PLASMID ANALYSIS AND LOCATION OF THE OXA-40 CARBAPENEMASE GENE IN MULTIDRUG-RESISTANT ENDEMIC CLONES OF *Acinetobacter baumannii*

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OBJECTIVE

1. INVESTIGATE SEQUENTIAL ISOLATES OF TWO ENDEMIC MULTIDRUG-RESISTANT CLONES PRODUCING THE OXA-40 CARBAPENEMASE OBTAINED IN A HOSPITAL FROM NORTHERN SPAIN FROM 1999 T0 2005

2. EVOLUTIONAL GENETIC EVENTS

3. ANALYSE THE PRESENCE OF PLASMIDS AND ITS RELATION WITH THE *bla* $_{\text{OXA-40}}$ GENE

BACKGROUND

1. TOTAL ISOLATES: 102, 82 and 30 *A. baumannii*

(years 1999, 2002 and 2005 respectively)

2. HOSPITAL of Osakidetza (Bilbao, Northern Spain):

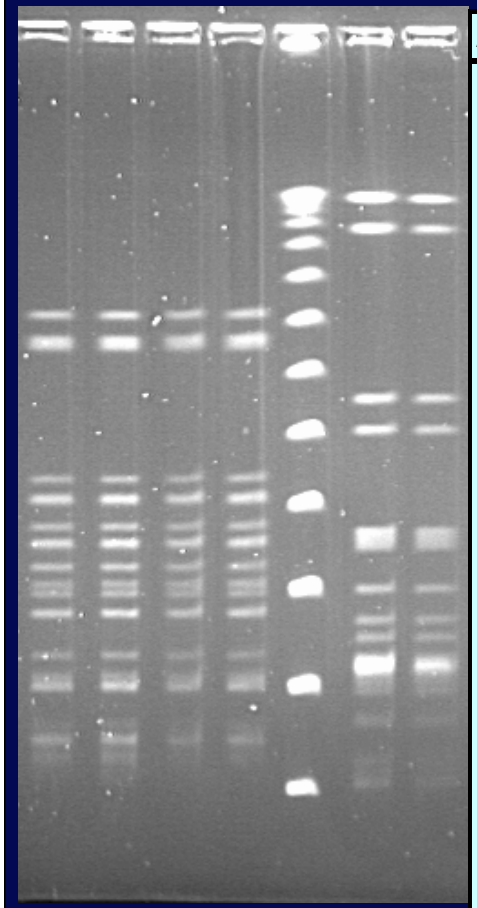
- **A 240-bed respiratory illness-specialized institution**
- **Prevalence of elderly patients**
- **Hospitalized for long periods of time (median, 1 month).**

3. SUSCEPTIBILITY ASSAYS

4. CLONAL RELATEDNESS investigation:

- **RAPD-PCR fingerprinting primers M13 and ERIC2**
- **Pulsed-field gel electrophoresis (PFGE) with *Apa* I**

5. DETECTION OF CARBAPENEMASES



ANTIBIOTIC	CLONE I			CLONE II			OTHERS		
	1999 n=28	2002 n=52	2005 n=25	1999 n=50	2002 n=17	2005 n=5	1999 n=24	2002 n=13	2005 n=0
CTX	85%	91%	100%	100%	100%	100%	70%	58%	-
CAZ	68%	85%	96%	90%	100%	80%	58%	66%	-
ATM	85%	-	100%	84%	-	100%	96%	-	-
IPM	32%	75%	100%	84%	71%	40%	41%	33%	-
MEM	25%	85%	100%	88%	82%	60%	33%	20%	-
AMK	18%	85%	76%	52%	65%	0%	8%	66%	-
GEN	75%	83%	95%	94%	71%	80%	95%	45%	-
TOB	10%	88%	96%	70%	27%	0%	60%	69%	-
CIP	93%	98&	100%	90%	100%	100%	84%	92%	-
OFX	96%	-	100%	100%	-	100%	80%	-	-
SAM	60%	42%	21%	6%	83%	20%	33%	15%	-
TZP	92%	94%	100%	6%	100%	100%	30%	69%	-
CRO	-	98%	100%	-	100%	100%	-	92%	-
FEP	82%	96%	96%	80%	94%	100%	72%	77%	-
TET	-	98%	96%	-	100%	100%	-	92%	-
CHL	-	94%	92%	-	72%	100%	-	100%	-
CT	0%	0%	0%	0%	0%	0%	0%	0%	-
SXT	-	88%	96%	-	94%	80%	-	54%	-

-TWO ENDEMIC CLONES WHOSE PREVALENCE HAD CHANGED

-MULTIDRUG-RESISTANCE PHENOTYPE

- OXA-40 PRODUCERS (CLONE I FROM 22% IN 1999 TO 96% IN 2005)

- NO METALLO-β-LACTAMASES

METHODS

-BACTERIAL ISOLATES: 15 *A. baumannii* selected as representatives including bla_{OXA-40} positive and negative isolates per year and clone.

-SUSCEPTIBILITY ASSAYS: Minimun Inhibitory Concentration to cefotaxime, ceftazidime, imipenem, meropenem, amikacin and gentamicin by means of the agar dilution method

-MULTIPLEX-PCR: to search for $bla_{OXA-23-like}$, $bla_{OXA-51-like}$, and $bla_{OXA-24-like}$ and *Int*1 genes. A PCR was also designed to selectively amplify the *ompA*, *csuE* and $bla_{OXA-51-like}$ (Laboratory of HealthCare Associated Infection, Colindale, UK)

- CLASS 1 INTEGRONS

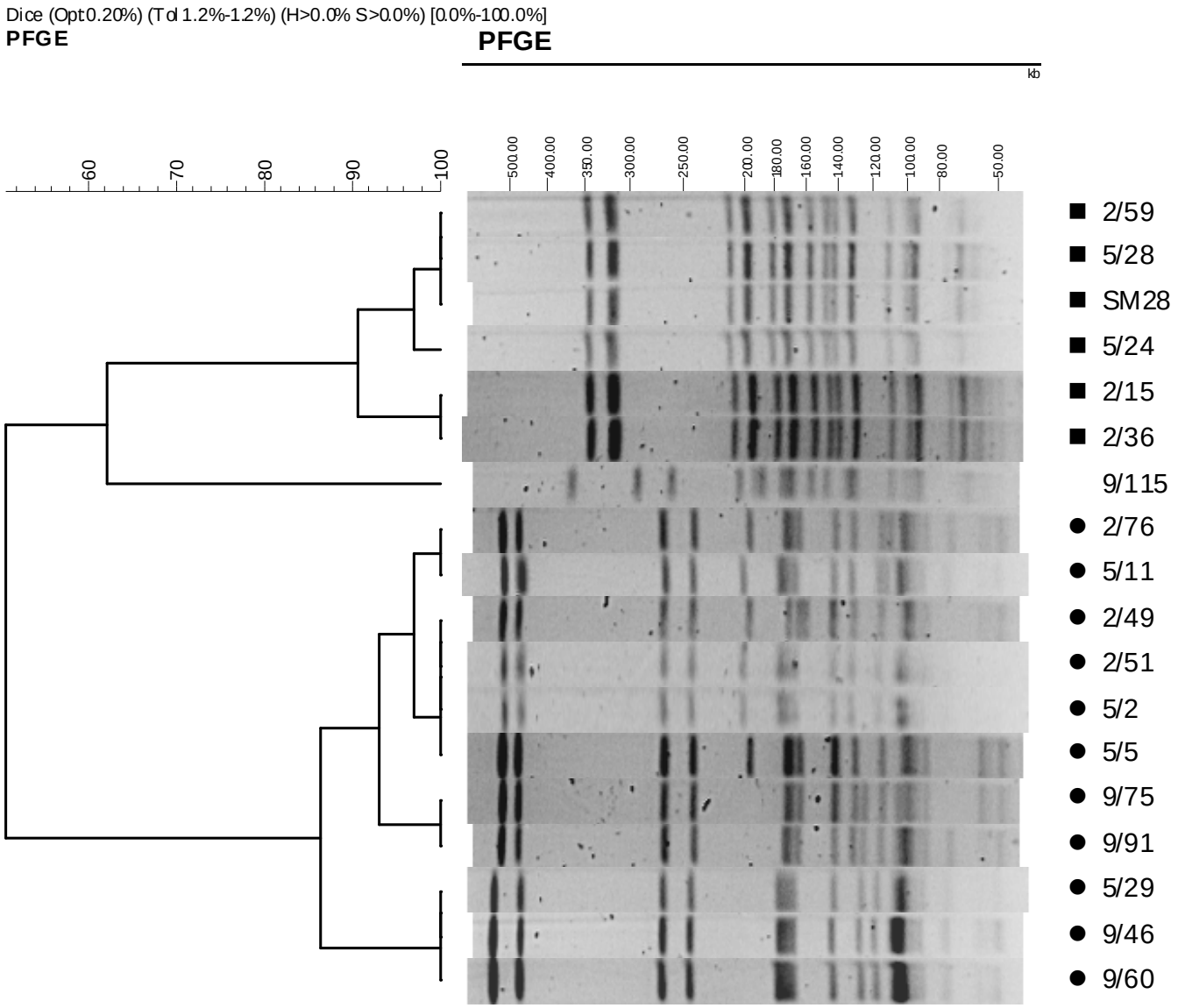
METHODS

- **PLASMID ANALYSIS:** Plasmid DNA was extracted by with a comercial plasmid extraction kit
  - Size was determined by comparison to plasmid DNAs from the standard strains *E.coli* NCTC 50193 and NCTC 59192

-**ENDONUCLEASE MAPPING:** About 1 μg of plasmid DNA was used for digestions with restriction enzymes *Eco*RI, *Pst*I and *Hind*III endonucleases

-**HYBRIDIZATION EXPERIMENTS:** Southern transfer of plasmid DNA and the corresponding digestions with an OXA-40 specific probe labelled with dUTP-digoxigenin.

PFGE *Apa I*


Dice (Opt 0.20%) (Tol 1.2%-1.2%) (H>0.0% S>0.0%) [0.0%-100.0%]
PFGE
PFGE
kb
60
70
80
90
100
500.00
400.00
350.00
300.00
250.00
200.00
180.00
160.00
140.00
120.00
100.00
80.00
50.00
2/59
5/28
SM28
5/24
2/15
2/36
9/115
2/76
5/11
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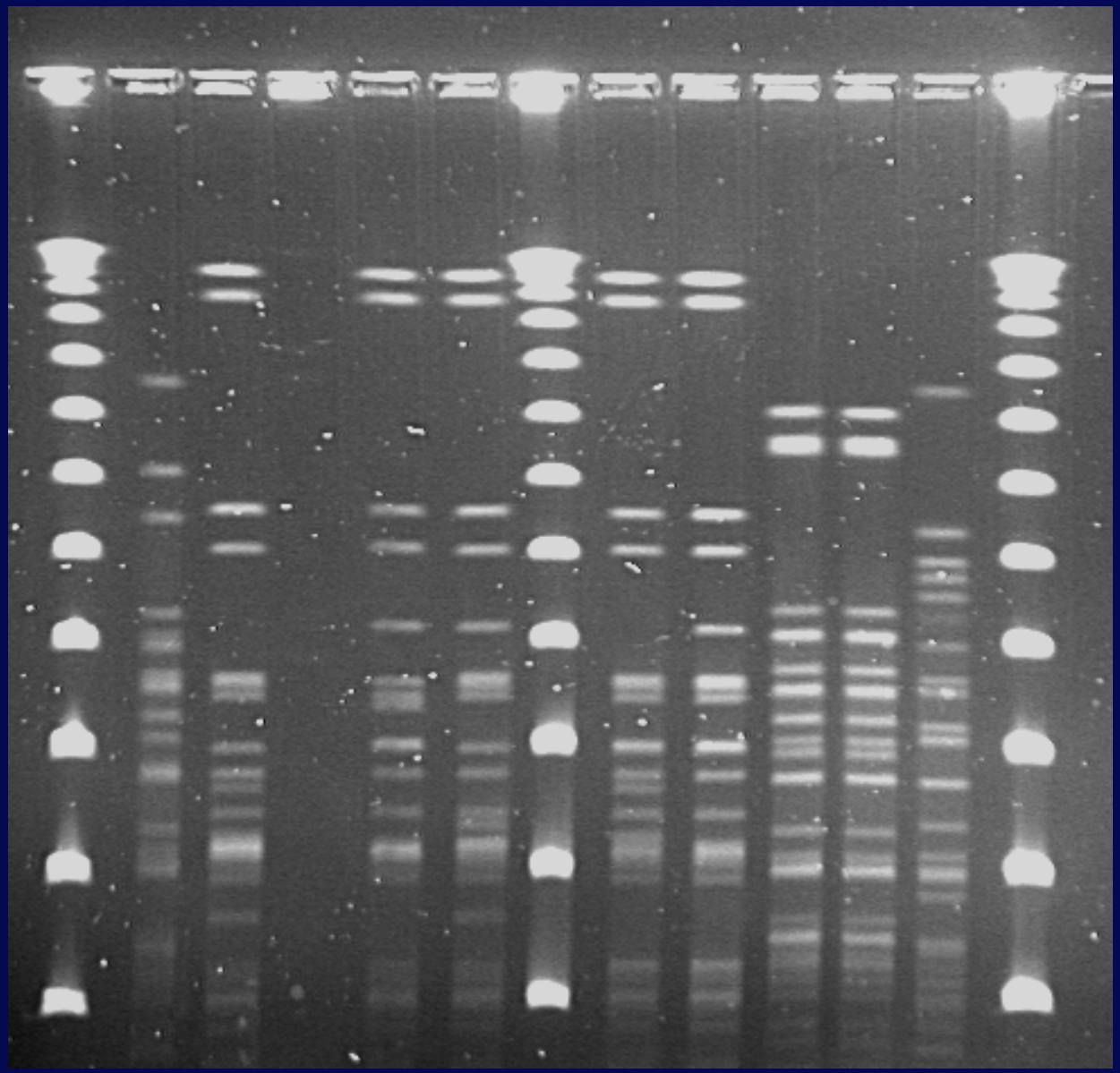
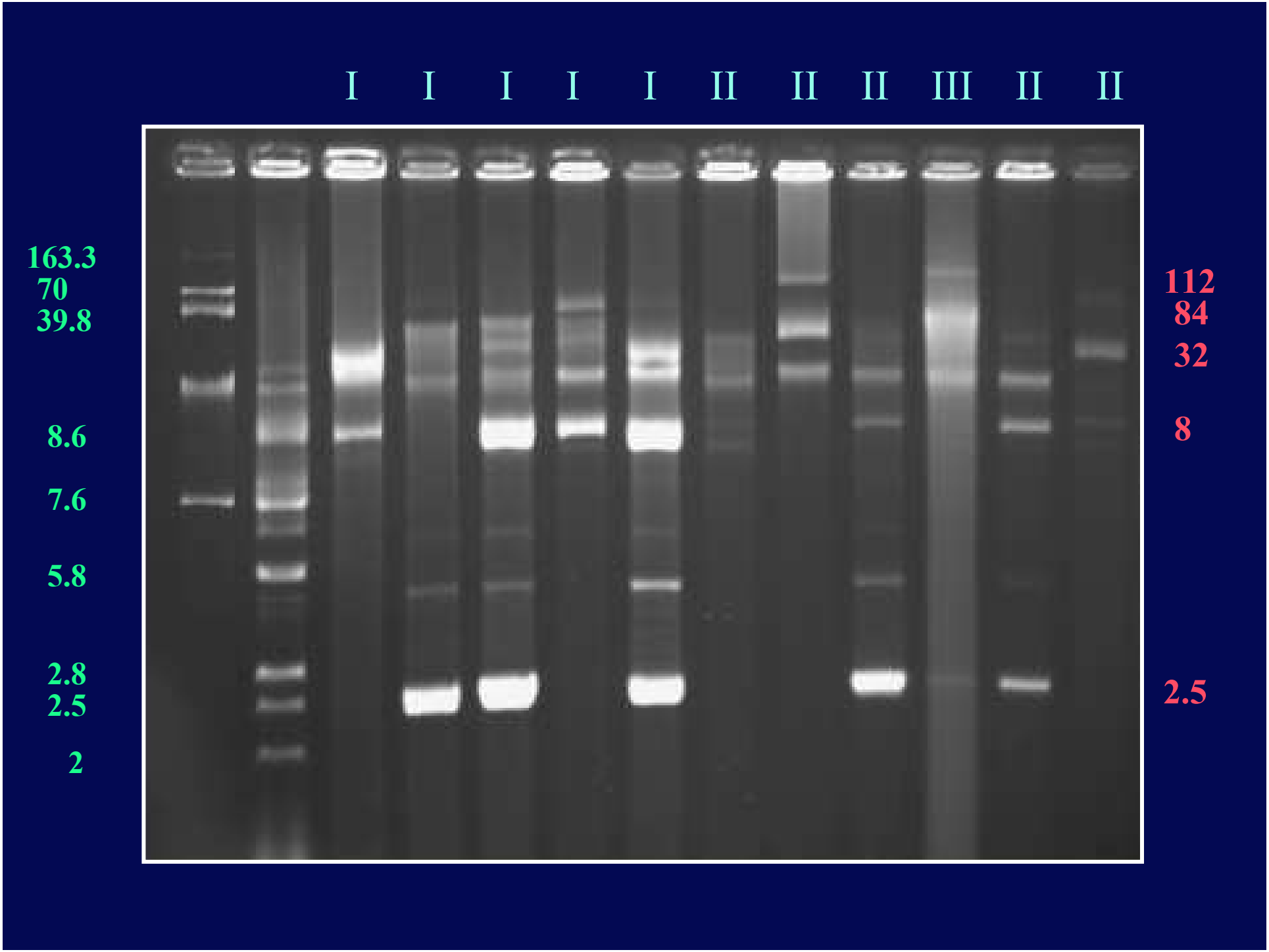
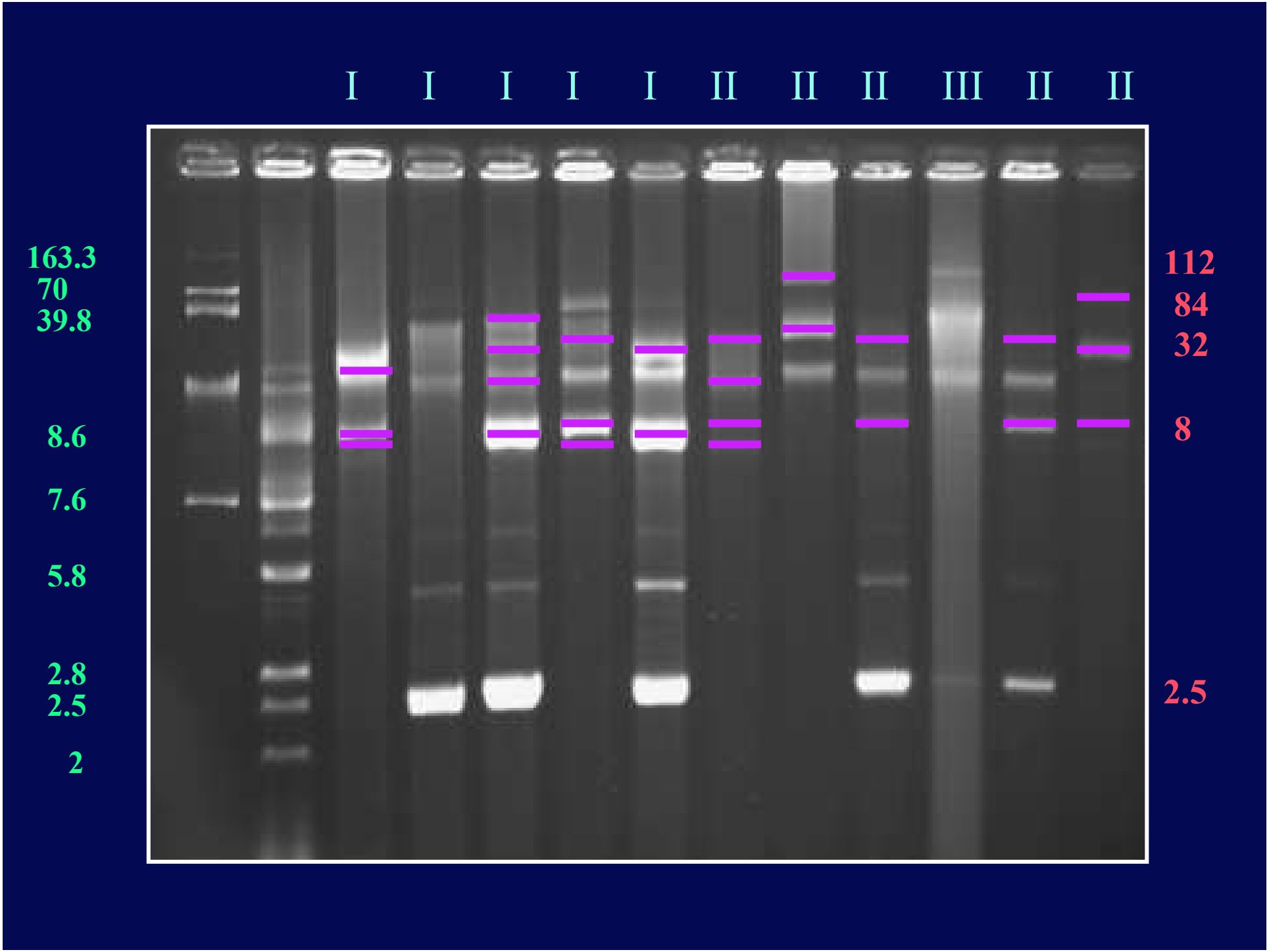
2/51
5/2
5/5
9/75
9/91
5/29
9/46
9/60



				OXA-type carbapenemase			Multiplex-PCR			
Isolate	**YEAR**	**SAMPLE**	**PFGE**	**OXA-40**	**OXA-51**	**OXA-23**	**GP1***	**GP2***	**INTEGRONS (bp)**	**PLASMIDS (Kb)**
1	1999	sputum	I	+	+	-	-	+	760	2.5, 8, 32
2	1999	sputum	I	+	+	-	-	+	550, 1200	8
3	2002	sputum	I	+	+	-	-	+	760	2.5, 8, 29, 84
4	2002	sputum	I	+	+	-	-	+	760	8, 32
5	2005	sputum	I	+	+	-	-	+	760, 1500	2.5, 8, 32
6	2005	sputum	I	+	+	-	-	+	760, 1500	2.5, 8, 29
7	1999	urine	I	-	+	-	-	+	760	2.5, 70
8	2002	sputum	I	-	+	-	-	+	760	2.,5, 30
9	2005	urine	I	-	+	-	-	+	760, 1500	2.5, 8, 30
10	1999	sputum	II	+	+	-	+	-	550, 1200	32, 125
11	1999	wound	II	+	+	-	+	-	550, 760, 1200	8, 32, 84, 112
12	2002	sputum	II	+	+	-	+	-	550, 760, 1200	2.5, 8, 32
13	2002	sputum	II	+	+	-	+	-	550, 760, 1200	84, 125
14	2005	sputum	II	+	+	-	+	-	550	8, 32
15	2005	sputum	II	+	+	-	+	-	550	8, 32, 84


I I I I I II II II III II II
163.3
70
39.8
8.6
7.6
5.8
2.8
2.5
2
112
84
32
8
2.5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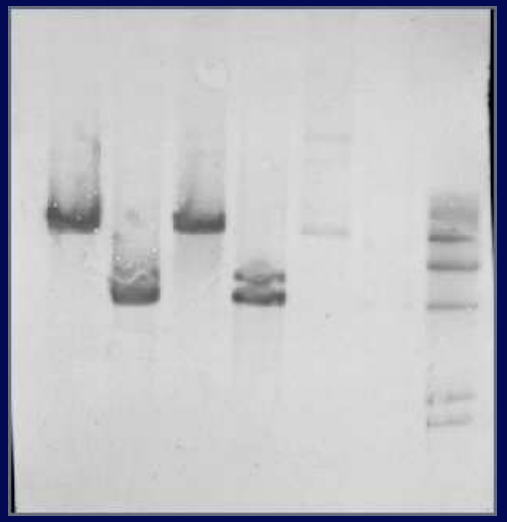

A. baumannii SM28 (OXA-40)

CONCLUSIONS

1. **CLONE I:**
   - **INCREASEMENT IN RESISTANCE**
   - **MODIFICATIONS IN PFGE PROFILE**
   - **OXA-40 SPREAD**
   - **DIFFERENT *ompA* AND *csuE* ALLELES**
   - ***bla*** $_{\text{OXA-40}}$ **AND** ***bla*** $_{\text{OXA-51-LIKE}}$ **(*bla*** $_{\text{OXA-71}}$ **)GENES**

2. **PLASMIDS:**
   - **DETECTED IN ALL ISOLATES TESTED**
   - **RANGING IN SIZE FROM 2.5 TO 125 Kb**
   - ***bla*** $_{\text{OXA-40}}$ **GENE LOCATION ON DIFFERENT STRUCTURES**