

A. baumannii β -Lactam

2002 8

A cinetobacter
baumannii β -Lactam

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**β -Lactam Antimicrobial Resistance of
Acinetobacter baumannii Isolated
from Clinical Specimens**

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ABSTRACT

Acinetobacter baumannii strains were isolated from clinical specimens at the D. university hospital from January, 2001 to December, 2001. The strains which showed the antibiotic resistance against all the antibiotics tested were subjected to the EDTA double disk synergy test and the modified Hodge test in order to identify the activity of metallo- β -lactamase. The strains showing the positive in those test were examined by polymerase chain reaction (PCR) to verify the mechanism of drug resistance. The results were summarized as follows:

1. The frequency of *Acinetobacter baumannii* isolated was 8.3%, which also showed *E. cloacae* (8.6%), *E. coli* (11.9%), *S. aureus* (15.4%) and *P. aeruginosa* (20.0%).
2. *Acinetobacter baumannii* isolated was from the sputum (43.2%),

and the rest was from pus (32.6%), urine (14.1%), and blood (5.7%).

3. The places of *Acinetobacter baumannii* isolated were Intensive Care Unit (32.8%), Internal Medicine ward (18.1%), Surgery ward (7.3%), Urology ward (4.7%), and Nursery (4.7%).
4. The 9.04 percent of *Acinetobacter baumannii* isolated the resistance against imipenem and others showed multi-drug resistance.
5. Among the 10 strains showing resistance against all the drug used, 3 strains were positive in the EDTA double disk synergy test and 2 strains were positive in the modified Hodge test.
6. The amplification of VIM-2 gene by the PCR using the strains showing the positive in the EDTA double disk synergy test and the modified Hodge test.

Conclusively. it is desirable to introduce the simple method like EDTA double disk synergy test or modified Hodge test to remove the important causative organism of hospital infection having drug resistance isolated frequently as *Acinetobacter baumannii*.

Acinetobacter baumannii

가

(17,18).

(29).

A. baumannii

가

가

가

ventilator

(4,23).

가

가

가

가

가

(24).

metallo-

β -lactamase

imipenem

Pseudomonas

aeruginosa(19)

*Serratia marcescens*가

(8)

carbapenem

가

A. cinetobacter baumannii

·
Metallo-β-lactamase β-lactamase 가
가 , penicillin, cephalosporin, cephamycin
carbapenem 가 (7).
carbapenem β-lactam

metallo-β-lactamase IMP 1-8 VIM 1-3 family
11가 (16). Metallo-β-lactamase plasmid

A. baumannii imipenem 가

·
D · *A. baumannii*
β-lactam

1.

2001 1 1 2001 12 31 1 D
가

A cinetobacter baumannii

2.

가

5% Sheep Blood Agar() MacConkey agar(OXOID LTD.,
BASINGSTOKE, HAMPSHIRE, ENGLAND) (pus)
(fluid) Thioglycollate broth(OXOID LTD., BASINGSTOKE,
HAMPSHIRE, ENGLAND) 35°C

18 Gram

MacConkey agar Gram positive Gram
negative 5% Sheep Blood Agar

1.8Mℓ 0.45%

McFarland No. 1.0 200μℓ

Vitek
system (BioMerieux Vitek Inc, Hazelwood, Mo., U.S.A) Gram
Negative Identification (GNI) card

3.

1)

Bauer(2) NCCLS(20)(National Committee for Clinical Laboratory Standards)

Amikacin, Ampicillin, Cefazolin, Cefotetan, Ceftriaxone, Ciprofloxacin, Gentamicin, Imipenem, Piperacillin, Tobramycin, Trimeth/Sulfamethoxazole(SXT) (BBL, Cockeysville, Mi., U.S.A) (Tryptic soy broth) McFarland No. 0.5 Kirby-Bauer Mueller-Hinton agar (OXOID LTD., BASINGSTOKE, HAMPSHIRE, ENGLAND)

35°C

18

Caliper mm

2) Vitek GNS-LH card

가

5% Sheep Blood Agar

1.8Mℓ 0.45%

McFarland No. 0.5

200μℓ

1.8Mℓ 0.45%

Gram

Negative Susceptibility (GNS-LH) card

Vitek system

3) (minimal inhibitory concentration)
 MacConkey agar
 McFarland No. 0.5
 Steers replicator (Craft Machine, Chester, PA., USA)
 가 0.06- 128 $\mu\text{g}/\text{Ml}$ Mueller-Hinton
 agar . 35°C 18
 NCCLS (21) (MIC)
 . *E. coli* ATCC 25922

amikacin, amoxicillin/clavulanic acid, ampicillin, ce-
 fotaxime, cefoxitin, ceftazidime, ciprofloxacin, gentamicin, imipenem,
 piperacillin-tazobactam, ticarcillin, ticarcillin/clavulanic acid, tobra-
 mycin trimethprim/sulfamethoxazole .

4. Metallo- β -lactamase

Lee (15)
 EDTA double disk synergy . ,
 Trypticae soy broth(TSB)
 McFarland No. 0.5 .
 Mueller-Hinton agar EDTA (5 μg)
 imipenem (10 μg , BBL) meropenem (10 μg , BBL) 가
 1.0cm가 . 35°C

5. Carbapenemase

Modified Hodge

test(15) Mueller-Hinton agar *E. coli* ATCC 25922

McFaland No. 0.5

imipenem (10 μ g, BBL)

. positive control negative control 가

control Y 35 $^{\circ}$ C

18

가

6. (PCR) VIM gene

1) DNA

tube 500 μ l TE
 buffer, 30 μ l 10% SDS, 20mg/ml 3 μ l proteinase K 가 37 $^{\circ}$ C
 1 . 100 μ l 5M NaCl 80 μ l
 CTAB/NaCl 65 $^{\circ}$ C 10 .
 chloroform/isoamyl alcohol 4 $^{\circ}$ C 5 12,000 rpm
 . phenol/chloroform/iso-
 amyl alcohol 가 12,000 rpm 5 0.6
 volume isopropanol DNA가 가
 . 70% 1ml washing 5 4 $^{\circ}$ C
 12,000 rpm 100 μ l TE buffer -70 $^{\circ}$ C

2) PCR

10 \times PCR buffer 2.5 μ l, 50ng/ μ l DNA 1 μ l, 25mM MgCl₂ 2.5 μ l,
 2.5mM dNTP(Takara Shuzo Co., Shiga, Japan) 2 μ l, VIM-F(5'-ATT
 GGT CTA TTT GAC CGC GTC-3'), VIM-R (5'-TGC TAC TCA
 ACG ACT GAG CG-3') primer (20pmol) 1 μ l(Table 9), *Taq*
 polymerase (promega) 0.5 unit 25 μ l가
 . Perkin Elmer 9600 94 $^{\circ}$ C 5
 94 $^{\circ}$ C 20 , 52 $^{\circ}$ C 20 , 72 $^{\circ}$ C 45 30
 72 $^{\circ}$ C 10 .

4)

PCR 8 μ l ethidium bromide 0.5 μ g/Ml 가 2% agarose
gel 20 UV transilluminator

1. *A. baumannii*

509 *A. baumannii*가 P.
aeruginosa, *S. aureus*, *E. coli*, *E. cloacae* 5
 1999 433 가 5 2000 446 가
 4 가 (Table 1).
 가 220 (43.2%)
 166 (32.6%), 72 (14.1%), 29 (5.7%)
 (Table 2). 가 167
 (32.8%) 가 41 (8.1%),
 37 (7.3%), 24 (4.7%), 24
 (4.7%) (Table 3). 1999 가
 , , ,
 . 2000

509 penicillin ampicillin
 cephalosporin 1, 2 cephalothin cefoxitine 100%
 , carbapenem imipenem 가 46
 (9.04%) (Table 4). 1999 (Table 5) 2000
 (Table 6) ampicillin cephalothin 100%
 , imipenem 가 18 (4.0%), 17 (4.0%)
 가 (Fig 1). (MIC)
A. baumannii imipenem MIC 16µg/Mℓ
 penicillin piperacillin ticarcillin 128µg/Mℓ, cephalosporin
 MIC 32µg/Mℓ 64µg/Mℓ Aminoglycoside

amikacin 64 $\mu\text{g}/\text{Ml}$, Gentamicin 16 $\mu\text{g}/\text{Ml}$, tobramycin 16 $\mu\text{g}/\text{Ml}$
(Table 7).

Table 1. The frequency of bacteria isolated from clinical specimens

Strains	'1999		'2000		'2001	
	Count	%	Count	%	Count	%
<i>P. aeruginosa</i>	1,160	19.0	1,057	19.6	1,233	20.0
<i>S. aureus</i>	972	15.9	771	14.3	950	15.4
<i>E. coli</i>	890	14.6	685	12.7	729	11.9
<i>E. cloacae</i>	417	6.8	377	7.0	531	8.6
<i>A. baumannii</i>	433	7.1	446	8.3	509	8.3
CNS*	158	2.6	217	4.0	317	5.2
<i>Kl. pneumoniae</i>	449	7.4	402	7.5	293	4.8
<i>S. epidermidis</i>	282	4.6	257	4.8	194	3.6
<i>E. faecalis</i>	89	1.4	156	2.9	180	2.9
<i>B. cepacia</i>	93	1.5	105	2.0	145	2.4
<i>S. marcescens</i>	102	1.7	101	1.9	144	2.3
<i>P. mirabilis</i>	97	1.6	96	1.8	125	2.0
Others	866	14.2	696	12.9	806	13.1
Total	6,106	100.0	5,386	100.0	6,156	100.0

*CNS: Coagulase Negative Staphylococcus

Table 2. The specimens of *Acinetobacter baumannii* isolated

Specimen	'1999		'2000		'2001	
	Isolate No.	%	Isolate No.	%	Isolate No.	%
Sputum	191	44.1	207	46.4	220	43.2
Pus	144	33.3	136	30.5	166	32.6
Urine	45	10.4	54	12.1	72	14.1
Blood	22	5.1	21	4.7	29	5.7
Bile	5	1.2	4	0.9	8	1.6
Fluid	18	4.2	19	4.3	8	1.6
Stool	8	1.8	5	1.1	6	1.2
Total	433	100	446	100	509	100

Table 3. The places of *A cinetobacter baumannii* isolated

Location	'1999		'2000		'2001	
	Isolate No.	%	Isolate No.	%	Isolate No.	%
ICU	129	29.8	103	23.1	167	32.8
IM	34	7.9	50	11.2	41	8.1
ER	14	3.2	7	1.6	41	8.1
GS	32	7.4	9	2.0	37	7.3
UR	11	2.5	15	3.4	24	4.7
NR	30	6.9	42	9.4	24	4.7
others	183	42.3	220	49.2	175	34.4
Total	433	100	446	100	509	100

ICU: Intensive Care Unit, IM: Internal Medicine, NR: Nursery

ER: Emergency Room GS: General Surgery UR: Urology

Table 4. Antimicrobial resistances of *A. baumannii* isolated from clinical specimen in 2001

Antimicrobial agents	%Resistance	Antimicrobial agents	%Resistance
Amikacin	74	Cephalothin	100
Ampicillin	100	Ciprofloxacin	69
Ampicillin/ Sulbactam	59	Gentamicin	77
Aztreonam	95	Imipenem	9
Cefoxitine	100	Ofloxacin	69
Cefepime	75	Piperacillin	77
Cefotaxime	80	Piperacillin/tazobact	64
Cefotetan	99	Ticarcillin/ Clavulanic	
Cefoxitin	99	acid	31
Ceftazidime	72	Tobramycin	80
Ceftriaxone	92	Trimeth/ Sulfa	58

Table 5. Antimicrobial resistances of *A. baumannii* isolated from clinical specimen in 1999

Antimicrobial agents	%Resistance	Antimicrobial agents	%Resistance
Amikacin	71	Ciprofloxacin	67
Ampicillin	100	Gentamicin	79
Ampicillin/ Sulbactam	58	Imipenem	4
Cephalothin	100	Ofloxacin	68
Cefotaxime	74	Piperacillin	69
Cefotetan	99	Ticarcillin/ Clavulanic acid	32
Ceftazidime	60	Tobramycin	78
Ceftriaxone	68	Trimeth/ Sulfa	57

Table 6. Antimicrobial resistances of *A. baumannii* isolated from clinical specimen in 2000

Antimicrobial agents	%Resistance	Antimicrobial agents	%Resistance
Amikacin	72	Ciprofloxacin	65
Ampicillin	100	Gentamicin	75
Ampicillin/ Sulbactam	50	Imipenem	4
Cephalothin	100	Ofloxacin	66
Cefotaxime	76	Piperacillin	73
Cefotetan	98	Ticarcillin/ Clavulanic acid	33
Ceftazidime	70	Tobramycin	76
Ceftriaxone	86	Trimeth/ Sulfa	55

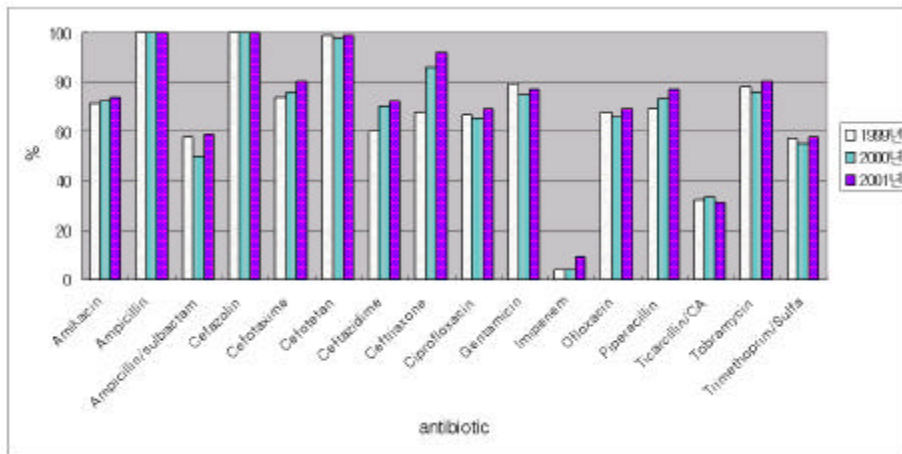


Fig. 1. Antimicrobial resistances of *A. baumannii* isolated from clinical specimen (1999-2001)

Table 7. MIC (Minimal inhibitory concentrations) of *A. baumannii* isolated

Amikacin	>= 64	R
Amoxicillin/Clavulanic Acid	>= 32	R
Ampicillin	>= 32	R
Cefotaxime	>= 64	R
Cefoxitin	>= 64	R
Ceftazidime	>= 64	R
Ciprofloxacin	>= 4	R
Gentamicin	>= 16	R
Imipenem	>= 16	R
Piperacillin/T azobactam	>=128	R
T icarcillin	>=128	R
T icarcillin/Clavulanic Acid	>=128	R
T obramycin	>= 16	R
Trimethoprim/ sulfamethoxazole	>=320	R

2. Metallo-β-lactamase

10

(Table 8), 3 가 EDTA double disk synergy ,
2 가 Hodge modified (Fig. 2,3)

3. (PCR)

EDTA double disk synergy test 3
(PCR) 1 VIM-2 gene
(Fig 4).

Table 8. Results of EDTA double disk synergy and Modified Hodge test of *A. baumannii* isolated

NO.	ID	Sex	Age	Location	EDTA test	Hodge test	PCR (VIM2)
42323	001	M	48	ICU	-	-	
42589	002	M	47	ICU	-	-	
24033	003	M	75	GS	-	-	
45423	004	M	59	ICU	-	-	
27361	005	F	76	ICU	-	-	
46271	006	M	70	IM	-	-	
46273	007	F	45	ICU	+	+	-
27851	008	M	46	ICU	+	+	780bp
46480	009	F	75	ICU	+	-	-
46495	010	M	70	IM	-	-	

Table 9. Sequences of primers used for detection of the VIM-2 gene from *A. baumannii* isolated

Gene	Primers	Oligonucleotide sequence (5' - 3')	expected product size (bp)
VIM-2	VIM-2-F	ATTGGTCTATTTGACCGCGTC	780
	VIM-2-R	TGCTACTCAACGACTGAGCG	



Fig. 2. EDTA double disk synergy test. The presence of an enlarged zone of inhibition was interpreted as the positive of the EDTA double disk synergy test.

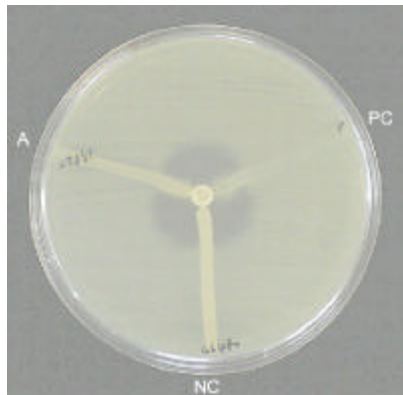


Fig. 3. Modified Hodge test. The presence of a distorted inhibition zone after overnight incubation was interpreted as the positive of the modified Hodge test. *A. baumannii* (A) showed positive reaction; portion of upper right was positive control (PC); low portion was negative control (NC).

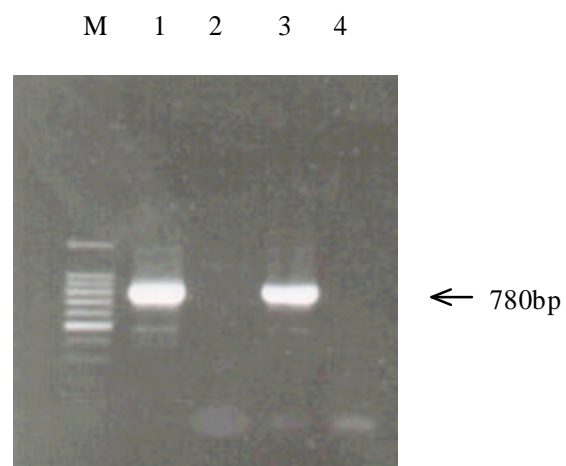


Fig. 4. Amplification of VIM-2 gene by PCR.

M; 100bp DNA ladder, 1; positive control, VIM-2-producing *P. aeruginosa* YMC 704 (GenBank accession No. AY029772), 2-4; Specimens.

IV.

A cinetobacter Bergey manual Family Nisseriaceae
Moraxellaceae . *A cinetobacter* *Micro-*
coccus aclacoaceticus, *Bacterium anitratum*, *M ima ploymorpha*,
A chromobacter anitratus ,
(4),
Brisou (10) *A cinetobacter* .
A cinetobacter anitratus
A cinetobacter lowffii , DNA DNA
21
16가 (23). geno-
mospecies 1, 2, 3 13 *A cinetobacter calcoaceticus-baumannii*
complex , *A cinetobacter*
A. calcoaceticus, *A. baumannii*, *A. haemolyticus*, *A. junii*, *A.*
johnsonii, *A. lowffii*, *A. radioresistens* 7 .
A cinetobacter 가 *A cinetobacter calcoaceticus-baumannii*
complex 가 가
. *A cinetobacter baumannii*가 가
,
(1,6).
가
가
, *A. baumannii* 가 8.3% ,
A. baumannii
7.3%

, *P. aeruginosa*, *S. aureus*, *E. coli*, *E. cloacae*

3

monitoring

*S. epidermidis*가

, *E. faecalis* *E. faecium*

가

Kl.

*pneumoniae*가

, *B.*

cepacia, *S. marcescens*

*A. baumannii*가

가

가

가 ventilator

가

가

(6).

(3).

가

(27)

sputum

68.4%

pus가 14.9%, blood 5.9%, urine

5.5%

sputum 3 43

46%

D

가

가

ventilator

(28)

*A. baumannii*가 45.2%

32.5 37.7%

가

가

가

가

A. baumannii β -lactam
 , β
 -lactam peptidoglycan penicillin
 binding protein (PBP)

(26).

penicillin ampicillin , β -lactam
 (22).

가

Gentamicin Ticarcillin/CA Vitek system
 가 (28) 1996
 1997 *A. baumannii* 9.2%, 4.2%

가 Imipenem resistant *A. baumannii* (IRAB) Lee

(12) 1998 25 KONSAR group
 imipenem resistant *A. baumannii*가 5%

1999

가 1999 4%, 2000 4%,
 2001 9.04% . 2000 6
 가 가 4%

Imipenem, meropenem carbapenem extended-
 spectrum β -lactamase(ESBL), AmpC β -lactamase
 β -lactamase , penicillin
 binding protein (PBP) 가 peptidoglycan
 (9). penicillin
 cephalosporin

metallo- β -lactamase
 carbapenem
 가 가 carbapenem
 β -lactam (11).
 metallo- β -lactamase 1988 (25),
 imipenem 가 IMP-1 .
 IMP P.
aeruginosa (5), 8가 IMP가
 . 1999 metallo- β -lactamase
 VIM-1 *P. aeruginosa*가 ,
 VIM-2 *P. aeruginosa*, VIM-3
*P. aeruginosa*가 . 1995 VIM-2
*P. aeruginosa*가 , P.
putida, *A. baumannii*, *A. cinetobacter* genomospecies 3 S.
*marcescens*가 (13). IMP-1
 가 (8)
 IMP-1 A.
baumannii 1 (14). 509 A.
baumannii 10
 metallo- β -lactamase 3
 (PCR) VIM-2 gene .
 primer VIM-2 IMP gene
 VIM-1 VIM-3 .
 carbapenem
 . metallo- β -lactamase

carbapenem MIC가 가
NCCLS ,
가 carbapenem
. carbapenem
, β -lactamase ,
,
.
. carbapenem
Hodge , metallo- β -lactamase 2-mercapto-
ptopropionic acid, EDTA(Ethylene Diamine Tetra Acetic acid)
가 double disk synergy test(1) 가
.
Metallo- β -lactamase carbapenem
EDTA double disk
synergy test Hodge modified test .
Metallo- β -lactamase 가
.
metallo- β -lactamase가
 β -lactamase Augmentin (Clavulanic acid + Amoxicillin)
Unasyn (Sulbactam + Ampicillin), Zosyn (Tazobactam +
Piperacillin) 가 가

A. baumannii

double disk synergy test modified Hodge

test

-

.

V.

		<i>A. baumannii</i>				
		β-lactam				
		2001	1	2001	12	D
<i>A. baumannii</i> 509						
		Metallo-β				
-lactamase		EDTA double disk synergy				
test	modified Hodge test	(PCR)				
1.	<i>A. baumannii</i> 가 509	<i>P.</i>				
	<i>aeruginosa, S. aureus, E. coli, E. cloacae</i>	5				
2.	<i>A. baumannii</i>	220 (43.2%)				
	가	166	(32.6%),	72	(14.1%),	
		29	(5.7%)			
3.	<i>A. baumannii</i>	167 (32.8%),				
		41	(18.1%),	37	(7.3%),	24 (4.7%),
		24	(4.7%)			
4.	509 <i>A. baumannii</i> imipenem	46				
	(9.04%)가	가				
5.		10	<i>A. baumannii</i>			
	EDTA double disk synergy test	3 가				
	, modified Hodge test	2 가				
6.	EDTA double disk synergy test modified Hodge test					

(PCR) 1 가 VIM-2

A. baumannii

EDTA double disk synergy test modified Hodge test

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