterion (17, 19) has been a cornerstone for describing a bacterial species. Quantitative measurement of DNA-DNA hybridization from renaturation rates has contributed to the determination of genetic relatedness among bacterial strains (66, 67, 68, 69). The methods used in this technique are either a free solution method in which S1 nuclease (65) or a method in which single-standed DNA is fixed on a solid support, such as nitrocellulose filters (70). However, to carry out these hybridization experiments, DNA must be labelled with radioactive substances by nick translation or random primed labelling (71).

Recent developments have made it possible to label DNA with nonradioactive materials without using enzymes (3, 69, 72). Biotinylation of DNA with photoreactive biotin (photobiotin) is one of these recent developments (72).

In 1989, Eizaki et al. (69) developed the fluorometric hybridization in microdilution well to determine genetic relatedness among microorganisms. The result showed that this method had been used successfully in the identification of clinical important human bacteria. Tanasupawat et al., 1992 (73), used the DNA-DNA hybridization technique in the identified of the coagulase-negative staphylococci isolated from fermented fish in Thailand. This investigation successfully discovered the new species for the name *Staphylococcus pisciferm*entans.

In 1994, Aoki and Yamada (74), applied the microplate DNA-DNA hybridization technique to identify the mycobacteria in the clinical samples and confirmed the utility of this technique for a fast and accurate diagnosis of mycobacterial infection. They result showed that with samples containing more than 10^8 colony-forming units (CFU)(5 cases), the species identification was successfully performed on the day of sample

preparation. With the samples of not more than 10^7 CFU (14 cases), although 4-21 day's primary culture were necessary, the species were also correctly identified by the microplated method. Furthermore, *M. avium* and *M. intracellulare* were distinctly identified. In conclusion, the microplate DNA-DNA hybridization was a dependable technique for rapid diagnosis of mycobacterial infection.

In 1997, Elomari et al. (75) used DNA-DNA hybridization in the identification of the *Pseudomonas* species isolated from clinical specimens. The result showed that they could identify the organism to species level. The investigators proposed a new species, as named *Peudomonas monteilii.*

The first DNA-DNA hybridization study in the identification of *Acinetobacter* species was performed by Johnson et al. (76), who showed that the genus contained of at least six relatedness groups.

In 1986, Bouvet and Grimont (8) published a DNA hybridization study of 85 *Acinetobacter* strains. They characterized 12 DNA hybridization groups, of which all but two could be differentiated by the chemical tests. Four new species, *A. baumannii*, *A. haemolyticus*, *A. johnsonii* and *A. junii* and the descriptions of *A. calcoaceticus* and *A. lwoffii* were emended. By using the DNA-DNA hybridization technique, another new species, *A. radioresistens*, was descriped by Nishimura et al. (32). The type strain of *A. radioresistens* was shown to be a member of DNA group 12 (77).

In 1989, Tjernberg and Ursing (18), the *Acinetobacter* strains conisisting of 168 consective clinical strains and 30 type and reference strains was studied using DNA-DNA hybridization and showed that most the strains could be identify as the member of the DNA groups described by Bouvet and Grimont. From this study, three new DNA

group were described. Tjernberg and Ursing numbered their DNA groups according to the classification of Bouvet and Grimont and the new group were numbered 13 through 15, however , they could not reproduce the results by Bouvet and Grimont concerning DNA groups 8 and 9 , they comitted DNA group 9 from their system. In 1989, Bouvet and Jeanjean (28) reported five DNA groups (which they named groups 13 through 17) of proteclytic *Acinetobacter* strains among these groups , number 13 corresponds to DNA group 14 from the study by Tjernberg and Ursing (10,12).

In 1992, Gerner-Smidt et al. (47) used the restriction enzymes for digested chromosomal DNA and then hybridized with a labelled cDNA probe derived from *E.coli* rRNA. Pattern generated by restriction with *Eco*RI, *Cla*I or *Sal*I have been used to investigate 70 strains that had been identified as *either A. calcoaceticus, A. baumannii,* or genomic species 2 or 13TU by DNA-DNA hybridization.

In 1997, Seifert et al. (14) used of DNA - DNA hybridization techniques for identification of *Acinetobactor* species on human skin.

The genomic species is one of the major concepts of a bacterial species and is based on the quantitative similarities between chromosomal DNAs of bacteria as determined by DNA-DNA hybridization. A genomic species can be defined as a species only when it has phenotypic traits that distinguish it from its nearest neighbour genomic species (17) The similarity of small subunit rRNA (16S rRNA) sequences is increasingly being used for the classification of bateria (65, 78). Yamamoto and Harayama, 1998 (65), performed the phylogenetic analysis of *Acinetobacter* strains based on the 16S rRNA gene sequence and on the *gyr*B-based. The results showed that the resolution of 16S rRNA sequence analysis was insufficient to distinguish closely related genomic

species. They concluded that this method could not replace the DNA-DNA hybridization method. Eventhough the *gyr*B-based analysis provides a greater degree of resolution than that based on 16S rRNA genes.

In 1999 Yamamoto et al. (79) interested in performing phylogenic analysis by protein-encoding genes in the identification of *Acinetobacter* strains. These investigators successfully the result from this technique was equivalent to that of DNA-DNA hybridization. The analysis of 49 *Acinetobacter* strians showed that they belonged to 18 different genomic species.

2. Species of Clinical Importance

Numerous studies have now supported the original observation (8) that *A. baumannii* is the species most prevalent in clinical specimens and the main genomic species associated with the outbreaks of nosocomial infection. In the study by Seifert et al. (4) of 584 *Acinetobacter* strains isolated from 420 patients at 12 different hospitals over a 12-month period, 426 (72.9 %) strains were identified as *A. baumannii*, with 208 isolates being recovered from respiratory tract specimens, 113 being recovered from blood cultures and central venous lines, 70 isolates being recovered from wound swabs, and 35 isolates being recovered from other miscellaneous specimens (1). This study also identified 158 isolates that belonged to species other than *A. baumannii*, of which the most common were *Acinetobacter* genomic species 3 (55 isolates), *A. johnsonii* (29 isolates), and *A. lwoffii* (21 isolates).

Further investigations were required to define the clinical significance of *Acinetobacter* species other than *A. baumannii*. Such isolates in clinical specimens were

often considered to be contaminants derived from the environment. Diagnosis of infection with " unusual" *Acinetobacter* genomic species therefore often depends on clinical indications and the repeated isolation of the same strain from a single patient. *Acinetobacter* genomic species 3 and 13 TU (18) have been implicated in nosocomial outbreaks of infection, while *A. johnsonii* has been associated with catheter-related bacteremia (80). A study in Sweden found that *Acinetobacter* genomic species 3 was predominant among clinical isolates (18).

In the European studies, the most common genospecies isolated from skin and mucous membranes of patients and controls was *A. lwoffii*, accounting for 44 to 61% of isolates, with genospecies 3 accounting for only 5 to 12%, DNA group 7 (up to 22%) and DNA group 5 (up to 10%) were reported in the German study but not found at all in the London study, which reported 15 BJ as the most common species after *A. lwoffii* (4, 20). The natural habitats of the most common clinical isolates as *A. baumannii* and genospecies 13 TU (4), while *A. haemolyticus* and *Acinetobacter* genospecies 6, other groups seem to be implicated only occasionally in human infections (1).

In conclusion, although *A. baumannii* appeared to be the *Acinetobacter* genomic species of greater clinical importance, repeated isolation of another genomic species (particularly one belonging to *A. calcoaceticus-A. baumannii* complex) from a patient shoud be a cause for suspicion of infection, especially if clinical symptom are also present.

3. Natural habitats

Acinetobacter species are ubiquitious in the environments (soil, water, milk,

frozen soups) and the hospital (ventilator, neulizer, respiratory therapy equipment, sink, humidifiers, catheter and contaminate medication). They are often respond poorly to chemical controls such as disinfectant or antimicrobial agent and also found as the normal flora of human skin as well as the colonizing microorganisms in mucous membrane and perinium in healthy people and are opportunistic pathogens involved in the outbreaks of the hospital infections (4, 81, 82). About 25% of adult have skin colonizatoin with *Acinetobacter* species while 7% carry the organism in their pharynx. Hospitalized patient may become easily colonized if they were not already harboring the organisms, and thus, *Acinetobacter* may be isolated non sigificantly from urine, feces, vaginal secretions, and many different types of respiratory specimens. As many as 45% of tracheostony site may be colonized (83, 84).

4. Virulence

Although *Acinetobacter* species are considered to be relatively low-grade pathogens (30), certain characteristic of these organism may enhance the virulence of strain involved in infections, these characteristics include the presence of the polysacchride capsule formed of L-rhamnose, D-glucose, D-glucuronic acid and D-mannose, which probably renders the surface of strains to be more hydrophilic, although hydrophobicity may be higher in *Acinetobacter* strains isolated from catheters or tracheal devices, the property of adhesion to human epitherial cells in the presence of fimbriae and/or capsular polysaccharide (31), the production of enzymes which may damage tissue lipids, and the potentially toxic role of the lipopolysaccharide component of the cell wall and the presence of lipid A (3). In common with other gram-negative bacteria,

Acinetobacter species produce a lipopolysaccharide responsible for lethal toxicity in mice, pyrogenicity in rabbits, and a positive reaction in the *Limulus* amoebocyte lysate test. The production of endotoxin *in vivo* is probably responsible for the disease symptoms observed during *Acinetobacter* septicemia. *A. baumannii* appears to have only limited virulence in mice (50% lethal dose, 10^6 to 10^8 CFU per mouse) when innoculated intraperitoneally, even in neutropenic mice. Experimentally , mixed infections combining other bacteria with *Acinetobacter* species are more virulent than infections with *Acinetobacter* species alone (85). Slime produced by the *Acinetobacter* strain studies was considered to be the main factor responsible for the enhancement of virulence in mixed infections, but few acinetobacters are slime producing, indeed, of 100 isolates tested, only 14 had slime producing ability (85). The same study demonstrated that slime was associated with cytotoxicity against neutrophils and the inhibition of neutrophils migration into peritoneal exudate of mice. No correlation was observed between the amount of slime produced and the degree of virulence.

The ability of bacterium to obtain the necessary iron for growth in the human body is also an important virulence determinant, and some *Acinetobacter* strains have been shown to produce siderophores, such as aerobactin, and iron-repressible outer membrane receptor protein (86, 87).

5. Nosocomial infections

Although *A. baumanniii* now recognized to be the *Acinetobacter* genomic species of greater clinical importance, it is difficult to extrapolate the older literature to say with certainty what a culture reported in 1980 as "*A. anitratus*" would now be called. Even

today, many reports of infection caused by "*A. baumannii*" do not include the necessary test for specific rather than presumtive identification. Given this qualification, *Acinetobacter* species have been isolated from various types of opportunistic infections, including septicemia, pneumonia, endocarditis, meningitis, skin and wound infection, and urinary tract infection (1). The distribution by site of *Acinetobacter* infection does not differ from that of other nosocomial gram-negative bacteria, with the main site of infection in several surveys (1, 88) being the lower respiratory tract and the urinary tract. *Acinetobacter* species have emerged as particurlarly important organism in ICUs (1, 89, 90) , and this is probably related, at least in part, to the increasingly invasive diagnostic and therapeutic procedures used in hospital ICUs over the last two decades.

The true frequency of nosocomial infection caused by *Acinetobacter* species is not easy to assess, partly because the isolation of these organisms from clinical specimens may not neccessary reflected infection but rather, may result from colonization (1). *Acinetobacter* species accounted for 1.4% of all nosocomial infections during a 10 year period (1971 to 1981) in a university hospital in the United States (1), with the principle site and types of infection including the respiratory tract, bacteriamia, peritoneum, urinary tract infection, surgical wound, menigitis, and skin or eye infection. A more recent study in university hospital found that hospitalization in an ICU and previous administration of antibiotics were associated with *Acinetobacter* colonization at various body site in 3.2 to 10.6 per 1,000 patients, with *Acinetobacter* infection accounting for 0.3% of endemic nosocomial infections in critically ill patients and for 1% of nosocomial bacteremia hospital-wide (1). These figures are in agreement with previous observations (91). One study has reported a seasonal incidence of

Acinetobacter infection (92), with an increase in infection rates in late summer and early winter a difference that could be related to temperature and humidity changes but one that has no yet been confirmed by other studies. The example of nosocomial infections caused by *Acinetobacter* species included.

5.1 Respiratory Infection

Numerous outbreak of nosocomial pulmonary infection caused by *Acinetobacter* species have been reported particularity in the role played by *Acinetobacter* species (1, 93). In ventilator associated pneumonia appeared to be increasing. Regardless of the bacteriological method used to define the cause of pneumonia precisely, several studies have reported that about 3 to 5 nosocomial pneumonias are caused by *Acinetobacter* species (91). In patients with pneumonia enrolled in the National Nosocomial Infection Study (1), this organism accounted for 4% of the total number of pulmonary infections during 1990 to 1992.

To try to better define the link between the use of ventilators and pneumonia caused by *A. baumann*ii, 159 consecutive patients who received mechanical ventilation for >72 h in a medical-surgical ICU over a 13 months period were studied (1). Fiberotic bronchoscopy with protected-specimen brush and bronchoalveolar lavage was performed on each patient suspected of having pneumonia because of the presence of a new pulmonary infiltrate, fever, and purulent tracheal secretion, but the diagnosis of pulmonary infection was retained only if protected-specimen brush and bronchoalveolar lavage specimens grew $>10^3$and 10^4 CFU of at least one microorganism per ml, respectively. By the criteria used in such study, nosocomial pneumonia associated directly with *A. baumannii* occurred in 19 of the 159 study patients (12%), while the

organism was present in 27% of ventilator-associated pneumonia cases diagnosed during this period.

Crude mortality rates of 30 to75% have been reported for nosocomial pneumonia caused by *Acinetobacter* species with the highest rates reported in ventilator-dependent patients (1). It is therefore clear that the prognosis asscociated with this type of infection is considerably worse than that associated with other gram- negative or gram-positive bacteria, with the exception of *P. aeruginosa*. In a study in the patients which the diagnosis was retained only if protected-specimen brush specimens grew $>10^3$ CFU of at least one organism per ml (3), mortality associated with *Pseudomonas* or *Acinetobacter* pneumonia was >75%, compared with only 55% for pneumonia caused by other organisms ($P<0.05$) (1).

Although these statistics indicating that nosocomial pneumonia could be caused by *Acinetobacter* species especially in the ventilator-dependent patients, it is difficult to established whether such critically ill patients would have survived if nosocomial pneumonia had not occurred.

5. 2 Bacteremia

The most common *Acinetobacter* species causing significant bacteremia is now identified as *A. baumannii* in most series of adult patients in whom proper species identification is made (1). *Acinetobacter* species may be found either as a sigle pathogen or as part of polymicrobial bacteremia. Immunocompromised patients make up the largest group of adult patients. In these patients, the source of bacteremia is often a respiratory tract infection, with the highest rate of nosocomial bacteremia occurring during the second week of hospitalization. Malignant disease, trauma, and burns seem to

be among the most common predispoising factors.

A second important group of patients may consist of neonates. One report from Japan described 19 neonates with *Acinetobacter* septicemia in the neonatal ICU over aperiod of 30 months. All cases were of late-onset type septicemia in infants hospitalized for long period, with a mortality rate of 11% (1). The predispoing risk factor septicemia were low birth weight, previous antibiotic therapy, mechanical ventilation, and the presence of neonatal convulsions. A second report (3) described an outbreak of septicemia in a neonatal ICUs.

As far as risk factors for adult are concerned, surgical wound infections caused by *Acinetobacter* species have been described, and such wound infections may lead to bacteremia. There are also a number of reports in the literature describing *Acinetobacter* bacteremia in burn patients (1). Several studies have shown that there is a correlation between vascular catheterization and *Acinetobacter* infection (94). Changing the catheter insertion site every 48 h and appropriate adherence to aseptic protocols may reduce the risk. An association between *Acinetobacter* bacteremia and the use of tranducers for pressure monitoring has been reported (1), and prompt attention to sterilization techniques when handing equipment such as tranducer may also reduce the infection rate. In general, the underlying disease seem to determine the prognosis of the patient. The prognosis of patients with malignant disease and burn is rather poor, but trauma patients have a better prognosis. Previous antibiotic treatment is associated with the selection of the resistant strain (95).

5. 3 Miningitis

Secondary menigitis is the predominant form of *Acinetobacter* meningitis,

although sporadic cases of primary meningitis have been reported, particularly following neurosurgical proceures or head trauma. Until 1967, there were about 60 reported incidents of *Acinetobacter* meningitis, most of which were community aquired. However, since 1979, the vast majority of cases were nosocomial infections, with almost all caused probably by *A. baumannii*. Mortality rate from different series range from 20 to 27%. Most patients were men adult who had undergone lumbar punctures, myelgraphy, ventriculography, or orhter neurosurgical procedures, except one patient who had posttraumatic otorrhea without intervention (90). A case of *Acinetobacter* meningitis associated with a ventriculoperitoneal shunt with concominant tunnel infection in which *A. baumannii* was isolated from cerebrospinal fluid has been described (1). Risk factor included the presence of a continuous connection between the ventricles and the external environment, a ventriculostomy, or a cerebrospinal fluid fistula. In addition, the presence of an indwelling ventricular catheter for more than 5 days is an important risk factor. Another important predisposing factor is the heavy use of antimicrobial agents in the neurosurgical ICU. One outbreak subsided spontaneously only when the selective pressure of antibiotics was reduced (90).

5. 4 Urinary Tract Infection

Nosocomial urinary tract infection is caused only infrequently by *Acinetobacter* species. It occurred most commonly in elderly debilitated patients confined to ICUs, and in patients (80%) tend to be male (1), perhaps reflecting the higher prevalence of indwelling urinary catheters in this population as a result of prostatic enlargement. It should, however, be noted that not every isolation of *Acinetobacter* from the urinary tract of patients with an indwelling urinary catheter can be correlated with actualy

infection (96).

6. Epidemiology

Acinetobacters can form part of the bacterial flora of the skin, particularly in moist regions such as the axillae, groin, and toe webs, and it has been suggested that at least 25% of normal individuals carry *Acinetobacter* species on their skin. *Acinetobacter* species have also been found occasionally in the oral cavity and respiratory tract of healthy adults (1), but the carraige rate of *Acinetobacter* species in nonhospitalized patients, apart from on the skin, is normally low.

In contrast, the carriage rate may be much higher in hospitalized patients, especially during outbreak of infection. Throat swab have been found to be positive for *Acinetobacter* spp. in 7 to 18% of patients, while tracheostomy swabs were positive in 45% of hospitalized patients. High colonization rate of the skin ,throat, respiratory system, or digestive tract, of various degrees of importance, have been documented in a several outbreaks. In particular, outbreaks involving mechanically ventilated ICU patients are associated with a high colonization rate of the respiratory therapy tract (1), which may indicate contamination of respiratory therapy equipment as the possible source of an outbreaks. In addition, patients often have skin colonization during outbreaks. Such colonization of patients plays an important role in subsequent contamination of the hands of hospital staff during trivial contracts, thereby contributing to the spread and persistence of outbreaks. Colonization of the digestive tract of patients with *Acinetobacter* species is unusual (97), but several studies have documented oropharyngeal colonization of patients with respiratory tract colonization, and digestive

tract colonization has been reported to be a major reservoir of resistant strains.

Several conclusions regarding colonization in hospitalized patients can be drawn from the published studies, a high rate of colonization can be found in debilitated hospitalized patients, especially during outbreak situations, a predominant site of colonization is the skin, but other sites, such as the respiratory or digestive tract, may also be involved and may predominate on certain occasions, and the observed discrepancies between carraige rates for outpatients and hospitalized patients suggests that infecting or colonizing organisms in hospital-acquired infections may derive more often from cross-tranmission or hospital environmental sources rather than from endogenous sources in patients.

The large proportion of colonized patients in a given hospital setting means that the differentiation between colonization and infection may not be straightforward. Nosocomial *Acinetobacter* infections may involve any site, but they predominate in the respiratory tract, urinary tract, and wounds. Many isolates from the skin and the respiratory tract should still be considered to be colonizing rather than infection organisms. A steady increase (from 25 to 45%) in the proportion of *Acinetobacter* isolates from superficial wounds has been recorded over the past decade (1), and the skin, respiratory tract, and superficial wounds should therefore be considered to be potential important reserviors of infecting organism during outbreak situations.

CHAPTER III

MATERIALS AND METHODS

1. Chemical reagents, media, and instruments.

Most of the chemical agents in this study were molecular biology grade. Name list of all media, chemical reagents, materials, instruments and reagents were shown in Appendix I.

2. Organisms.

2.1. Tested isolates.

Of 350 isolates, 75 isolates of the nonfermentative gram-negative bacteria were kindly provided from the Department of Microbiology, Faculty of Medicine, King Chulalongkorn Memorial Hospital and 285 isolates were obtained from Division Bacteriology, Department of Microbiology, Faculty of Medicine, Siriraj Hospital, Mahidol University. They were obtained during 6 months period between July to September 1999 from the clinical specimen were blood, sputum, pus, urine, and wound.

2.2.Type strains.

Total nine type strains of *Acinetobacter* species were included in this study. The details of their designation and source of isolation were shown in Table 3.

3. Identification methods

The presumptive phenotypic characteristics of all the isolates were studied in order to identify the genus *Acinetobacter* according to the recommended method by

Bergey's Manual of Determinative Bacteriology and Bailey & Scott's. Briefly as followed .

Table. 3 Type strains of *Acinetobacter* species used in this study

Species	Strain designation			Source of isolation	Reference
	ATCC	DMST	JCM		
A. baumannii	17909^T	2271^T	6841 ^{T}	Urine	98, 99
A. calcoaceticus	23055^T	2270 ^{T}	-	Soil	98, 99
A. haemolyticus	17906^T	2273 ^{T}	-	Sputum	8, 99
A. lwoffii	15309^T	2277^T	6840 ^{T}	Urine	98, 99
A. junii	17908^T	2274 ^{T}	-	Urine	8, 99
A. johnsonii	17909^T	2276 ^{T}	-	Duodenum	8, 99
A. radioresistens	43998^T	2281^T	9326 ^{T}	Cotton tampon	98, 99
Acinetobacter genospecies 3	19004^T	2272 ^{T}	-	Cerebrospinal fluid	8, 99
Acinetobacter genospecies 6	17979^T	2275 ^{T}	-	Throat	8, 99

JCM, Japan Collection of Microorganisms, Saitama, Japan.

DMST, Department of Medical Sciences, National Institute of Health, Bangkok.

ATCC, American Type Culture Collection ; Manassas, VA, U.S.A.

3.1 Morphological and cultural characteristics

The colonies on the tryptose blood agar base medium and MacConkey agar after 24 hours incubation at 37 ^{0}C were examined for their characteristics.

Gram staining; Thin smear of bacterial colony was prepared on a clean slide. Slide was fixed by passing through flame. The smear was covered with crystal violet for 30 sec, then rinsed with water and drained. Next, the smear was covered with iodine for 30 sec, then with rinsed water. Decolorized with ethanol 95% and washed with water, then was couter stained about 30 sec with safanin. Blot slide dried and examine under oil immersion (1,000 x). *Acinetobacter* species are gram-negative coccobacillary cells often appear as diplococci.

Motility test; The isolates were stabed into the center of the tube of motility medium to a depth of about 2 mm. and incubated at 37 ^{0}C. A initial reading was made within 4 to 6 hours of inoculation. Motile organisms could grow away from the inoculation site into the surrounding medium, while non-motile organisms remained localized at the inoculation site.

3.2. Physiological and biochemical characteristics

3.2.1. Oxidative and fementative test (O-F)

The 24 hours old colony on the tryptic soy agar were picked up with sterile needle and deep stabed into O-F base medium. Each of the two identical tubes was overlayed with a sterile melted parafin (approximately 1 cm. tall). The tubes were then incubated at 37 ^{0} C for 24 hours and examined for the production of acid as indicated by a change in the bromthymol blue indicator from green to yellow.

3.2.2. Growth on Triple Sugar Iron agar (TSI)

The 24 hours old isolates from tryptose blood agar base medium plate was streaked on the sloped surface and inoculated the butt of the same tube by a central stab. The tubes was incubated at 37 ^{0}C for 24 hours and examined for the acid production from butt and slant. Acid production are detected by the pH indicator phenolred which changes its colour from red-orange to yellow, on alkalinization it become deep red.

3.2.3. Oxidase test

Each colony was streaked on nutrient agar and incubated at 37 ^{0}C for 24 hours. A few drops of 1% tetramethyl-p-phenylenediamine were dropped on sterile filter paper disc. The test culture was then smeared across the moist disc with sterlie loop. The apperance of dark-purple colour on the paper within 30 seconds denoted a positive reaction .

3.2.4. Catalast test.

With a sterile loop, transfer a small amount of pure growth was transfered from agar onto the slide. Immediately place a loop of 3% hydrogen peroxide (H_2O_2) was immediately placed onto a portion of a colony on the slide. The evolution of gas bubbles indicating a positive test was observed.

3.2.5. Growth at 37 ^{0}C, 41 ^{0}C and 44 ^{0} C

Tube containing 2 ml of tryptocasein soy broth (TSB) was inoculated with a drops of bacterial cell suspension in saline prepared from an overnight culture and incubated at 37 ^{0} C for 24 hours (9). After inoculation each tube were incubated at 37 ^{0}C, 41 ^{0}C and 44 ^{0} C. The growth examination was performed after 24 hours.

3.2.6. Production of Acid from carbohydrates

Tube of peptone water was supplemented with 1% (wt/vol) D-glucose, maltose, mannitol, lactose and sucrose. All media contained bromthymol blue as indicator for acid production. Each carbohydrate was inoculated with two drops of bacterial cell suspension in saline which was an overnight culture and incubated at 37 ^{0} C for 24 hours. Acid production was detected by the change of indicator from blue to yellow.

3.2.7. Utilization of amino acid and carbon sources.

The carbon source utilization tests were performed in liquid medium as described by Gordon and Mihm (100) with the addition of an appropriate carbon source at final concentration 0.2% (wt/vol). The following 16 different carbon sources were used including L-histidine, L-arginine, β-alanine, L-leucine, L-tartrate, L-ornithine, malonate, succinate, benzoate, glutaric, DL-lactate, citrate, acetate, L-phenylalanine, D-malate and aspartate. The media were adjusted to pH 6.8 and phenolred 0.2% solution (wt/vol) was added as an indicator solution. The liquid

medium was dispensed into 2 ml tubes with 11 mm diameter . The tubes were inoculated with two drops of 24 hours of bacterial cell suspension in saline (8) and incubated at 30 ^{0}C. Growth was recorded daily for up to 6 days. The positive result were shown by colour change of the indicator from yellow to red.

3.3. DNA-DNA hybridization labelled with photobiotin.

3.3.1. Isolation and purification of DNA

Isolation of DNA from *Acinetobacter* species was done according to the method recommened by Tanasupawat (73). Briefly , the bacterial inoculated in TSB 50 ml was shaked at 200 rpm for 24 hour in order to obtain the growth during logarithmic phase. The cell were harvested by centrifugation at 7,000 rpm and washed twice with 10 ml of saline-EDTA. Bacteria cell lysis was done by adding the 10% sodium dodecyl sulfate (SDS) and Tris-buffer pH 9.0. After well mixing the suspension was heated at 60 ^{0} C for 10 min. After the cells were lysed, the cell suspension was change from turbid to opalescent and become very viscous. Following the addition of 4 ml of phenol-chloroform (1:1 v/v), the mixure was vortexed for at least 30 sec. It was then centrifuged at 12,000 rpm for 10 min. The supernatant was then transferred into a small beaker. After adding of cold 95% ethanol into supernatant to precipitate DNA, DNA was spooled with a glass-rod, and rinsed with 70% then 95% (v/v) ethanol and air dried. DNA was then dissolved in 5 ml of 0.1 x SSC. RNase A solution (0.3 ml) was added and the DNA solution was incubated at 37 ^{0}C about 20 min for the purification. After adding 0.5 ml of 10 x SSC, 2 ml of phenol-chloroform were mixed by vortexed for 1 min and centrifuged at 12,000 rpm for 10 min. The upper layer was transferred to another tube. The DNA was precipitated by adding cold 95% ethanol and DNA was spooled with a glass-rod then rinsed with 70% then 95% (v/v) ethanol. After air dried, DNA was dissolved in

5 ml of 0.1 x SSC. The purity and quality of DNA solution were determined from the ratio between absorbance value at 260 and 280 nm (A_{260}/A_{280}) as described by Marmur and Doty (101).

3.3.2. DNA-DNA hybridization

Under optimal conditions, 100 μl portion of a heat-denatured, purified DNA solution of unknown and type strains (10 μg of DNA /1 ml) in phosphate-buffered saline containing 0.1 M $MgCl_2$ were incubated for 2 hours at 37 ^{0}C in microdilution plates (Nunc Corp., Denmark). Photobiotinylation of DNA was performed, by meaning that 10 μg /1 ml of photobiotin and an equal volume of DNA solution (10 μg of DNA /1 ml) were mixed in an eppendorf tube and then irradiated with sunlamp (500W) for 25 min. After irradiation, free photobiotin was removed by n-butanol extraction. The biotinylation of DNA was used immediately for hybridization experiment.

For quantitative detection of biotinylated DNA in microdilution wells, 200 μl of a prehybridize solution (20xSSC, 5% Denhardt solution, 50% formamide) containing 10 μg of denatured salmon sperm DNA / ml were added to microdilution wells which had been coated with the reference DNA. The microdilution plates were then incubated at 43 ^{0}C for 30 min. The prehybridize solution was discarded and replaced with 100 μl portions of hybridization mixure (20xSSC, 5% Denhardt solution, 3% dextrane sulfate, 50% formamide, 10 μg of denatured salmon sperm DNA / ml) containing 10 μg of bitinylated DNA. The microplates were then covered with aluminium foil, and incubated for overnight (15 hours) at 43 ^{0}C. After hybridization, the microdilution well were washed three times with 200 μl of 0.2 x SSC buffer. A 100 μl of streptavidin peroxidase conjugate solution (Boehringer

Mannheim Germany) was added to the wells, and the preparations were incubated at 37 ^{0}C for 30 min. After incubation, the wells were washed three times with PBS-buffer. Then the enzyme solution was discarded and100 μl of substrate 3,3',5,5'-tetramethylbenzidine-H_2O_2 solution (Wako, Japan) was added to each well. The plate were incubated at 37 ^{0}C for 10 min. The reaction was stopped with 2 M H_2SO_4 and the colour intensity was measured with Microplate Reader Model 3550 (Bio-Rad, CA, USA) at a wavelength 450 nm (69, 75). The homology values for the DNA-DNA hybridization were calculated. In practice, a DNA-homology above 70% indicates a relationship in the species level, while values between 20% to 60% give evidence for the genus (17).

3.4. Ubiquinone analysis

Fifteen clinical isolates represented various genomic species based on the DNA relatedness were grown on tryptic soy broth 300 ml by shaking in a rotary shaker at 200 rpm for 18 hour at 30 ^{0}C. Cell were harvested by centrifugulation at 7,000 rpm and washed with sterile water. The isoprenoid quinone were extracted from intact cells by using chloroform-methanol (2:1,v/v) in flask and shked for 3 hours. The residual cells were separated by filtration. The combined filtrate was concentrated to dryness under a reduced pressure on the rotary evaporator. Crude quinone was dissolved in a small amout of acetone. Acetone solution was applied to thin-layer chromatography on a siliga-gel glass plate (20x20 cm, E. Merk, Silica gel 60F254, Art. 5715) and developed with a solvent system of pure benzene. Standard quinones should also be included. The quinone spots can be visualized by UV light at 254 nm. The R_f of ubiquinone was 0.4. The band of ubiquinone was scraped off and extracted with acetone. The purified quinones were examined by HPLC (Shimadzu

model LC-3A). The abbreviation (e.g. Q-9,Q-8, etc.) used for ubiquinone indicated the number of isoprene units in the side chain.

3.5. Identification by Vitek 32 Automatic System.

Fifty-two clinical isolates which had been identified as various genomic groups of *Acinetobacter* species based on DNA relatedness were selected for the identification by Vitek 32 Automatic System (bioMerieux Vitek, Inc.). The bacterial isolates were cultured on tryptic soy agar plate with 5% sheep blood and incubated at 37 ^{0} C for 24 hours. A 1.8 ml of sterile 0.45% saline were aseptically added to the tube. The colonies from agar plate were picked with a sterile cotton swab which was then inserted into the test tube and swirled in the saline until a uniform suspension was achieved. This suspension was adjusted until it was equivalent to the appropriate color zone on the Vitek Colorimeter between the blue and green scale ranges, indicating a concentration of not less than 1.0 McFarland standard. The transfer tube was firmly inserted into the Card port with the end of the tube pointing towards the notches of the Card. The mated Card/Transfer Tube unit were placed onto the Vitek Filling Stand, with the long part of the Transfer Tube inserted into the test tube. The Transfer Tube which had already been inserted into the Card properly, would extended well into the sample, and took it onto the Filling Module. Following, the mated Card/Transfer Tube was inserted into the Sealer Module for seals the Card port, the Card was loaded into the Reader/Incubator tray and the test was proceeded. The GNI Card biochemical tests were analyzed and stored automatically by the programmed computer.

CHAPER IV

RESULTS

1. Identification

All 350 clinical isolates were obtained during the study period of 6 months. The presumtive phenotypic characteristics (TSI, oxidase test, motility, oxidative and fermentative test) of all the isolates were studied. A total of 150 isolates out of the 350 isolates were identified as the genus *Acinetobacter* based on the presumptive phenotypic characteristics as shown in Table 4. Among the 150 isolates, 127 isolates were oxidizer and 23 isolates were nonoxidizer groups as shown in Figure 2.

1.1. Morphological and Cultural characteristics

All 150 isolates were gram-negative rods, 0.5-0.8 μm in diameter and 1.0-1.9 μm in length. They occured in singly, in pair or in chains. They were nonmotile and nonsporing. Colonies on blood agar were convex and entire, gray to white, 2-3 mm in diameter and on MacConkey agar were slightly buish tint colonies.

1.2. Physiological and biochemical characteristics

All isolates were characterized by physiological and biochemical characteristics and were separated into five groups (Groups A to E) on the basis of physiological and biochemical characteristics as shown in Table 5. All isolates in Group A (73 isolates) oxidized glucose grew at 44 ^{0}C utilized of malonate, succinate and produced acid from D-glucose. They were identified as *A. baumannii*. The isolates in Group B (8 isolates) oxidized glucose, but they could not grow at 44 ^{0}C, then they were identified as *A. calcoaceticus*. The 46 isolates in Group C did not oxidized glucose. Half of the isolates (23 isolates) utilized malonate and most of

them utilized succinate and produced acid from D-glucose and showed variable reactions as shown in Table 5. This group could not be identified to the species level and were designated as unidentified group. The 8 isolates in Group D did not oxidized glucose, not grow at 41 ^{0}C and 44 ^{0}C, and could neither produce acids from D-glucose, nor utilize malonate and succinate. They were identified as *A. lwoffii*. Group E were nonoxidizer. All 15 isolates could not produce acid from D-glucose and not utilize succinate, but half of isolates utilized malonate. The utilization of carbon sources of there isolates were variable. All strains of Group E could not be identified to the species level.

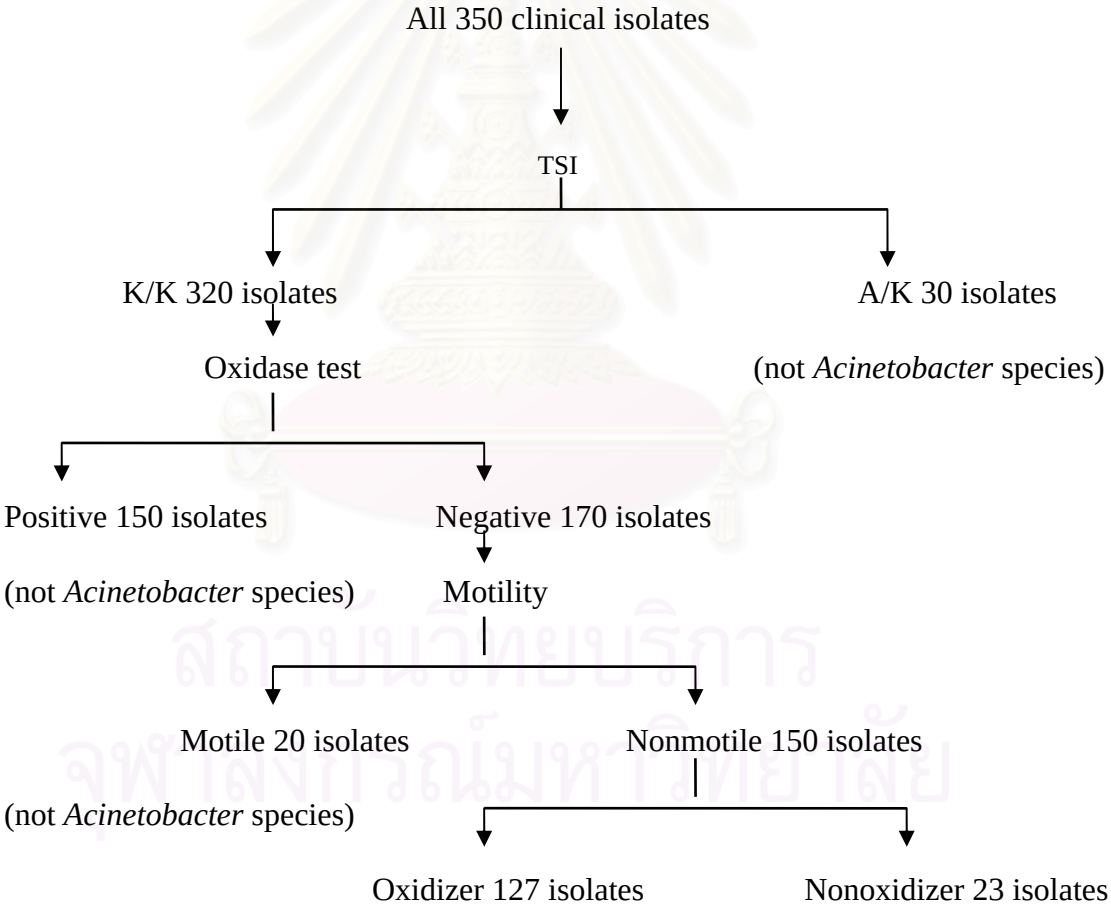


Figure 2 Presumptive identification for *Acinetobacter* species.

Note: A, acid; K, alkaline.

The *Acinetobacter* strains were isolated from various clinical specimens was shown in Table 6. Out of the 150, 73 isolates were identified by phenotypic characteristics as *A. baumannii*, 8 as *A. calcoaceticus*, 8 as *A. lwoffii* and 61 as unidentified strains. *A. baumannii* strains from patients were mainly isolated from pus, urine, sputum, and blood (29, 16, 11 and 7 isolates, respectively).

2. DNA-DNA hybridization labelled with photobiotin

All of 150 isolates were separated into 7 groups on the basis of DNA relatedness as shown in Tables 7, 8 and 9 respectively. The tested strains of Group I (105 isolates) showed high degrees of DNA homology (over 70.11%) with *A. baumannii* JCM 6841^T (Table 7), thus they were identified as *A. baumannii*. Most of the isolates of Group I grew at 41 ^{0}C and 44 ^{0}C. They could utilize L-arginine, L-histidine, β-alanine, DL-lactate, L-ornithine, malonate, succinate, glutarate, citrate, and D-malate. All of the isolates could produce acid from glucose, but some of them also produced acids from D-maltose, D-mannitol, lactose and sucrose. Forty-two isolates were isolated from pus, 19 isolates from urine, 15 isolated from sputum, 11 isolated from blood, 2 isolated from blood, and 16 were isolated from the unknown sources.

Group II contained 5 isolates which exhibited high degrees of DNA homology (over 71.22%) with *A. calcoaceticus* DMST 2270^T (Table 8). They were identified as *A. calcoaceticus* . The phenotypic characteristics of Group II were similar to Group I, except that the isolates in this group could not grow at 41 ^{0}C and 44 ^{0}C. For the types of specimen it was found that 3 isolates were from pus, one isolate from blood, and one isolate from urine.

Group III contained 3 isolates which showed high degrees of DNA homology (over 70.33%) with *A. lwoffii* JCM 6840^{T} (Table 8). They were identified as *A. lwoffii*. All isolates were nonoxidizer glucose, no growth at 41^{0} C and 44^{0}C, could not utilize glutarate, citrate, L-arginine and not produced acid from D-glucose. They were all isolated from pus.

Group IV consisted of 10 isolates. They showed high degrees of DNA homology (over 72.08%) with *A. junii* DMST 2274^{T} (Table 8) and were identified as *A. junii* . All isolates did not produce acid from D-glucose. Most of isolates utilized L-arginine, citrate and grew at 41 ^{0}C. The isolates in this group, 5 isolates were distributed in pus, 1 isolate in sputum, 1 isolate in urine and 3 isolates in the unknown specimens.

Group V contained 4 isolates, which showed high degrees of DNA homology (over 74.19%) with *A. haemolyticus* DMST 2273^{T} (Table 8). They were identified as *A. haemolyticus*. All isolates oxidized glucose and did not grow at 41 ^{0}C . Most of isolates utilized L-arginine, malonate, succinate. Few isolates produced acid from D-glucose.They were all isolated from pus.

Group VI contained 3 isolates, which showed high degrees of DNA homology (over 81.22%) with *Acinetobacter* genospecies 3 DMST 2272^{T} (Table 8) and they were identified as *Acinetobacter* genospecies 3. Few isolates grew at 41 ^{0}C and produced acid from D-glucose. The isolates in this group were all isolated from cerebrospinal fluid (CSF).

Group VII contained 21 isolates showed low degrees of DNA homology(lower 69.67%) with with *A. baumannii* JCM 6841^{T}, *A. calcoaceticus* DMST 2270^{T}, *A. lwoffii* JCM 6840^{T}, *A. junii* DMST 2274^{T}, *A. haemolyticus* DMST 2273^{T}, *Acinetobacter* genospecies 3 DMST 2272^{T}, *Acinetobacter* genospecies 6

DMST 2275^T, and *A. radioresistens* JCM 9326^T (Table 9). However, some strains showed quite some strains high degree of DNA homology but still less than 70%. The strains CBF3-6, CP4-5, SU13-5, and Un11-31 showed as low as 63.60% of DNA homology with *A. calcoaceticus* DMST 2270^T. The strain CP4-3 showed 60.32% of DNA homology with *A. baumannii* JCM 6841^T and strain Un11-29 showed 66.06% of DNA homology with *A. lwoffii* JCM 6840^T. The biochemical reactions of the isolates in all 7 DNA relatedness groups were variable as shown in Table10 and 11.

The comparatives identification results between phenotypic testes and DNA-DNA hybridization were shown in Table 12. All 150 isolates were separated into 5 different groups by phenotypic tests. Group A, contained 73 isolates which were identified as *A. baumannii*, but only 71 isolates were identified by DNA relatedness as *A. baumannii*. The remaining 2 isolates was *A. haemolyticus* and unidentified *Acinetobacter* species. The strains in Group B contained 8 isolates which were identified as *A. calcoaceticus* by phenotypic tests, but only 1 isolates was confirmed *A. calcoaceticus* by DNA relatedness, while 4 isolates was *A. baumannii* , 1 isolate was *Acinetobacter* genospecies 3 and 2 isolates unidentified *Acinetobacter* species by DNA relatedness. The Group C strains, contained 46 isolates and were unidentified by the phenotypic characteristics, but they were identified by DNA relatedness as *A. baumannii* (28 isolates), as *A. calcoaceticus* (4 isolates), *Acinetobacter* genospecies 3 (1 isolate), *A. junii* (3 isolates), and *A. haemolyticus* (2 isolates). The remaining 8 isolates were still unidentified *Acinetobacter* species even by DNA relatedness. The Group D strains contained 8 isolates which were identified as *A. lwoffii* by phenotypic characteristics. In contrast, only 2 isolates were identified as *A. lwoffii*, while based on DNA relatedness 2 isolates as *A. junii*, 1 isolate as *Acinetobacter* genospecies 3 and 3 isolates unidentified *Acinetobacter* species. The Group E strains

contained 15 isolates which were unidentified by phenotypic characteristics, but they could be identified by DNA relatedness as *A. junii*(5 isolates), as *Acinetobacter* genospecies 3 (2 isolates) and 8 isolates were still unidentified *Acinetobacter* species.

Types of specimens and various *Acinetobacter* strains according to DNA relatedness were shown in Table. 13. Of total 150 isolates, 105 isolates were identified by DNA-DNA hybridization as *A. baumannii*. Nineteen isolates were isolated from urine, 15 isolates from sputum, 11 isolates from blood, 41 isolates from pus, 2 isolated from body fluid and 16 isolates were isolated from unknown sources. Total of 5 *A. calcoaceticus* isolates were found. Among these, 3 isolates were from pus while one isolate was from urine and one from blood. Only 2 *A. lwoffii* were isolated from pus. As among as 5 out of 10 *A. junii* isolates were from pus. Four *A. haemolyticus* were also from pus.

3. Ubiquinone analysis

Fifteen isolates which had already been identified and grouped by DNA relatedness as in the 7 groups of *Acinetobacter* species were selected for the determination of ubiquinone component as shown in Table 14. Group I (SU1-1, SP9-9, SS4-9, CP5-1, CP1-5, CBF4-25, CP3-5 and SS1-3) had Q-9 ranged from 77.5 to 85.4% as major ubiquinone and Q-8 (6.2-19.1%), Q-7 (1.0- 6.3%), Q-10 (1.7-4.5%) as minor component. Group II (CP5-20) had Q-8 (79.8%) as major ubiquinone and Q-9, Q-7, and Q-10 (3.0-11.0%) as minor ubiquinones. Group III (CP3-20) had Q-9 (78.6 %) as major ubiquinone and Q-7, and Q-8 as minor ubiquinone. Group IV (CP3-12) contained Q-9 (79.6%) as a major ubiquinone. Group VI (CCSF3-23) had Q-9 (89.6%) as a major ubiquinone and Q-7, Q-8, Q-10 as minor ubiquinones. Group VII (SU4-3, CP3-14) had Q-8 ranged from 90.7 to 96.5% as a major

ubiquinone and Q-7 (3.5-9.3%) as minor ubiquinone, while the strains CP4-23 had Q-9 (88.1%) as a major ubiquinone and Q-8, Q-10 as minor ubiquinone.

4. Identification by Vitek 32 Automatic System.

Fifty-two isolates which had already been identified as different group of *Acinetobacter* strains according to the DNA relatedness were selected for the identification of *Acinetobacter* species by Vitek 32 Automatic System. The result of the 52 isolates tested were listed in Table 15.

5. Comparison of the results obtained from 3 different tests (conventional biochemical tests, Vitek 32 system, and DNA relatedness).

According to Group I (*A. baumannii*) based on DNA relatedness tests, 16 isolates were agreed by all 3 tests, 8 isolates were identified as *A. baumannii* by conventional biochemical tests but as *A. calcoaceticus-A. baumannii* complex (A*bc* complex) by Vitek 32 system, 4 isolates were identified as *A. calcoaceticus* by conventional biochemical tests but as A*bc* complex by Vitek 32 system, 10 isolates were unidentified by conventional biochemical tests but were identified as *A. baumannii* (6 isolates) and A*bc* complex (4 isolates) and one isolate was identified as *A. baumannii* by conventional biochemical tests but as *A. lwoffii*/ *A. junii* by Vitek 32 system. According to Group II (*A. calcoaceticus*) they were unidentified by conventional biochemical tests but as A*bc* complex by Vitek 32 system. Group III (*A. lwoffii*) they were identified by conventional biochemical tests as *A. lwoffii* and as *A. lwoffii*/*A. junii* by Vitek 32 system. According to Group IV (*A. junii*) based on DNA relatedness tests, 2 isolates were identified as *A. lwoffii* by conventional biochemical tests and *A. lwoffii* / *A. junii* by Vitek 32 system, one isolate was

unidentified by conventional biochemical tests but as *A. lwoffii* /*A. junii* by Vitek 32 system. Group V (*A. haemolyticus*), the isolate was unidentified by conventional biochemical tests but as A*bc* complex by by Vitek 32 system. According to Group VI (*Acinetobacter* genospecies 3), one isolate was identified as *A. calcoaceticus* by conventional biochemical tests but as A*bc* complex by by Vitek 32 system, and one isolate was identified as *A. lwoffii* by conventional biochemical tests and *A.lwoffii* / *A. junii* by Vitek 32 system. According to Group VII (unidentified *Acinetobacter* species) based on DNA relatedness, 2 isolates were unidentified by conventional biochemical tests but one isolate as A*bc* complex and one isolate as *A. lwoffii* /*A. junii* by Vitek 32 system, 2 isolates were identified as *A. calcoaceticus* by conventional biochemical tests but were identified by Vitek 32 system as *A.lwoffii* /*A. junii*.

In this study, among 52 isolates tested by Vitek 32 system (Table 16). The species identification of 17 isolates (34%) were agreed by all phenotypic tests, DNA relatedness and Vitek 32 Automatic System, 10 isolates (54%) were agreed by phenotypic tests and DNA relatedness, 3 isolates (40%) were agreed by phenotypic test and Vitek 32 Automatic System and 10 isolates (54%) were agreed by DNA DNA relatedness and Vitek 32 Automatic System (Table 17).

Table 4 Number of *Acinetobacter* species isolated from various clinical specimens.

Sources	No.of isolates from							Total
	Urine	Sputum	Blood	Pus	Body fluid	CSF	Unknown	
King Chulalongkorn Memmorial hospital	4	0	0	47	0	1	0	55
Siriraj Hospital	23	16	11	17	3	0	28	95
Total	27	16	11	64	3	1	28	150

Table 5 Phenotypic characteristics of 5 Groups of *Acinetobacter*species according to conventional biochemical tests.

Characteristics	Group A (73)[a]	Group B (8)	Group C (46)	Group D (8)	Group E (15)
O-F Glucose	+	+	+	-	-
Growth at					
44 °C	+	-	-	-	-
41 °C	+	+(-4)	+(-11)	+	+(-5)
Utilization of					
L-Arginine	+(-1)	+(-2)	+(-10)	+(-4)	+(-5)
Malonate	+(-11)	+(-1)	+(-23)	-	-(+6)
Succinate	+	+(-1)	+(-8)	-	+
Citrate	+(-5)	+	+(-6)	+(-4)	+(-6)
Tratrate	+(-11)	-	+(-23)	-	-
Acid from					
Glucose	+	+	+(-7)	-	-

[a]Number of tested strains. Number in parentheses indicated the number of strains showing positive or negative reaction..

Table 6 *Acinetobacter* strains according to the type of specimens.

Species	No. of isolates from								Group
	Urine	Sputum	Blood	Pus	Unknown	Body fluid	CSF	Total	
A.baumannii	16	11	7	29	9	1	0	73	A
A.calcoaceticus	3	1	0	2	2	0	0	8	B
A.lwoffii	1	1	0	4	1	0	1	8	D
unidentified	7	4	4	28	16	2	0	61	C, E
Total	27	17	11	63	28	3	1	150	

Table 7 *Acinetobacter* Group I according to DNA relatedness.

Isolate no.	Code	% homology with labelled strains	
		JCM 6841^T	DMST 2270^T
Group I			
001	SU1-1	**97.66**	39.75
002	SU7-1	**90.78**	ND
003	SU7-2	**78.74**	ND
004	SU9-3	**86.17**	ND
005	SU11-12	**87.80**	ND
006	SU1-2	**99.09**	ND
007	SU1-4	**99.76**	ND
012	SU13-9	**75.88**	ND
013	SS5-3	**79.40**	ND
014	SS6-5	**91.64**	ND
015	SS6-6	**89.86**	ND
016	SS6-7	**98.62**	ND
017	SS4-2	**89.61**	14.73
018	SS4-7	**76.87**	ND
020	SS4-9	**79.29**	33.84
021	SS6-3	**89.40**	ND
022	SS4-3	**70.11**	66.43
023	SS11-20	**82.11**	ND
024	SB7-1	**93.09**	ND
025	SB4-1	**88.13**	ND
026	SB6-15	**86.17**	ND
027	SB1-3	**98.93**	34.04
028	SB4-5	**76.58**	35.05
029	SB7-2	**91.70**	ND
030	SB6-13	**70.20**	60.61
031	SB6-14	**88.94**	ND
032	SB7-3	**89.40**	ND
033	SP5-6	**79.97**	ND
034	SP8-2	**75.60**	ND
035	SP8-5	**83.73**	ND

Table 7 (Cont.) *Acinetobacter* Group I according to DNA relatedness.

Isolate no.	Code	% homology with labelled strains	
		JCM 6841^T	DMST 2270^T
036	SP9-9	**89.43**	ND
037	SP1-1	**98.73**	39.52
038	SP1-2	**78.93**	22.41
040	SP13-12	**73.53**	ND
041	SP11-5	**81.62**	22.47
043	SP3-5	**97.93**	ND
044	SP3-6	**82.11**	ND
045	Un3-4	**82.11**	ND
046	Un3-1	**77.23**	ND
047	Un11-6	**80.48**	ND
048	Un11-20	**82.11**	ND
049	Un11-34	**85.36**	ND
050	Un11-36	**79.67**	ND
051	Un12-1	**95.34**	ND
052	Un12-4	**91.67**	ND
053	Un12-5	**72.06**	ND
057	Un10-31	**71.54**	14.45
058	Un10-35	**77.06**	21.42
062	Un10-5	**80.00**	21.24
063	SU2-1	**85.83**	64.07
064	CU3-22	**86.45**	ND
066	CP3-4	**74.48**	ND
067	CP5-1	**81.97**	ND
069	CP4-9	**93.09**	ND
071	CP5-2	**98.37**	ND
072	CP5-3	**84.55**	ND
073	CP5-5	**77.24**	ND
075	CP3-5	**82.99**	ND
076	CP5-10	**94.01**	ND
077	CP5-16	**96.77**	14.73
078	CP5-7	**87.56**	ND

Table 7 (Cont.) *Acinetobacter* Group I according to DNA relatedness.

Isolate no.	Code	% homology with labelled strains	
		JCM 6841^T	DMST 2270^T
079	CP5-9	**94.36**	14.03
080	CP4-3	**99.20**	16.14
081	CP5-13	**97.54**	16.14
082	CP4-1	**73.61**	38.1
083	CP4-2	**73.35**	20
085	CP2-4	**77.22**	46.56
086	CP3-8	**71.25**	24.00
087	CP4-4	**75.01**	33.53
093	CU3-21	**95.71**	ND
094	CP1-5	**92.90**	ND
095	CP4-27	**87.80**	ND
097	CBF4-26	**78.04**	ND
098	CBF4-25	**81.44**	ND
101	CP3-25	**89.77**	ND
103	CP3-1	**72.65**	64.04
106	CP3-24	**83.95**	ND
107	CP4-21	**85.78**	19.65
110	CP5-17	**70.32**	65.68
112	CP4-15	**68.40**	29.19
113	CP3-13	**70.58**	33.07
116	SS11-19	**87.8**	ND
117	SU11-16	**80.48**	ND
118	SU11-10	**77.36**	ND
120	SU6-22	**88.54**	27.59
121	SS11-19	**76.78**	19.65
122	SU11-16	**99.45**	ND
124	CP2-10	**87.10**	24.00
125	CP1-15	**72.29**	47.06
129	Un10-39	**81.22**	31.73
132	SS1-3	**98.29**	ND
133	SB1-4	**99.05**	ND

Table 7 (Cont.) *Acinetobacter* Group I according to DNA relatedness.

Isolate no.	Code	% homology with labelled strains	
		JCM 6841^T	DMST 2270^T
134	CP4-10	**89.40**	16.43
135	CU3-3	**74.81**	29.60
136	Un10-19	**86.99**	ND
137	Un10-25	**87.80**	ND
140	CP5-4	**94.30**	ND
141	SU13-2	**95.83**	ND
142	SU11-25	**83.51**	24.40
143	SU11-27	**80.23**	27.28
145	SS4-8	**90.69**	29.18
146	SS5-8	**88.13**	24.29
147	SP6-8	**73.6**	29.89
148	SP5-6	**79.97**	18.12
150	Un10-30	**71.58**	17.33
A. baumannii JCM 6841^T		**100.0**	25.22
A. calcoaceticus DMST 2270^T		36.8	**100.0**

Table 8 *Acinetobacter* Group II, III, IV,V, and VI according to DNA relatedness.

Isolate no.	Code	% homology with labelled strains	
		2270^T	6841^T
Group II			
068	CP5-20	**99.39**	18.89
092	CP4-8	**76.92**	30.00
096	CP4-24	**72.33**	21.80
144	SU11-33	**71.22**	ND
149	SB5-4	**72.93**	25.91
A. calcoaceticus DMST 2270^T		**100.0**	36.8
A. baumannii JCM 6841^T		25.22	**100.0**
		JCM 6840^T	DMST 2274^T
Group III			
088	CP3-20	**70.52**	30.52
114	CP3-2	**70.33**	28.79
A. lwoffii JCM 6840^T		**100.0**	22.62
A. junii DMST 2274^T		24.83	**100.0**
		DMST 2274^T	JCM 6840^T
Group IV			
019	SS6-4	**98.74**	29.30
039	SP9-8	**85.32**	25.26
055	Un10-21	**85.95**	26.24
056	Un10-22	**82.64**	22.25
065	CU3-11	**83.51**	24.18
084	CP4-13	**72.08**	29.70
090	CP4-12	**78.73**	54.92
102	CP3-12	**81.57**	60.95
108	SP9-8	**88.40**	33.47
131	Un11-27	**83.70**	49.72
A. junii DMST 2274^T		**100.0**	24.83
A. lwoffii JCM 6840^T		22.62	**100.0**

Table 8 (Cont.) *Acinetobacter* Group II, III, IV,V, and VI according to DNA relatedness.

Isolate no.	Code	% homology with labelled strains	
		DMST 2273^T	DMST 2275^T
Group V			
070	CP4-5	**74.19**	32.48
074	CP5-8	**80.19**	36.42
091	CP5-12	**82.45**	32.36
111	CP4-20	**78.98**	36.95
A. haemolyticus DMST 2273^T		**100.0**	36.49
Acinetobacter genospecies 6 DMST 2275^T 57.8			**100.0**

		DMST2270^T	DMST2270^T	JCM 6841^T	
Group VI					
054	Un3-10	**84.90**	20.21	20.38	
059	Un10-39	**81.22**	31.73	18.85	
104	CCSF3-23	**81.40**	31.51	57.48	
Acinetobacter genospecies 3 DMST 2272^T	**100.0**		28.06		20.61
A. calcoaceticus DMST 2270^T		27.43	**100.0**	26.80	
A. baumannii JCM 6841^T		27.04	25.22	**100.0**	

Table 9 *Acinetobacter* Group VII according to DNA relatedness.

Isolate no.	Code	% homology with labelled strains								
		JCM 6841^T	DMST 2270^T	DMST 2272^T	DMST 2273^T	DMST 2274^T	DMST 2276^T	JCM 6840^T	DMST 2275^T	JCM 9326^T
Group VII										
008	SU11-15	41.46	15.02	19.95	27.85	12.06	18.9	21.58	16.83	18.62
009	SU11-9	49.39	21.71	25.47	34.26	11.43	10.0	15.62	13.22	3.62
010	SU4-3	37.51	15.38	14.28	19.19	13.22	14.28	15.62	37.51	3.62
011	SU13-6	49.39	19.79	20.32	20.18	15.24	17.81	14.79	12.12	16.63
042	SP6-18	16.67	26.61	19.19	34.42	17.11	20.49	35.14	22.8	18.62
060	Un10-23	16.05	16.67	26.87	4.57	40.98	10.41	22.02	12.97	6.05
061	SP11-4	13.35	14.86	12.03	12.25	21.71	22.47	56.34	11.65	13.17
089	CP4-14	29.60	41.15	23.17	9.82	12.96	12.28	15.86	15.94	15.11
099	CP4-18	14.06	19.24	17.82	15.34	49.72	10.14	12.89	10.27	12.72
100	CBF4-6	69.67	48.32	14.14	15.24	11.33	18.47	46.11	16.68	30.29
105	CP4-23	19.65	60.32	23.23	38.99	15.86	17.44	55.93	23.76	14.19
109	CP3-14	24.46	17.45	16.46	11.66	15.73	11.94	17.97	13.52	13.13
112	CP4-15	68.40	29.19	27.21	47.31	33.10	28.82	27.62	18.96	10.38
115	SU13-7	66.00	23.79	23.13	21.42	15.68	17.63	13.97	19.28	10.23
119	Un10-9	18.38	30.42	37.55	25.61	25.61	42.7	52.53	35.46	24.65
123	SU10-30	47.01	23.13	55.61	18.96	13.44	14.69	11.87	19.69	16.42
126	Un10-4	19.24	22.75	9.32	55.73	8.12	12.87	12.25	28.15	10.34
127	Un10-24	11.93	19.72	12.83	16.18	20.32	17.84	40.96	19.24	25.76
128	Un10-34	22.47	42.38	57.19	12.48	11.10	8.94	31.57	16.91	12.75
138	Un11-31	63.60	21.58	16.39	32.84	16.63	18.96	47.31	20.66	35.93
139	Un11-29	16.42	21.58	25.75	34.79	68.27	24.26	66.06	24.18	18.52
A. baumannii JCM 6841^T		**100.0**	38.22	17.04	25.32	24.43	20.27	11.76	25.83	30.65
A. calcoaceticus DMST 2270^T		36.80	**100.0**	17.43	21.94	20.36	21.62	20.72	22.92	ND
Acinetobacter genospecies 3										
DMST 2272^T		20.61	28.06	**100.0**	26.58	23.07	22.97	16.83	28.33	29.14
A. haemolyticus DMST 2273^T		19.81	24.35	ND	**100.0**	38.91	36.49	ND	58.33	23.61

Table 9 (Cont.) *Acinetobacter* Group VII according to DNA relatedness.

Isolate no.	% homology with labelled strains								
	JCM 6841^T	DMST 2270^T	DMST 2272^T	DMST 2273^T	DMST 2274^T	DMST 2276^T	JCM 6840^T	DMST 2275^T	JCM 9326^T
Acinetobacter genospecies 6									
DMST 2275^T	21.66	26.09	ND	57.8	38.91	35.13	ND	**100.0**	30.65
A. junii DMST 2274^T	23.04	26.96	ND	45.57	**100.0**	27.03	ND	43.75	26.53
A. lwoffii JCM 6840^T	15.46	30.87	18.35	26.58	22.62	33.78	**100.0**	30.83	38.69
A. johnsonii DMST 2276^T	17.51	26.09	ND	40.08	24.43	**100.0**	ND	42.5	30.65
A. radioresistens JCM 9326^T	17.05	17.39	ND	15.06	13.62	14.05	ND	15.02	**100.0**

Table 10 Phenotypic characteristics of 150 clinical isolates according to the genomic groups.

No.	Characteristics		O-F		Growth	Growth at		Utilization of carbohydrate and amino acid																Acid form				
	Organisms	Oxidase	glucose	Motility	on MAC	41°C	44°C	Arg	His	Ala	Leu	Lac	Tar	Orn	Mal	Suc	Ben	Glut	Cit	Ace	Phe	Mal	Asp	Glu	Mlt	Man	Lac	Suc
Group I *A.baumannii*																												
001	SU1-1	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	-	+	-	-	-	-
002	SU7-1	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
003	SU7-2	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
004	SU9-3	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
005	SU11-12	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	-	-	-	-	-
006	SU1-2	-	+	-	+	+	+	+	+	+	-	+	-	-	-	-	+	+	+	+	+	+	-	+	-	-	-	-
007	SU1-4	-	+	-	+	+	+	+	+	+	-	+	-	-	-	-	+	+	+	+	+	+	-	+	-	-	-	-
012	SU13-9	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
013	SS5-3	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
014	SS6-5	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	+	-
015	SS6-6	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	+	-
016	SS6-7	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
017	SS4-2	-	+	-	+	+	-	+	-	-	-	+	-	+	+	+	-	+	+	+	-	+	-	+	-	-	-	-
018	SS4-7	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
020	SS4-9	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
021	SS6-3	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	-	+	+	-	+	-	+	-	-	-	-
022	SS4-3	-	+	-	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	+	-	+	-	+	-	-	-	-

Table 10 (Cont.) Phenotypic characteristics of 150 clinical isolates according to the genomic groups.

No.	Characteristics		O-F		Growth	Growth at		Utilization of carbohydrate and amino acid																Acid form				
	Organisms	Oxidase	glucose	Motility	on MAC	41°C	44°C	Arg	His	Ala	Leu	Lac	Tar	Orn	Mal	Suc	Ben	Glut	Cit	Ace	Phe	Mal	Asp	Glu	Mlt	Man	Lac	Suc
023	SS11-20	-	+	-	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	+	-	+	-	+	-	-	-	-
024	SB7-1	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	+	-
025	SB4-1	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	-	+	-	+	-	-	-	-
026	SB6-15	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
027	SB1-3	-	+	-	+	+	+	-	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	+	+	+	+
028	SB4-5	-	+	-	+	+	+	-	-	-	-	+	-	-	-	+	-	+	+	+	+	+	-	-	-	-	-	-
029	SB7-2	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
030	SB6-13	-	+	-	+	+	+	+	+	+	-	+	-	-	-	+	+	+	+	+	+	+	-	+	-	-	-	-
031	SB6-14	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
032	SB7-3	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
033	SP5-6	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
034	SP8-2	-	+	-	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+	+	+	-	+	+	-	+	-
035	SP8-5	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	+	-
036	SP9-9	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
037	SP1-1	-	+	-	+	+	+	+	+	+	-	+	-	-	-	-	+	+	+	+	-	+	-	+	+	+	+	+
038	SP1-2	-	+	-	+	+	+	+	-	+	-	+	-	-	-	-	+	+	+	+	+	+	-	+	-	-	-	-
040	SP13-12	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
041	SP11-5	-	+	-	+	+	-	+	+	-	-	+	+	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-

Table 10 (Cont.) Phenotypic characteristics of 150 clinical isolates according to the genomic groups.

No.	Characteristics		O-F		Growth	Growth at		Utilization of carbohydrate and amino acid																Acid form				
	Organisms	Oxidase	glucose	Motility	on MAC	41°C	44°C	Arg	His	Ala	Leu	Lac	Tar	Orn	Mal	Suc	Ben	Glut	Cit	Ace	Phe	Mal	Asp	Glu	Mlt	Man	Lac	Suc
043	SP3-5	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+
044	SP3-6	-	+	-	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	-	+	-	+	-	-	-	-
045	Un3-4	-	+	-	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	-	+	-	+	+	+	+	+
046	Un3-1	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	-	+	-	+	-	-	-	-
047	Un11-6	-	+	-	+	+	+	+	+	-	-	+	-	-	-	+	-	+	+	+	+	+	-	+	-	-	-	-
048	Un11-20	-	+	-	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	+	+	+	-	+	-	-	-	-
049	Un11-34	-	+	-	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
050	Un11-36	-	+	-	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
051	Un12-1	-	+	-	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
052	Un12-4	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
053	Un12-5	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
057	Un10-31	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	-	+	+	-	+	-	+	+	+	-	+
058	Un10-35	-	+	-	+	-	-	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
062	Un10-5	-	+	-	+	+	+	+	+	-	-	+	+	-	+	+	-	+	+	+	-	+	-	+	+	+	+	+
063	SU2-1	-	+	-	+	+	-	+	+	+	-	+	-	-	-	-	+	+	+	+	+	+	-	+	-	-	-	-
064	CU3-22	-	+	-	+	+	+	+	+	+	-	+	-	-	-	+	+	+	+	+	+	+	-	+	-	-	-	-
066	CP3-4	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	-	+	+	-	+	-	-	-	-
067	CP5-1	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	-	+	-	+	-	-	-	-

Table 10 (Cont.) Phenotypic characteristics of 150 clinical isolates according to the genomic groups.

No.	Characteristics		O-F		Growth	Growth at		Utilization of carbohydrate and amino acid																Acid form				
	Organisms	Oxidase	glucose	Motility	on MAC	41°C	44°C	Arg	His	Ala	Leu	Lac	Tar	Orn	Mal	Suc	Ben	Glut	Cit	Ace	Phe	Mal	Asp	Glu	Mlt	Man	Lac	Suc
069	CP4-9	-	+	-	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+	-	+	-	+	-	-	+	+
071	CP5-2	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
072	CP5-3	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
073	CP5-5	-	+	-	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	+	+	+	-	+	-	-	-	-
075	CP3-5	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	-	+	-	-	-	-
076	CP5-10	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	-	+	-	+	-	-	-	-
077	CP5-16	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	-	+	-	+	-	-	-	-
078	CP5-7	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
079	CP5-9	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
080	CP4-3	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+
081	CP5-13	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+	+	-	+	-	+	-	-	-	-
082	CP4-1	-	+	-	+	+	+	+	+	+	-	+	-	-	-	-	-	+	-	+	+	+	-	+	+	+	+	+
083	CP4-2	-	+	-	+	+	-	+	-	+	-	+	-	-	-	+	-	+	+	+	-	-	-	+	-	-	-	-
085	CP2-4	-	-	-	+	+	-	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	-	-	-	-	-
086	CP3-8	-	+	-	+	+	-	+	-	+	-	-	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
087	CP4-4	-	+	-	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	+	+	+	-	+	+	+	+	+
093	CU3-21	-	+	-	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	+	-	+	-	+	-	-	-	-
094	CP1-5	-	+	-	+	+	+	+	+	+	-	+	-	-	-	+	+	+	+	+	+	+	-	+	-	-	-	-

Table 10 (Cont.) Phenotypic characteristics of 150 clinical isolates according to the genomic groups.

No.	Characteristics		O-F		Growth	Growth at		Utilization of carbohydrate and amino acid																Acid form				
	Organisms	Oxidase	glucose	Motility	on MAC	41°C	44°C	Arg	His	Ala	Leu	Lac	Tar	Orn	Mal	Suc	Ben	Glut	Cit	Ace	Phe	Mal	Asp	Glu	Mlt	Man	Lac	Suc
095	CP4-27	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
097	CBF4-26	-	+	-	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	-	+	-	+	-	-	-	-
098	CBF4-25	-	+	-	+	+	+	+	+	+	-	+	-	-	+	-	-	+	+	+	+	+	-	+	+	+	+	+
101	CP3-25	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
103	CP3-1	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
106	CP3-24	-	+	-	+	+	+	-	+	+	-	+	-	-	+	+	+	+	+	+	-	+	-	+	-	-	-	-
107	CP4-21	-	+	-	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
110	CP5-17	-	+	-	+	+	+	+	-	-	-	+	-	-	+	+	-	+	+	+	-	-	-	+	-	-	-	-
112	CP4-15	-	-	-	+	+	+	+	+	-	-	+	-	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
113	CP3-13	-	+	-	+	+	-	+	+	+	-	+	-	-	-	-	-	-	+	+	-	+	-	+	+	-	+	+
116	SS11-19	-	+	-	+	+	+	-	+	-	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
117	SU11-16	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	-	+	+	-	+	-	+	-	-	-	-
118	SU11-10	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	-	+	+	-	+	-	+	-	-	-	-
120	SU6-22	-	-	-	+	+	-	+	+	+	-	+	-	-	-	+	-	+	+	+	-	+	-	-	-	-	-	-
121	SS11-19	-	+	-	+	+	-	-	+	-	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
122	SU11-16	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
124	CP2-10	-	-	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	+	-
125	CP1-15	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-

Table 10 (Cont.) Phenotypic characteristics of 150 clinical isolates according to the genomic groups.

No.	Characteristics		O-F		Growth	Growth at		Utilization of carbohydrate and amino acid																Acid form				
	Organisms	Oxidase	glucose	Motility	on MAC	41°C	44°C	Arg	His	Ala	Leu	Lac	Tar	Orn	Mal	Suc	Ben	Glut	Cit	Ace	Phe	Mal	Asp	Glu	Mlt	Man	Lac	Suc
129	Un10-39	-	+	-	+	+	-	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
132	SS1-3	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
133	SB1-4	-	+	-	+	+	+	-	+	-	-	+	-	-	-	+	-	+	+	+	-	-	-	+	-	+	-	-
134	CP4-10	-	+	-	+	+	-	+	+	+	-	+	-	-	-	+	-	+	+	+	-	+	-	+	-	-	-	-
135	CU3-3	-	+	-	+	+	+	+	+	-	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
136	Un10-19	-	+	-	+	+	+	+	+	-	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
137	Un10-25	-	+	-	+	+	+	+	-	-	+	-	-	-	+	+	-	+	-	+	+	+	-	+	-	-	-	-
140	CP5-4	-	+	-	+	+	+	+	+	+	-	+	-	-	-	+	-	+	+	+	-	+	-	+	-	-	-	-
141	SU13-2	-	+	-	+	+	+	+	+	-	-	+	-	-	+	+	-	+	+	+	+	-	-	+	-	-	-	-
142	SU11-25	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	-	+	+	+	+	-	+	-	-	-	-
143	SU11-27	-	+	-	+	+	+	+	-	+	-	+	-	-	-	+	-	-	-	+	-	+	-	+	-	-	-	-
145	SS4-8	-	+	-	+	+	+	+	+	+	-	+	+	-	+	+	-	-	+	+	-	+	-	+	-	-	+	+
146	SS5-8	-	+	-	+	+	+	+	+	+	-	+	+	-	+	+	-	-	+	+	+	+	-	+	-	-	-	-
147	SS6-8	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
148	SP5-6	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
150	Un10-30	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-

Table 10 (Cont.) Phenotypic characteristics of 150 clinical isolates according to the genomic groups.

No.	Characteristics					Growth at		Utilization of carbohydrate and amino acid																Acid form				
	Organisms	Oxidase	O-F glucose	Motility	Growth on MAC	41°C	44°C	Arg	His	Ala	Leu	Lac	Tar	Orn	Mal	Suc	Ben	Glut	Cit	Ace	Phe	Mal	Asp	Glu	Mlt	Man	Lac	Suc
Group II *A. calcoaceticus*																												
144	SU11-33	-	+	-	+	-	-	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
068	CP5-20	-	+	-	+	-	-	-	+	+	-	+	-	-	+	+	-	-	+	-	+	+	-	+	-	-	-	-
092	CP4-8	-	+	-	+	-	-	-	+	+	-	+	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-
096	CP4-24	-	+	-	+	-	-	-	+	+	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
149	SB5-4	-	+	-	+	-	-	+	+	+	-	+	+	-	-	+	-	-	+	+	+	+	+	+	-	-	-	-
Group III *A.lwoffii*																												
088	CP3-20	-	-	-	+	-	-	-	+	+	-	+	-	-	-	+	-	+	-	+	-	+	-	-	-	-	-	-
114	CP3-2	-	-	-	+	-	-	-	-	+	-	+	-	-	-	+	-	-	-	+	-	+	-	-	-	-	-	-
Group IV *A. junii*																												
039	SP9-8	-	+	-	+	+	-	+	+	-	-	+	-	-	-	-	-	+	+	+	-	+	-	+	+	+	+	+
102	CP3-12	-	+	-	+	+	-	+	+	+	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-
108	SP9-8	-	+	-	+	+	-	+	+	-	-	+	-	-	-	+	-	-	+	+	-	+	-	-	-	-	-	-
019	SS6-4	-	-	-	+	-	-	+	+	+	-	+	-	-	-	+	-	+	+	+	-	+	-	-	-	-	-	-
065	CU3-11	-	-	-	+	-	-	+	+	+	-	+	-	-	-	+	+	+	+	+	-	+	-	-	-	-	-	-
055	Un10-21	-	-	-	+	+	-	+	+	-	-	+	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-
056	Un10-22	-	-	-	+	+	-	+	+	-	-	+	-	-	-	+	-	+	+	+	-	+	-	-	-	-	-	-
084	CP4-13	-	-	-	+	-	-	+	+	+	-	+	-	-	+	+	-	+	+	+	-	+	-	-	-	-	-	-

Table 10 (Cont.) Phenotypic characteristics of 150 clinical isolates according to the genomic groups.

No.	Characteristics		O-F		Growth	Growth at		Utilization of carbohydrate and amino acid																Acid form				
	Organisms	Oxidase	glucose	Motility	on MAC	41°C	44°C	Arg	His	Ala	Leu	Lac	Tar	Orn	Mal	Suc	Ben	Glut	Cit	Ace	Phe	Mal	Asp	Glu	Mlt	Man	Lac	Suc
090	CP4-12	-	-	-	+	+	-	+	+	+	-	+	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-
131	Un11-27	-	-	-	+	-	-	-	+	-	-	+	-	-	+	+	-	+	-	-	-	+	-	-	-	-	-	-
Group V *A. haemolyticus*																												
070	CP4-5	-	+	-	+	+	+	+	+	+	-	+	-	-	+	+	+	-	+	-	-	+	-	+	-	-	+	+
074	CP5-8	-	+	-	+	+	+	+	+	+	-	+	-	-	-	+	-	+	-	+	-	+	-	+	-	-	-	-
111	CP4-20	-	-	-	+	+	-	+	+	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-
Group VI *Acinetobacter* genospecies 3																												
054	Un3-10	-	+	-	+	+	-	+	+	+	-	+	+	-	-	+	-	+	+	+	-	-	-	+	+	+	+	+
059	Un10-39	-	+	-	+	-	-	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
104	CCSF3-23	-	-	-	+	-	-	+	+	+	-	+	-	-	-	+	-	-	+	+	-	+	-	-	-	-	-	-
Group VII unidentified																												
128	Un10-34	-	+	-	+	+	+	+	+	-	-	+	+	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
105	CP4-23	-	+	-	+	-	-	-	-	+	-	+	-	-	+	+	+	+	+	+	+	+	-	+	-	-	-	-
115	SU13-5	-	+	-	+	+	-	+	+	+	-	+	+	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
008	SU11-15	-	+	-	+	-	-	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
009	SU11-9	-	+	-	+	-	-	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	-	-
010	SU4-3	-	-	-	+	-	-	-	-	-	-	+	-	-	+	+	-	-	-	+	-	+	-	-	-	-	-	-
011	SU13-6	-	+	-	+	-	-	+	+	+	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-

Table 10 (Cont.) Phenotypic characteristics of 150 clinical isolates according to the genomic groups.

No.	Characteristics				Growth	Growth at		Utilization of carbohydrate and amino acid																Acid form				
	Organisms	Oxidase	O-F glucose	Motility	on MAC	41°C	44°C	Arg	His	Ala	Leu	Lac	Tar	Orn	Mal	Suc	Ben	Glut	Cit	Ace	Phe	Mal	Asp	Glu	Mlt	Man	Lac	Suc
042	SP6-18	-	+	-	+	-	-	-	+	-	+	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-
091	CP5-12	-	+	-	+	-	-	-	+	+	-	-	-	-	-	-	-	+	+	+	-	-	-	+	-	-	-	-
099	CP4-18	-	+	-	+	-	-	+	+	+	-	+	-	-	+	+	-	+	+	+	+	+	-	-	-	-	+	-
119	Un10-9	-	+	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	+	-	-	-	-	-	-
123	SU10-30	-	+	-	+	+	-	+	-	-	-	+	-	-	-	-	-	+	-	+	+	+	-	+	-	-	-	-
126	Un10-4	-	+	-	+	+	-	+	+	-	-	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	-
060	Un10-23	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-
061	SP11-4	-	-	-	+	-	-	+	+	-	-	+	-	-	+	+	-	+	+	+	-	+	-	-	-	-	-	-
089	CP4-14	-	-	-	+	-	-	-	+	+	-	+	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-
100	CBF3-6	-	-	-	+	+	+	+	+	-	-	+	-	-	+	+	-	+	+	+	-	+	-	-	-	-	-	-
109	CP3-14	-	-	-	+	+	-	+	+	+	-	+	-	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-
112	CP4-15	-	-	-	+	+	+	+	+	-	-	+	-	-	+	+	+	+	-	+	-	-	-	-	-	-	+	-
127	Un10-24	-	-	-	+	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
138	Un11-31	-	-	-	+	+	-	-	+	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
139	Un11-29	-	-	-	+	-	-	-	+	-	-	+	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-

Table 11 Summary of the general characteristics of all *Acinetobacter* isolates according to the genomic groups.

Characteristics	*A. baumannii* JCM 6841^T	Group I (105)[a]	*A. calcoaceticus* DMST 2270^T	Group II (5)	*A. lwoffii* JCM 6840^T	Group III (2)	*A. junii* DMST 2274^T	Group IV (10)	*A. haemolyticus* DMST 2273^T	Group V (4)	*Acinetobacter* genospecies 3 DMST 2272^T	Group VI (3)	Group VII (21)
Oxidases	-	-	-	-	-	-	-	-	-	-	-	-	-
O-F Glucose	+	+(-4)	+	+	-	-	-	-(+3)	+	+	+	2(-1)	+(-10)
Motility	-	+	-	-	-	-	-	-	-	-	-	-	-
Growth on MAC	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at													
44 °C	+	+(-11)	-	-	-	-	-	-	-	+(-2)	-	-(+1)	-(+3)
41 °C	+	+(-1)	-	-	-	-	+	+(-4)	-	+(-1)	w	-(-2)	-(+8)
37 °C	+	+	+	+	+	+	+	+	+	+	+	+	+
Utilization of													
L-Arginine	+	+(-6)	+	-(+2)	-	-	+	+(-1)	+	+(-1)	+	+	+(-8)
L-Histidine	+	+(-8)	+	+	-	+(-1)	+	+	+	+	+	+	+(-4)
β-Alanine	+	+(-14)	+	+	-	+	-	+(-5)	-	+	+	+	+(-10)
L-Leucine	+	-(+1)	-	-	-	-	-	-	-	-	+	-	-
DL-Lactate	+	+(-2)	+	+	+	+	+	+	-	+(-2)	+	+	+(-4)
L-Tartrate	-	-(+10)	-	+(-1)	-	-	+	-	-	-	+	-(+1)	+(+2)
L-Ornithine	+	-(+2)	-	-	-	-	-	-	-	-	-	-	-

Table 11 (Cont.) Summary of the general characteristics of all *Acinetobacter* isolates according to the genomic groups.

Characteristics	*A. baumannii* JCM 6841^T	Group I (105)[a]	*A. calcoaceticus* DMST 2270^T	Group II (5)	*A. lwoffii* JCM 6840^T	Group III (2)	*A. junii* DMST 2274^T	Group IV (10)	*A. haemolyticus* DMST 2273^T	Group V (4)	*Acinetobacter* genospecies 3 DMST 2272^T	Group VI (3)	Group VII (21)
Malonate	+	+(-24)	-	+(-2)	-	-	-	-(+2)	-	-(+1)	+	-(+1)	+(-9)
Succinate	+	+(-8)	-	+	-	+	-	+(-1)	+	+(-1)	+	+	+(-2)
Benzoate	+	-(+24)	-	-	-	-	-	-(+2)	-	-(+1)	-	-	-(+2)
Glutarate	+(-9)	+(-9)	+	3(-2)	-	+(-1)	-	+(-2)	-	+(-2)	+	+(-1)	+(-6)
Citrate	+	+(-4)	+	+	-	-	-	+(-1)	+	+(-2)		+	+(-7)
Acetate	+	+	+	+(-1)	+	+	-	+(-1)	+	+(-2)		+	+(-2)
L-Phenylalanine	+	+(-52)	+	+(-2)	-	-	-	-(2)	-	-	+	-(+1)	+(-16)
D-Malate	+	+(-5)	+	+(-1)	+	+	+	+(-2)	+	+(-2)	+	+(-1)	+(-6)
Aspartate	-	-	-	-(+1)	-	+	-	-	-	-		-	-
Acid form													
D-Glucose	+	+	+	+(-1)	-	-	-	-	+	+(-1)	+	+(-1)	-(+8)
D-Maltose	-	-(+12)	-	-	-	-	-	-	-	-		-(+1)	-
D-Mannitol	-	-(+11)	-	-	-	-	-	-	-	-		-(+1)	-
Lactose	-	-(+19)	-	-	-	-	-	-	-	-(+1)		-(+1)	+(-12)
Sucroce	-	-(+13)	-	-	-	-	-	-	-	-(+1)		-(+1)	-

[a]Number of tested strains. Number in parentheses indicated the number of strains showing positive or negative reaction.

Table 12 Grouping of *Acinetobacter* species based on phenotypic characteristics and DNA relatedness.

Code	Phenotypic group	DNA group
Group A (73 isolates)		
SU1-1 , SU7-1 , SU7-2 , SU9-3 , SU11-12 , SU1-2 , SU1-4 , SU13-9 , SS5-3 , SS6-5 , SS6-6 , SS6-7 , SS4-2 , SS4-7 , SS4-9 , SS6-3 , SS11-20 , SB7-1 , SB4-1 , SB6-15 , SB7-2 , SB6-13 , SB6-14 , SB7-3 , SP5-6 , SP8-2 , SP8-5 , SP9-9 , SP13-12 , Un3-1 , Un11-6 , Un11-20 , Un12-4 , Un12-5 , CU3-22 , CP3-4 , CP5-1 , CP4-9 , CP5-2 , CP5-3 , CP5-5 , CP3-5 , CP5-10 , CP5-16 , CP5-7 , CP5-9, CP5-13, CU3-21, CP1-5, CP4-27, CBF4-26, CP3-25, CP3-1, CP3-24, CP4-21, CP5-17, SU11-16, SU11-10, SU11-17, CP2-10, CP1-15, SS1-3,CU3-3, Un10-19, Un10-25, CP5-4, SU13-2, SU11-25, SS6-8, SP5-6, Un10-30	A (71)[a]	I (71)[a]
CP4-5	A(1)	V(1)
Un10-34	A(1)	VII(1)
Group B (8 isolates)		
SU11-33	B (1)	II (1)
Un10-35 , SU2-1 , CP3-8 , SS11-19	B(4)	I(4)
Un10-39	B(1)	V(1)
CP4-23 , SU13-5	B (2)	VII(2)
Group C (46 isolates)		
SU11-15 , SU11-9 , SU13-6 , SP6-18 , CP4-18 , SP8-8 , Un10-9 , SS10-30 , Un10-4 , Un10-24 , SP11-3	C(11)	VII(11)
SS4-3 , SB1-3 , SB4-5 , SP1-1 , SP1-2 , SP11-5 , SP3-5 , SP3-6 , Un3-4 , Un11-34 , Un11-36 , Un12-1 , Un10-31 , Un10-5 , CP4-3 , CP4-1 , CP4-2 , CP4-4 , CBF4-25 , CP3-13 Un10-39 , SB1-4 , CP4-10 , SU11-27 , SS4-8 , SS5-8	C(26)	I(26)
Un3-10	C(1)	VI(1)
CP3-20 , CP4-8 , CP4-24 , SB5-4	C(4)	VII(4)

Table 12 (Cont.) Grouping of *Acinetobacter* species based on phenotypic characteristics and DNA relatedness.

Code	Phenotypic group	DNA group
CP5-8 , CP5-12 ,	C(2)	V(2)
SP9-8 , CP3-12 ,	C(2)	IV (2)
Group D (8 isolates)		
CP3-20 , CP3-2	D(2)	III(2)
SS6-4 , CU3-11	D(2)	IV (2)
CCSF3-23	D(2)	VI(1)
Un10-23 , SP11-4 , CP4-14	D(3)	VII(3)
Group E (15 isolates)		
SU4-3 , CBF3-6 , CP3-14 , Un10-23 , Un11-31 , Un11-29	E(6)	VII(6)
Un10-21 , Un10-22 , CP4-13 , CP4-12 , Un11-27	E(5)	IV (5)
CP2-4 , CP4-15 , SU6-22	E(3)	I(3)
CP4-20	E(1)	V(1)

Number in parentheses indicated the number of strains.

Table 13 Identification of *Acinetobacter* strains according to DNA relatedness.

Source / Species	Urine	Sputum	Blood	Pus	Body fluid	CSF	unknown	Total	Group
A.baumannii	19	15	11	42	2	0	16	105	I
A.calcoaceticus	1	0	1	3	0	0	0	5	II
A.lwoffii	0	0	0	2	0	0	0	2	III
A.junii	1	1	0	5	0	0	3	10	IV
A.haemolyticus	0	0	0	4	0	0	0	4	V
Acinetobacter genospecies 3	0	0	0	0	0	1	2	3	VI
unidentified	6	0	0	7	1	0	7	21	VII
Total	27	16	12	63	3	1	28	150	

Table 14 Ubiquinone system of *Acinetobacter* strains.

DNA	Strains	Ubiquinone (%)			
		Q-7	Q-8	Q-9	Q-10
Group I	SU1-1	5.3	14.4	**77.5**	2.8
	SP9-9	2.6	10.4	**82.5**	4.3
	SS4-9		19.1	**79.2**	1.7
	CP5-1	5.0	10.0	**81.8**	3.2
	CP1-5	3.8	10.3	**81.6**	4.1
	CBF4-25	1.0	11.2	**85.4**	2.4
	CP3-25	2.4	11.6	**83.5**	2.5
	SS1-3	6.3	6.2	**83.0**	4.5
	A. baumannii JCM 6841^T	3.2	16.3	**77.6**	2.9
	A. baumannii DMST 2271^T	5.9	20.7	**71.4**	2.0
Group II	CP5-20	6.1	**79.8**	11.0	3.0
	A. calcoaceticus DMST 2270^T	3.8	**87.6**	8.6	
Group III	CP3-20	15.7	5.6	**78.6**	
	A. lwoffii JCM 6840^T	8.2	7.5	**82.8**	1.3
Group IV	CP3-12	3.3	15.4	**79.6**	1.6
	A. junii DMST 2274^T	20.2	31.0	**47.6**	1.0
Group VI	CCSF3-23	3.2	4.8	**89.6**	2.3
Group VII	SU4-3	9.3	**90.7**		
	CP3-14	3.5	**96.5**		
	CP4-23		9.0	**88.1**	2.9

Table 15 Phenotypic chararacteristic of *Acinetobacter* strains in Vitek 32 system.

Characteristics / Organisms	DP3	OFG	GC	ACE	ESC	PLI	URE	CIT	MAL	TDA	PXB	LAC	MLT	MAN	XYL	RAF	SOR	SUC	INO	ADO	COU	H2S	ONP	RHA	ARA	GLU	ARG	LYS	ORN	OXI	TLA	Identification
Group I																																
SU1-1	-	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
SU1-2	-	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	*A.baumannii*
SS6-5	-	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
SS6-7	-	+	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
SB7-3	-	+	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
SP8-2	-	+	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
SP8-5	-	+	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
SP9-9	-	+	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
Un11-20	-	+	+	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
CP4-9	-	+	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
CP3-5	-	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
CU3-21	-	+	+	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
CP1-5	-	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
CP4-27	-	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	*A.baumannii*
CBF4-26	-	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
CP3-25	-	+	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
SU11-12	-	+	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	A*bc* complex
SB6-13	-	+	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	A*bc* complex
CU3-22	-	+	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	A*bc* complex
CP5-2	-	+	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	A*bc* complex
CP5-3	-	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	A*bc* complex

Table 15 (Cont.) Phenotypic chararacteristic of *Acinetobacter* strains in Vitek 32 system.

Characteristics Organisms	DP3	OFG	GC	ACE	ESC	PLI	URE	CIT	MAL	TDA	PXB	LAC	MLT	MAN	XYL	RAF	SOR	SUC	INO	ADO	COU	H2S	ONP	RHA	ARA	GLU	ARG	LYS	ORN	OXI	TLA	Identification
CP4-21	-	+	+	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	A*bc* complex
CP3-24	-	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	A*bc* complex
CP5-5	-	+	+	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	A*bc* complex
SP1-2	-	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
SP11-5	-	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
SP3-5	-	+	+	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
CP4-3	-	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
CP4-4	-	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
CP3-13	-	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	*A.baumannii*
CP4-10	-	+	+	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A*bc* complex
SS11-19	-	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	A*bc* complex
SP3-6	-	+	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	A*bc* complex
CP4-1	-	+	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	A*bc* complex
CP2-4	-	+	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	A*bc* complex
CBF4-25	-	+	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	A*bc* complex
Un10-35	-	+	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	A*bc* complex
SU2-1	-	-	+	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	A*bc* complex
Un10-5	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*A.lwoffii/A.junii*
Group II																																
CP5-20	-	+	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	A*bc* complex
Group III																																
CP3-20	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*A.lwoffii/A.junii*

Table 15 (Cont.) Phenotypic chararacteristic of *Acinetobacter* strains in Vitek 32 system.

Characteristics / Organisms	DP3	OFG	GC	ACE	ESC	PLI	URE	CIT	MAL	TDA	PXB	LAC	MLT	MAN	XYL	RAF	SOR	SUC	INO	ADO	COU	H2S	ONP	RHA	ARA	GLU	ARG	LYS	ORN	OXI	TLA	Identification
Group IV																																
SS6-4	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*A.lwoffii/A.junii*
CU3-11	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	*A.lwoffii/A.junii*
Un10-21	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*A.lwoffii/A.junii*
Group V																																
CP5-12	-	+	+	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	A*bc* complex
Group VI																																
Un10-39	-	+	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	A*bc* complex
CCSF-23	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*A.lwoffii/A.junii*
Group VII																																
CBF3-6	-	+	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	A*bc* complex
CP4-23	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*A.lwoffii/A.junii*
CP4-15	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*A.lwoffii/A.junii*
SU13-5	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*A.lwoffii/A.junii*

Table 16 Identification of *Acinetobacter* base on phenotypic characteristics, DNA relatedness, and Vitek 32 system.

Code	DNA relatedness	Identification: Conventional biochemical tests	Identification: Vitek
Group I (*A.baumannii*)			
SU1-1 , SU1-2 , SS6-5 , SS6-7 , SB7-3 , SP8-2 , SP8-5 , SP9-9 , Un11-20 , CP4-9 , CP3-5 , CU3-21 , CP1-5 , CP4-27 , CBF4-26 , CP3-25 (Total 16 isolates)		*A.baumannii*	*A.baumannii*
SU11-12 , SB6-13 , CU3-22 , CP5-2 , CP5-3 , CP4-21 , CP3-24 , CP5-5 (Total 8 isolates)		*A.baumannii*	A*bc* Complex
SP3-6 , CP4-1 , CP2-4 , CBF4-25 (Total 4 isolates)		*A.calcoaceticus*	A*bc* Complex
SP1-2 , SP11-5 , SP3-5 , CP4-3 , CP4-4 , CP13-3 (Total 6 isolates)		unidentified	*A.baumannii*
CP4-10 , SS11-19 (Total 2 isolates)		unidentified	A*bc* Complex
Un10-35 , SU2-1 (Total 2 isolates)		unidentified	A*bc* Complex
Un10-5 (Total 1 isolates)		*A.baumannii*	*A.lwoffii* / *A.junnii*
Group II (*A. calcoaceticus*)			
CP5-20 (Total 1 isolates)		unidentified	A*bc* Complex
Group III (*A.lwoffii*)			
CP3-20 (Total 1 isolates)		*A.lwoffii*	*A.lwoffii* / *A.junnii*
Group IV (*A.junnii*)			
SS6-4 , CU3-11 (Total 2 isolates)		*A.lwoffii*	*A.lwoffii* / *A.junnii*
Un10-21 (Total 1 isolates)		unidentified	*A.lwoffii* / *A.junnii*
Group V (*A.haemolyticus*)			
CP5-12 (Total 8 isolates)		unidentified	A*bc* Complex
Group VI (*Acinetobacter* genospecies 3)			
Un10-39		*A.calcoaceticus*	A*bc* Complex
CCSF3-23		*A.lwoffii*	*A.lwoffii* / *A.junnii*

Table 16 (Cont.) Identification of *Acinetobacter* base on phenotypic characteristics, DNA relatedness, and Vitek 32 system.

		Identification	
Code	DNA relatedness	Conventional biochemical tests	Vitek
Group VII (Unidentified)			
CBF3-6		unidentified	A*bc* Complex
CP4-23		*A.calcoaceticus*	*A.lwoffii* / *A.junnii*
CP4-15		unidentified	*A.lwoffii* / *A.junnii*
SU13-5		*A.calcoaceticus*	*A.lwoffii* / *A.junnii*

*A*bc* complex; *A. calcoaceticus-A. baumannii* (according to Vitek 32 system).

Table 17 Correlation of the identifcation using the phenotypic tests, DNA-DNA hybridization, and Vitek 32 system.

Species agreement based on method used	No. of isolate (Total 52 isolates)	Correlation (%)
Three methods	17	34%
Phenotypic characteristics and DNA-DNA hybridization	27	54%
Phenotypic tests tests and Vitek 32 system	20	40%
DNA-DNA hybridization and Vitek 32 system	27	54%

CHAPTER V

DISCUSSION

The total of 350 clinical isolates obtained from King Chulalongkorn Memorial Hospital and Siriraj Hospital, 150 isolates were presumptive grouping as *Acinetobacter* species. The presumptive screening tests including TSI, oxidase test, motility, oxidation and fermentation test were done according to Bailey and Scott's Dignostic Microbiology 1994 (36) in order to separate the glucose oxidizing *Acinetobacter* species from the nonoxidizing species. Among these, 127 isolates were glucose oxidizing and 23 isolates were glucose nonoxidizing groups.

The twenty-three tests on the utilization of the carbon sources were carried out , in order to further identify the species of *Acinetobacter* strains. It was clearly shown that all the tests were not significant for separating the *Acinetobacter* species . However, the growth ability at 44 ^{0}C was still useful for differentiating *A . baumannii* strains from the other species. Among the 150 isolates, *Acinetobacter* were separated into five groups on the basis of phenotypic characteristics from conventional biochemical tests. The isolates of Group A (73 isolates) were identified as *A. baumannii* , Group B (8 isolates) belonged to *A. calcoaceticus*, Group C (46 isolates) and Group E (15 isolates) were unidentified while the isolates of Group D (8 isolates) were *A. lwoffii*. The phenotypic characteristics results for *Acinetobacter* isolates showed many variable reactions and had no significant for the differentiation such isolates. The identification of *Acinetobacter* to species level was not possible due to the inconsistency of the phenotypic characteristics. However, a presumptive identification based upon growth at 44 ^{0}C , 41 ^{0}C and 37 ^{0}C along

with the acid production from glucose should be helpful in the identification of *A. baumannii* (20, 102) .

On the basis of DNA relatedness, total of 150 isolates were separated into 7 groups (Group I to VII) when 70% or greater DNA relatedness with the type strains of *Acinetobacter* species were shown (17). Only one group showed low degree homologies with all type strains of *Acinetobacter* species included in this study.

Group I (105 isolates) showed high degree of DNA homologies (over 70.11%) with *A. baumanii* JCM 6841^{T}. They were identified as *A. baumannii.* The most isolates of this group oxidized glucose, grew at 41 ^{0}C and 44 ^{0}C, and utilized malonate, citrate, and malate, and produced acid from D-glucose the same as previuos reported (8, 11, 12). These characteristics should be useful in the separation of *A. baumannii* from the other species. The growth at 41 ^{0}C 44 ^{0}C were confirmed to be useful in the separation of *A. baumannii, A. calcoaceticus* and *Acinetobacter* genospecies 3 as shown in the study by Kampfer et al. (103). Bouvet and Grimont (18) reported that *A. baumannii* (genospecies 2) produced acid from D-glucose and utilized citrate by the most strains while only auxotrophic strains utilized citrate when the medium was supplemented with a growth factor. All tested isolates in this study utilized DL- lactate, glutarate, but did not utilized L- leucine, L-ornithine, and L- aspartate. These results were different from the results of the study by Bouvet and Grimont (8), who showed that all *A. baumannii* had to utilize the three amino acids. In addition, most strains showed the typically biochemical characteristics of *A. baumannii* by the ability to utilize L-phenylalanine, L-histidine, D-malate, L- leucine, L- arginine, and L-ornithine.

The growth at 44 ^{0}C had long been classified as one key characters of *A. baumannii* (8). Surprisingly, 11 isolates (SS4-2, SB4-5, SB1-3, SU2-1, CP4-2,

CP3-8, CP3-13, SS11-19, Un10-39, CP4-10) with DNA relatedness (over 70.58 %) with *A. baumanii* JCM 6841^T and the phenotypic characteristics were similar to the other isolates of this group could not grow at 44 ^{0}C. In addition, eight isolates from the Group I and *A. baumannii* JCM 6841^T had Q-9 as major ubiquinone component which were different from the study by Yokota. et al. (55) who reported that *A. baumannii* ATCC 9955 (ATCC 17961) contained Q-8 as major component. However, the strain in such study was not the type strain and its taxonomic position should be confirmed. In this study, *A. baumannii* was distributed in many clinical specimens particularly in pus. Of 105 isolates 42 isolates were found in pus, 19 in urine, 15 in sputum, 11 in blood, 2 in body fluid, and 16 isolates were from the unknown sources.

Group II consisted of 5 isolates (SU11-33, CP5-20, CP4-8, CP4-24, SB5-4) (Table 9). They had high degree of DNA homologies with *A. calcoaceticus* (over 71.22%) and were identified as *A. calcoaceticus* . They could not grow at 41^{0}C and 44^{0}C which can be differentiated from Group I isolates. This was similar to the previous reports (8, 11). All isolates utilized L-histidine, β-alanine, lactate, succinate, and citrate but did not utilized L-leucine, and L-ornithine. Most of them utilized D-malate which was the same as the result obtaind by Gerner-Smidt et al. (11) but was different from the report done by Bouvet and Grimont (8), who showed that some strains utilized L-tartrate, malonate, glutarate, phenylalanine and acetate. The strain designated as CP5-20 and *A. calcoaceticus* DMST 2270^T had Q-8 as major ubiquinone, while in Yokota. et al., reported that *A. calcoaceticus* IAM 12087 contained Q-9 as major ubiquinone. Then the taxonomic position of strain IAM 12087 should be confirmed. The majority of isolates in Group II were in pus (3 isolates were isolated from pus, 1 isolate from blood and 1 isolate from urine).

Group III isolates (CP3-20 and CP3-2) showed high degree of DNA homologies with *A. lwoffii* JCM 6840^T (over 70.33%) and were identified as *A. lwoffii*. All isolates were nonoxidizer and no growth at 41 ^{0}C. They did not utilized L-arginine, histidine, malonate, and citrate which were distinguishing them from *A. junii* and *A. jonhsonii* as reported by Bouvet and Grimont and Gerner-Smidt et al. (8, 11). In this study, the isolates could utilized L-histidine, β-alanine and glutarate but in the study by Bouvet and Grimont indicated that *A. lwoffii* could not utilize these three amino acids. The strain tested, CP3-20 and *A. lwoffii* JCM 6840^T had Q-9 as a major ubiquinone component which agreed with the result in the report by Yokota et al., (55). All two isolates were from pus .

The isolates of Group IV consisted 10 isolates (SP9-8, CP3-12, SP9-8, SS6-4, CU3-11, Un10-21, Un10-22, CP4-13, CP4-12, Un11-27). They showed high degrees of DNA homology (over 72.08%) with *A. junii* DMST 2274^T and were identified as *A. junii*. All isolates did not produce acid from D-glucose. This group can be differentiated from Group III by the utilization of L-arginine, citrate and growth at 41^{0}C. They utilized citrate, DL-malate and produced acid from D-glucose. In the study by Bouvet and Grimont (8), *A. junii* was differentiated from the other glucose nonoxidizing by the utilizing lactate, and L-histidine and lack of utilization of glutarate, but in the present study the isolates failed to do so. The strain tested, CP3-12 and *A. junii* DMST 2274^T had Q-9 as a major ubiquinone component which disagreed with the result in the report by Yokota et al. (55). Most of the isolates were also from pus (5 isolates), 1 isolate from sputum, 1 isolate from urine, and 3 isolates from unknown..

The isolates of Group V contained 4 isolates (CP4-5, Un10-39, CP4-20, which were from pus. They showed high degree of DNA homologies (over 74.19%) with

A. haemolyticus DMST 2273^T and were identified as *A. haemolyticus*. All isolates oxidized glucose and utilized L-arginine, L-histidine, D-malate, and citrate as reported by Bouvet and Grimont and Gerner-Smidt (8, 10). In this study, the isolates did not utilized L-leucine and L-aspartae, and utilized β-alanine which were different from the results of many other previous studies (8, 11, 18). In addition, all isolates could not hemolyse human red blood cells and few isolates grew at 41 ^{0}C. This was also different from the studies by Bouvet and Grimont, Gerner-Smidt, et al., and Tjernberg and Ursing who reported that all *A. haemolyticus* strains could hemolyzed human and sheep blood.

Group VI contained 3 isolates (Un3-10, Un10-39, CCSF3-23) which were from cerebrospinal fluid (CSF). All isolates showed high degree of DNA homologies (over 81.22%) with *Acinetobacter* genospecies 3 DMST 2272^T and were identified as *Acinetobacter* genospecies 3. In this study, the phenotypic characteristics of them could not be differentiated them from the Group II strains except for the ability of growth at 41^{0}C of few strains. All 3 isolates utilized L-arginine, histidine β-alanine, lactate, succinate, citrate, and acetate which similar to *Acinetobacter* genospecies 3 in the study by as Bouvet and Grimont. The isolates CSF3-23 contained Q-9 as a major ubiquinone while Yogota et al., reported *Acinetobacter* genospecies 3 ATCC 19004 contained Q-8 as a major ubiquinone. Therefore, the investigation of the isolates and the type strain should be reconfirmed.

Group VII (21 isolates) showed low degree of DNA homologies (10.0-69.67%) with all the type strains used including *A. baumannii* JCM 6841^T, *A. calcoaceticus* DMST 2270^T, *A. lwoffii* JCM 6840^T, *A. junii* DMST 2274^T, *A. haemolyticus* DMST 2273^T, *Acinetobacter* genospecies 3 DMST 2272^T, *Acinetobacter* genospecies 6 DMST 2272^T, and *A. radioresistens* JCM 9326^T. The

isolates SU4-3, CP3-14 had Q-8 as major ubiquinone, while CP4-23 had Q-9 as a major component. The major sources of this species were pus (7 isolates), urine (6 isolates), body fluid (1 isolates) and unkown (7 isolates).

In this study, the isolates belonged to the genus *Acinetobacter* could be separated into 7 groups. They were clearly identified as *A. baumannii* (105 isolates), *A. calcoaceticus, A. lwoffii , A. junii , A. haemolyticus,* and *Acinetobacter* genospecies 3 based on the DNA similarity. The remaining one group could not be identified. The DNA relatedness was useful for the differentiation of the *Acinetobacter* strains as previous studied by Bouvet and Grimont (8) which 12 DNA groups of *Acinetobacter* as *A. baumannii , A. haemolyticus, A. johnsonii, A. junii, A. calcoaceticus, A. lwoffii, Acinetobacter* genospecies 3 , 6, 9, 10, 11 and 12 were identified. In 1988, Nishimura et al.(32) identified the *Acinetobacter* genospecies 12 from cotton and soil as *A. radioresistens*. Tjernberg and Ursing (18) confirmed the remaining isolated reported by Bouvet and Grimont and proposed the new group of *Acinetobacter* genospecies 13, 14 and 15. Fox et al., and Stackebrandt and Goebel (78, 104) reported the resolution of 16S rRNA sequence analysis was insufficient to distinguish closely related genomic species and can not replace the DNA-DNA hybridization method. As mentioned above, the DNA-DNA hybridization was significant for the separation of *Acinetobacter* species. and the photobiotin labelling method is simple for studying the DNA similarity.

In the identification of 52 isolated selected from the 7 DNA groups, using the Vitek 32 system. The result shown in Table 15, that 22 isolates were identified as *A. baumannii* that agreed with the DNA relatedness results. However, the Vitek 32 system also provided different results from DNA-DNA hybridization because among the eleven isolates identified as *A. lwoffii* / *A. junii* by Vitek 32 system, 3 isolates

(Un10-35, SU2-1 and UN10-5) were *A. baumannii* based on DNA relatedness. Nineteen isolates were *A. calcoaceticus* - *A. baumannii* complex (A*bc* complex) by Vitek 32 system but 15 from 19 isolates were *A. baumannii* on the basis of DNA homology and the others, 1 was *A. calcoaceticus*, 1 as *A. haemolyticus* and 1 as *Acinetobacter* genospecies 3, and 1 as unidentified based on DNA relatedness.

From the results previously mentioned , it was indicated that the use of the Vitek 32 system alone could misidentify genus *Acinetobacter* at the species level when comparing to the identification by DNA-DNA hybridization. The Vitek 32 system would be more useful when the additional tests such as the growth at 44 ^{o}C for separating *A. baumannii* from A*bc* complex and the growth at 41^{o}C and citrate utilization for separating between *A. lwoffii* and *A. junii* were performed. The previous investigators showed that none of the isolates were correctly identified to the species level at initial testing by Vitek 32 system, especially, *A. lwoffii* (48, 47, 53). However, Vitek 32 system are simplifly and rapid but required additional testing for identification of *Acinetobater* species. The type of major ubiquinone from *Acinetobacter* species in different genomic species was still inconclusivable because of the results obtained were insimilar to those from previous studies. Therefore, more isolates showed be included in the ubiquinone study in order to give more informations on the postselecty of using this component in classification of *Acinetobacter* species.

Thus, the reliability of DNA -DNA hybridization for identification of *Acinetobacter* species may be superior to study of the phenotypic characteristics using conventional biochemical tests and Vitek 32 system. The photobiotin labelling DNA-DNA hybridization in the microdilution wells has some advantages over the other techniques. It is possible to automated the procedure and the

microdilution plates on which reference DNAs are fixed can be stored in a desiccator at room temperature. The microdilution plates are prepared in which the reference DNAs of medically important bacteria are fixed under dry condition . When an unknown organism is isolated, its DNA is extracted from 1 to 3 ml of an overnight culture broth by using a small- scale DNA extraction method (69). The DNA is labelled with photobiotin, and hybridization is carried out to identify the most closely related organism among the organisms whose reference DNAs are fixed in microdilution wells (69, 76). This method is used successfully for identification of clinical important human bacteria , and for identification of strain in the *A. calcoaceticus* - *A. baumannii* complex that could not be identified.

However, there are *Acinetobacter* genospecies that have no specific epithets as any species which may not be possible for a clinical bacteriology laboratory to carry out the DNA -DNA hybridization techniques for their identification. The need for the presumptive tests such as growth at 44 ^{0}C, 41 ^{0}C and 37 ^{0}C along with the acid production from glucose and utilization of carbon source tests would help in the identification of *Acinetobacter* species for the nosocomial pathogen especially in the compromised patients and intensive care units.

CHAPTER VI

CONCLUSION

On the basis of the phenotypic characteristics (TSI, oxidase test, motility, oxidatation and fermentation test, growth at 41 ^{0}C, 44 ^{0}C, utilization of malonate, succinate and produce acid from D-glucose), 150 isolates were separated into five groups. The isolates in Group A contained 73 isolates were identified as *A. baumannii*, 8 isolates belonged to Group B were *A. calcoaceticus*. The isolates in Group C and E contained 46 and 15 isolates, respectively and they were left unidentified. The isolates in Group D contained 8 isolates were *A. lwoffii*. The differentiation of *Acinetobacter* based on presumptive phenotypic characteristics were not clearly discriminating to species level.

On the basis of DNA relatedness, total 150 isolates were separated into 7 groups. The isolates of Group I (105 isolates) showed a high degree of homologies (over 70.11%) with *A. baumannii* JCM 6841^T. They were identified as *A. buamannii*. The isolates of Group II consisted of 8 isolates, showed a high degrees of homologies (over71.22%)with *A. calcoaceticus* DMST 2270^T. They were identified as *A. calcoaceticus*. The isolates of Group III, 2 isolates showed a high degrees of homologies (over 70.33%) with *A. lwoffii* JCM 6840^T. They were identified as *A. lwoffii*. Group IV contained 10 isolates, showed a high degree of homologies (over 72.08%) with *A. junii* DMST 2274^T and they were identified as *A. junii*. The 4 isolates which belonged to Group V, showed a high degrees of homologies (over 74.19%) with *A. haemolyticus* DMST 2273^T and they were identified as *A. haemolyticus*. Group VI contained 3 isolates, showed high degrees of homology

(over 81.22%) with *Acinetobacter* genospecies 3 DMST 2272^T and they were identified as *Acinetobacter* genospecies 3. The isolate in Group VII contained 21 isolated, showed low degrees of homology (10.0 to 69.67%) with *A. baumannii* JCM 6841^T, *A. calcoaceticus* DMST 2270^T, *A. lwoffii* JCM 6840^T, *A. junii* DMST 2274^T, *A. haemolyticus* DMST 2273^T, *A. johnsonii* DMST 2276, *Acinetobacter* genospecies 3 DMST 2272^T *Acinetobacter* genospecies 6 DMST 2275^T, and *A. radioresistens* JCM 9326^T. This group was left unidentified.

Among the15 isolates select for the ubiquinone component determination. The isolates in Group I, II, IV, and VI had Q-9 as a major ubiquinone, while Group II had Q-8 as a major component. The isolates of Group VII contained ubiquinone with Q-8 or Q-9 as a major component.

In the identification of 52 isolated selected from the 7 DNA groups, using the Vitek 32 system, 22 isolates were identified as *A. baumannii* that agreed with the DNA relatedness results. Eleven isolates were *A. lwoffii* / *A. junii* but 3 isolates (Un10-35, SU2-1 and UN10-5) were *A. baumannii* based on DNA relatedness. Nineteen isolates were *A. calcoaceticus* - *A. baumannii* complex (A*bc* complex) but 15 isolates were *A. baumannii* while the others, 1 was *A. calcoaceticus*, 1 as *A. haemolyticus* and 1 as *Acinetobacter* genospecies 3, and 1 was unidentified based on DNA relatedness.

The reliability of DNA-DNA hybridization for the identification of *Acinetobacter* species may be superior to the study of the phenotypic characteristics by both conventional biochemical tests and Vitek 32 system. However, the presumtive phenotypic characteistics such as the growth at 44 ^{0}C and glucose oxidation should be helpful in the routine identification of *Acinetobacter* species.

From non-fermentative gram-negative rods in the clinical specimen. *A. baumannii* was showed to be the species most prevalent among the nonfermentative bacteria isolated from clinical specimens (70.0%). The other species found included *A. calcoaceticus* (3.33%), *A. lwoffii* (1.33%), *A. junii* (6.66%), *A. haemolyticus* (2.66%), *Acinetobacter* genospecies 3 (2.0%), and unidentified (14.0%).

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APPENDICES

APPENDIX I

Media , Chemical agents , Materials, Instruments and Regents

A. Media

1. Trypticase soy broth

Casein peptone	17.0 g.
Soy peptone	3.0 g.
Glucose (dextrose)	2.5 g.
Sodium chloride	5.0 g.
Dipotassium phosphate	2.5 g.
Distilled water	1000.0 g.

2. Trypticase soy agar

Casein peptone	15.0 g
Soy peptone	5.0 g.
Sodium cholride	5.0 g.
Agar	15.0 g
Distilled water	1000.0 g.

3. Nutrient agar

Meat (beef) extract	10.0 g.
Peptone	10.0 g.
Sodium chloride	5.0 g.
Nutrient agar	15.0 g.

4. Tryptose blood agar base

Bacto tryptose	10.0 g.
Bacto beef extract	3.0 g.
Sodium chloride	5.0 g.
Bacto agar	15.0 g.
Distilled water	950.0 g.

Media preparation

All of ingredients were dissolved in distilled water and then steriled by autoclaving at 121 ^{0}C, 15 pounds/inch^{2} pressure, for 15 minutes. The steriled medium was cooled to 55 ^{0}C, and dispensed into sterile plates or tubes. For sterile tryptose blood agar base medium, 50 ml of sterile defibrinated blood was added aseptically into sterile plates before dispension.

5. Tryple sugar iron agar (TSI)

Meat extract	3.0 g.
Yeast extract	3.0 g.
Peptone	20.0 g.
Glucose	1.0 g.
Lactose	10.0 g.
Sucrose	10.0 g.
$FeSO_4$ $7H_2O$	0.2 g.
NaCl	5.0 g.
$Na_2S_2O_3 5H_2O$	0.3 g.
Agar	15.0 g.

Distilled water	1000.0 ml.
Phenol red 0.2% aq. Solution	12.0 ml.

All of ingredients except indicator were heated to dissolve the solids in distilled water. The indicator solution was added and then mixed and dispensed into tubes. The tubes were steriled by autoclaving at 115 ^{0}C , 15 pounds/inch^2 , for 20 minutes and then cooled to form slopes with deep butts abouts 3 cm. along.

7. O-F carbohydrate base medium

7.1 10% aqueous carbohydrate solution (glucose and lactose)

Carbohydrate (glucose and lactose) 2.0 g. was added into distilled water 20 ml. And then immediately steriled by passing them through a 0.2 μm membrane filter.

7.2 O-F carbohydrate base medium

Peptone or tryptone	2.0 g.
Sodium chloride	5.0 g.
Agar	2.0 g.
Dipotassium phosphate	0.3 g.
Bromthymol blue	0.03 g.
Distilled water	1000.0 ml.

All of ingredients were dissolved in distilled water and divided the solution into several smaller flasks with known volume and then steriled by autoclaving at 121 ^{0}C, 15 pounds/inch^2 pressure, for 15 minutes. The steriled medium was cooled to 55 ^{0}C.

Twenty ml of the sterile 10% carbohydrate solution was added aseptically into the flask of O-F base. The O-F carbohydrate base medium was dispensed into 16-

125 mm. Screw cap test tubes. The tube was allowed to solidify upright, tighten caps, and refrigerated.

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B. Chemical agents

EDTA (Amresco , USA)

Tris (Wako, Japan)

Sodium dodecyl sulfate (Fluka, Swizerland)

Phenol (Carlo, USA)

Chloroform (Mallinckrodt, USA)

Acetone (Merck, Germany)

Methanol (Merck, Germany)

Ethanol (Carlo, USA)

Sodium chloride (Carlo, USA)

Sodium citrate (Cica Kanto, Japan)

di- Potassium hydrogen orthophosphatate (Carlo, USA)

di-Sodium hydrogen phosphate (M&B, England)

L-arginine monohydrochloride (Fluka, Swizerland)

Sodium succinate (BDH Chemical, England)

Sodium tartrate (BDH Chemical, England)

L- histidine (Merck, Germany)

DL-leucine (BDH Chemical, England)

Sodium benzoate (BDH Chemical, England)

L-ornithine monohydrochloride (BDH Chemical, England)

L- phenylalanine (Sigma, USA)

β- alanine (Sigma, USA)

Sodium malonate (BDH Chemical, England)

L-glutamic acid sodium salt (BDH Chemical, England)

L-aspartate acid monosodium salt hydrate (Sigma, USA)

Malic acid sodium salt (Sigma, USA)

N, N, N', N'-tetramethyl-1, 4-phenylene diamine dihydrochloride (Fluka, Swizerland)

Sulfuric acid (Mallinckrodt, USA.)

Bovine serum albumin (Fraction V) (Sigma, USA)

Polyrinylpyrolidone (Fluka, Swizerland)

Triton X-100 (J.T. Baker, USA)

Deoxyribonucleic acid sodium salt fibrous from almon spermary (Wako, Japan)

Formamide (Carlo, USA)

Dextrane sulfate (Sigma, USA)

Citric acid (Ajax, Australia)

di-Sodium Hydrogen Phosphate (Carlo, USA)

N,N, dimethylformamide (Ajax, Australia)

3,3',5,5'Tetramethylbenzidine (Wako, Japan)

Hydrogen peroxide (Carlo, USA)

Ficoll 400 (Sigma , USA)

Streptavidin-POD conjugate (Bochringer Mannheim , Germany)

Rnase A (Sigma , USA)

Photobiotin (Vector, USA.)

Magnesium Choride (Carlo, USA)

n-Butanol (J.T. Baker, USA)

D-glucose (Difco, USA)

Sucrose (Merck, Germany)

D-mannitol (Merck, Germany)

Maltose (Sigma , USA)

Lactose (Difco, USA)

C. Materials

11x3 mm. , test tube (Pyrex , USA)

Eppendorf (Axygen)

Microdilution plates (Nunc Corp , Denmark)

Petridish (Anumbra)

Pipette Measuring

Beaker (Pyrex , USA)

Glass rod

Centrifuge Tube round bottum

D. Instrument

Incubator (Memmert , Germany)

Shaker

Mix Vortex (Scientifix , USA)

Refrigerator centifuge (4 ^{0}C) (Sigma , USA)

Microautometic pipette, p20 / p200 / p1000 (Gilson Medical Electronic , France)

pH meter (Beckman , USA)

Sunlamp (500w)

UV-Spectrophotometer

Microplate Reader Model 3550 (Bio-Rad , CA.,USA)

Vitek 32 Automatic System (bioMericur Vitek,Inc.)

E. Reagents

1. Saline-EDTA (0.15 M NaCl + 0.1 M EDTA)

NaCl	8.76 g
EDTA	37.22 g

NaCl and EDTA were dissolved in 1000 ml ultra pure water and adjusted the pH to 8.0 by adding 1 N HCL and then steriled by autoclaving at 121^0C , 15 pounds / inch pressure , for 15 minutes.

2. Phosphate-buffer saline (PBS)

NaCl	8.00 g.
KCl	0.20 g.
KH_2PO4	0.12 g.
Na_2HOP_4 (anhydrous)	0.91 g.
Distilled water	1000 ml.

Steriled by autoclaving at 121^0C , 15 pounds / inch2 pressure , for 15 minutes

3. 20 x SSC (20 x standard saline citrate)

NaCl	17.5 g
Sodium citrate	8.8 g
Distilled water	100 ml

Adjusted the pH to 7.0 and steriled by autoclaving at 121^0C 15 pounds / $inch^2$ pressure , for 15 minutes

4. 100 x Denhardt solution

Bovine serum albumin (Fraction V)	2 g
Polyvinylpyrrolidone	2 g
Ficoll 400	2 ml

Dissolved in 100 ml ultral pure water and was stored at 4 ^{0}C untill used.

5. Salmon sperm DNA

Salmon sperm DNA 10 mg per ml

Salmon sperm DNA 10 mg was dissolved in 10 mM Tris + EDTA buffer pH 7.6 volume 1 ml, boiled for 10 minutes and then immediately cooled in ice. Sonicated salmon sperm DNA solution for 3 min and was stored at 4 ^{0}C untill used.

6.Prehybridization solution

100 x Denhardt solution	2 ml
10 mg / ml Salmon sperm DNA	1 ml
20 x SCC	10 ml
Formamide	50 ml
Distilled water	34 ml

All of ingredients were dissolved in ultra pure water steriled and kept at 4 ^{0}C.

7. Hybridization solution

Prehybridization	100 ml
Dextran sulfate	5 g

Dissolved Dextran sulfate in Prehybridization solution and keep at 4 ^{0}C

8. Solution 1

Bovine serum albumin (Fraction V)	0.25 g
Triton X – 100	50 µl
PBS	50 ml

All of ingredients were mixed and keep at 4 ^{0}C

9. Solution 2

Streptavidin - POD conjugate	1 µl
Solution 1	4 ml

Dissolve Streptavidin -POD conjugate in solution 1 before used. The solution 2 was freshly prepared.

10. Solution 3

3 , 3$^{/}$, 5 , 5$^{/}$ Tetramenthylbenzidine (TMB)	
(10 mg/ ml in DMFO)	100 ml
0.3 % $H_2 O_2$	100 µl
0.1M citric + 0.2 M $Na_2 HPO_4$ buffer pH 6.2	
in 10 % DMFO	5 ml

All of ingredients were mixed and used. The Solution 3 was freshly prepared.

APPENDIX II

Table 18 Clinical isolates included in this study

Isolate no.	Code	Source
Siriraj Hospital		
001	SU1-1	Urine
002	SU7-1	Urine
003	SU7-2	Urine
004	SU9-3	Urine
005	SU11-12	Urine
006	SU1-2	Urine
007	SU1-4	Urine
008	SU11-15	Urine
009	SU11-9	Urine
010	SU4-3	Urine
011	SU13-6	Urine
012	SU13-9	Urine
013	SS5-3	Sputum
014	SS6-5	Sputum
015	SS6-6	Sputum
016	SS6-7	Sputum
017	SS4-2	Sputum
018	SS4-7	Sputum
019	SS6-4	Sputum
020	SS4-9	Sputum
021	SS6-3	Sputum
022	SS4-3	Sputum
023	SS11-20	Sputum
024	SB7-1	Blood
025	SB4-1	Blood
026	SB6-15	Blood
027	SB1-3	Blood
028	SB4-5	Blood
029	SB7-2	Blood
030	SB6-13	Blood
031	SB6-14	Blood
032	SB7-3	Blood

Table 18 (Cont.) Clinical isolates included in this study.

Isolate no.	Code	Source
Siriraj Hospital		
033	SP5-6	Pus
034	SP8-2	Pus
035	SP8-5	Pus
036	SP9-9	Pus
037	SP1-1	Pus
038	SP1-2	Pus
039	SP9-8	Pus
040	SP13-12	Pus
041	SP11-5	Pus
042	SP6-18	Pus
043	SP3-5	Pus
044	SP3-6	Pus
045	Un3-4	Unknown
046	Un3-1	Unknown
047	Un11-6	Unknown
048	Un11-20	Unknown
049	Un11-34	Unknown
050	Un11-36	Unknown
051	Un12-1	Unknown
052	Un12-4	Unknown
053	Un12-5	Unknown
054	Un3-10	Unknown
055	Un10-21	Unknown
056	Un10-22	Unknown
057	Un10-31	Unknown
058	Un10-35	Unknown
059	Un10-39	Unknown
060	Un10-23	Unknown
061	SP11-4	Pus
062	Un10-5	Unknown
063	SU2-1	Foley
108	SP9-8	Pus
115	SU13-7	Urine
116	SS11-19	Sputum
117	SU11-16	Urine

Table 18 (Cont.) Clinical isolates included in this study.

Isolate no.	Code	Source
Siriraj Hospital		
118	SU11-10	Urine
119	Un10-9	Unknown
120	SU6-22	Urine
121	SS11-19	Sputum
122	SU11-16	Urine
123	SU10-30	Urine
126	Un10-4	Unknown
127	Un10-24	Unknown
128	Un10-34	Unknown
129	Un10-39	Unknown
130	SP11-3	Pus
131	Un11-27	Unknown
132	SS1-3	Sputum
136	Un10-19	Unknown
137	Un10-25	Unknown
138	Un11-31	Unknown
139	Un11-29	Unknown
141	SU13-2	Urine
142	SU11-25	Urine
143	SU11-27	Urine
144	SU11-33	Urine
145	SS4-8	Sputum
146	SS5-8	Sputum
147	SP6-8	Pus
148	SP5-6	Pus
149	SB5-4	Blood
150	Un10-30	Unknown
King Chulalongkorn Memorial Hospital		
064	CU3-22	Urine
065	CU3-11	Urine
066	CP3-4	Pus
067	CP5-1	Pus
068	CP5-20	Pus
069	CP4-9	Pus
070	CP4-5	Pus

Table 18 (Cont.) Clinical isolates included in this study.

Isolate no.	Code	Source
King Chulalongkorn Memorial Hospital		
071	CP5-2	Pus
072	CP5-3	Pus
073	CP5-5	Pus
074	CP5-8	Pus
075	CP3-5	Pus
076	CP5-10	Pus
077	CP5-16	Pus
078	CP5-7	Pus
079	CP5-9	Pus
080	CP4-3	Pus
081	CP5-13	Pus
082	CP4-1	Pus
083	CP4-2	Pus
084	CP4-13	Pus
085	CP2-4	Pus
086	CP3-8	Pus
087	CP4-4	Pus
088	CP3-20	Pus
089	CP4-14	Pus
090	CP4-12	Pus
091	CP5-12	Pus
092	CP4-8	Pus
093	CU3-21	Urine
094	CP1-5	Pus
095	CP4-27	Pus
096	CP4-24	Pus
097	CBF4-26	Body fluid
098	CBF4-25	Body fluid
099	CP4-18	Pus
100	CBF4-6	Body fluid
101	CP3-25	Pus
102	CP3-12	Pus
103	CP3-1	Pus
104	CCSF3-23	CSF
105	CP4-23	Pus

Table 18 (Cont.) Clinical isolates included in this study.

Isolate no.	Code	Source
King Chulalongkorn Memorial Hospital		
106	CP3-24	Pus
107	CP4-21	Pus
109	CP3-14	Pus
110	CP5-17	Pus
111	CP4-20	Pus
112	CP4-15	Pus
113	CP3-13	Pus
114	CP3-2	Pus
124	CP2-10	Pus
125	CP1-15	Pus
133	SB1-4	Blood
134	CP4-10	Pus
135	CU3-3	Urine
140	CP5-4	Pus

BIOGRAPHY

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