

CHAPTER V

CONCLUSION

Ninety-four percent of ninety strains of B. fragilis group produced β -lactamase. The expression of the β -lactamases was constitutive. Only few strains resisted to cefoxitin where as more than 50 percent of the strains resisted to ampicillin and penicillin G.

From the study on minimum inhibitory concentration, 64 percent of B. fragilis group resisted to ampicillin, 63 percent resisted to penicillin, and 6 percent resisted to cefoxitin. The β -lactamase producing strains may not resist to ampicillin. There were many factors which affected the resistance of the bacteria.

The β -lactamase activity determined by chromogenic cephalosporin was not associated with the MIC of B. fragilis group to ampicillin and penicillin G.

β -lactamase mediated ampicillin resistance was transferable in 3 strains of B. ovatus using B. vulgatus as recipient. Transconjugants with higher levels of β -lactamase production can be found associated with higher ampicillin and penicillin MIC. The plasmid was not detectable in one strain of donor and all transconjugants. The resistant genes were not associated with extrachromosomal DNA but may associate with transposons or episome.

The isoelectric focusing study of β -lactamases from B. fragilis revealed the same pI as SAR-1 β -lactamase but no evidence of transference and with constitutive property therefore the β -lactamase of B. fragilis seemed to be chromosome borne. The isoelectric focusing study of β -lactamases from the species other than B. fragilis did not fit in the aerobic classification scheme.



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Table 1. Properties of Plasmid Mediated β -Lactamases by Various Investigators.

Type of β -lactamase ^a	Relative Rates of Hydrolysis ^b						Inhibition By ^d			pI ^e	M. W. (daltons)	Example Organisms which known to produce the enzyme	Ref.
	Pen	Amp	Carb	Oxa	Meth	Cep ^c	Clox	PCMB	NaCl				
TEM-1	100	106	10	5	0	76	S	R	R	5.4	28500	Enterobacteriaceae	[20]
TEM-2	100	107	10	5	0	74	S	R	R	5.6	28500		[20]
SHV-1	100	212	8	0	0	56	S	S/R	R	7.6	17000	<u>E. coli</u>	[20]
IMS-1	100	253	14	0	0	183	S	S	R	5.2	21000	<u>P. mirabilis</u>	[20]
TLE-1	100	67	13	4	5	52	S	-	R	5.55	19800	<u>E. coli</u>	[33]
ROB-1	100	186	25	6	-	24	S	-	R	8.1	-	<u>H. influenzae</u>	[37]
LCR-1	-	-	-	-	-	-	-	-	-	5.85	44000	<u>Ps. aeruginosa</u>	[35]
OXA-1	100	382	30	197	332	30	R	PS	S	7.4	23300	<u>E. coli</u>	[20]
OXA-2	100	179	15	646	23	37	R	R	S	7.45	44600	<u>P. mirabilis</u>	[20]
OXA-3	100	178	10	336	29	44	R	R	S	7.1	41200	<u>K. pneumoniae</u>	[20]
OXA-4	100	438	39	220	711	194	S	-	R	7.5	23000	<u>E. coli</u>	[33]
OXA-5	100	188	40	210	109	89	R	-	S	7.62	27000	<u>Ps. aeruginosa</u>	[33]
OXA-6	100	596	46	1048	585	149	S	-	R	7.68	40000	<u>Ps. aeruginosa</u>	[33]
OXA-7	100	545	48	702	424	136	R	-	S	7.65	25300	<u>E. coli</u>	[33]
PSE-1	100	90	97	0	0	18	R	S	-	5.7	28500	<u>Pseudomonas species</u>	[20]
PSE-2	100	267	121	317	803	32	R	S	S	6.1	12400		[20]
PSE-3	100	101	253	-	-	10	-	R	-	6.9	12000		[20]
PSE-4	100	88	150	8	16	40	-	R	-	5.3	32000		[20]
NPS-1	100	223	18	40	<0.1	3	-	-	-	6.5	25000	<u>Ps. aeruginosa</u>	[43]
AER-1	-	-	-	-	-	-	-	-	-	5.9	22000	<u>A. hydrophila</u>	[35]
CEP-1	100	5	-	0	-	325	-	R	R	8.1	31800	<u>P. mirabilis</u>	[20]
CEP-2	100	LOW	48	-	-	108	R	R	-	8.1	36200	<u>Achromobacter</u>	[20]
SAR-1	100	63	122	-	0	21	S	R	-	4.9	33700	<u>V. cholerae</u>	[46]

a TEM, Temoniera ; SHV, Sulphydryl variable ; IMS, Hedges Matthew and Smith ; TLE, TEM like enzyme ; ROB, Haemophilus influenzae type b strain ROB ; OXA, oxacillin hydrolyzing ; PSE, Pseudomonas specific enzyme ; NPS, New Pseudomonas specific enzyme ; AER, Aeromonas ; CEP, Cephalosporin hydrolyzing.

b The rates of hydrolysis are expressed as a function of the benzylpenicillin rate.

c Abbreviations : Pen, benzylpenicillin ; Amp, ampicillin ; Carb, carbenicillin ; Oxa, oxacillin ; Meth, methicillin ; Cep, cephaloridine ; Clox, cloxacillin ; PCMB, p-chloromercuribenzoate.

d S, Susceptible ; R, resistant ; PS, partially susceptible.

e pI, isoelectric point.

Table 2. The Isoelectric Points of β -Lactamases from Bacteroides fragilis Group.

Species	Isoelectric points	References
<u>B. fragilis</u>	4.6	[12]
	4.8	[9]
	4.9	[57, 82-86]
	4.9, 5.1	[9]
	4.7, 4.8	[112]
	6.9	[58]
<u>B. distasonis</u>	4.8	[12]
	4.9	[84]
	5.1	[83]
	5.4	[82]
	5.6	[9]
<u>B. ovatus</u>	4.3, 4.6, 4.8, 7.1	[82]
	4.0, 4.6	[12,85]
	5.1	[9]
	6.7	[83]
	6.3, 7.1	[84]
<u>B. thetaiotaomicron</u>	4.3	[9,82,84,85]
	4.5	[83]
	4.6	[12]
	4.3, 5.3, 6.7	[82]
<u>B. vulgatus</u>	4.4, 4.9	[82]
	4.5	[9]
	4.6	[12,83]
	4.7	[85]
	4.5, 4.9	[84]

Table 3. Properties of β -Lactamases from B. fragilis Group.

Species	No. of Strains	Relative Rates of Hydrolysis ^a						Inhibition by					pI	M. W. (daltons)	Ref.
		Pen	Amp	Clox	Cep	Fox	Com	Clox	Fox	PCMB	Clav	Sulb			
<u>B. fragilis</u>	1	100	60	10	13,800	-	4,100	S	S	S	S	-	5.55	-	} [113]
"	1	100	65	25	8,800	-	3,500	S	S	S	S	-	5.30	-	
"	1	100	55	20	12,100	0	3,500	S	S	S	S	-	5.25	30,000	
"	1	100	30	80	7,700	-	2,100	S	S	S	S	-	5.05	-	
"	1	100	20	-	160	-	-	S	-	-	-	-	-	-	[55]
"	2	100	-	-	3,000	0	1,400	-	-	-	-	-	4.9	-	[62]
"	3	100	40	0	5,000	-	-	S	-	S	-	-	4.9	-	} [57]
"	1	100	240	0	3,000	-	-	S	-	S	-	-	5.6	40,000	
"	1	100	350	270	70	0	-	-	S	S	S	-	6.9	41,500	[58]
"	3	100	500	-	360	70	-	S	S	S	-	-	-	-	} [14]
"	2	100	0	-	3,000	1,000	-	S	S	S	-	-	-	-	
"	1	100	120	120	40	20	30	-	-	S	R	R	4.8	26,000	} [112]
"	1	100	-	-	5,200	-	1,800	-	-	S	S	S	4.7	32,000	
"	3	100	50	3	3,100	-	-	S	-	S	-	-	-	30,000	[59]
<u>B. uniformis</u>	1	100	-	-	1,500	0	570	-	-	-	-	-	-	-	} [62]
"	1	100	-	-	3,000	0	5,700	-	-	-	-	-	-	-	
<u>B. ovatus</u>	1	-	-	-	-	-	-	S	-	-	S	S	-	-	} [97]
<u>B. vulgatus</u>	1	-	-	-	-	-	-	S	-	-	S	R	-	-	

a Abbreviations : Pen, benzylpenicillin ; Amp, ampicillin ; Clox, cloxacillin ;
 Cep, cephaloridine ; Fox, cefoxitin ; Com, cefuroxime ;
 PCMB, p-chloromercuribenzoate ; Clav, clavulanic acid ;
 Sulb, sulbactam.

Table 4. Test Concentrations of Antibiotics [96]

Antibiotic	disc concentration	No. of discs per tube	Calculated test conc./ml.
Penicillin G	10 units	1	2 units
Ampicillin	10 $\mu\text{g.}$	2	4 $\mu\text{g.}$
Cefoxitin	30 $\mu\text{g.}$	3	18 $\mu\text{g.}$

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Table 5. Sources of Bacteroides Strains in this Study.

Species	No. of Strains	Sources				
		Blood	Pus	Genito-Urinary	Oral cavity	other
<u>B. fragilis</u>	57	13	27	7	0	10
<u>B. ovatus</u>	18	6	4	3	0	5
<u>B. distasonis</u>	8	0	5	0	1	2
<u>B. vulgatus</u>	7	1	2	3	0	1
Total	90	20	38	13	1	18

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Table 6. β -Lactamase Activity of Bacteroides Strains Isolated from the Clinical Specimens.

Organisms	No. of tested strains	β -lactamase producing strains		No. of strains with indicated β -lactamase activity (degree)*			
		No. of strains	Percent	+++	++	+	-
<u>B. fragilis</u>	57	55	96	9	27	19	2
<u>B. ovatus</u>	18	18	100	2	8	8	0
<u>B. distasonis</u>	8	8	100	1	5	2	0
<u>B. vulgatus</u>	7	4	57	0	3	1	3
Total	90	85	94	12	43	30	5

- * +++, strongly β -lactamase detection
 ++, moderately β -lactamase detection
 +, slightly β -lactamase detection
 -, no β -lactamase detection

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Table 7. Minimum Inhibitory Concentration of Bacteroides fragilis Group from the Clinical Isolates.

Antibiotics	Species (No. of tested Strains)	No. of strains with indicated MIC ($\mu\text{g./ml.}$)								
		2	4	8	16	32	64	128	256	>256
Ampicillin	<u>B. fragilis</u> (57)		4	2	19	20	7	1		4
	<u>B. ovatus</u> (18)				1	5	10			2
	<u>B. distasonis</u> (8)		1		1	4	1			1
	<u>B. vulgatus</u> (7)		1	1	2	2				1
Penicillin G	<u>B. fragilis</u> (57)				18	27	3	3	1	5
	<u>B. ovatus</u> (18)				5	3	5	1		4
	<u>B. distasonis</u> (8)				4	2		1		1
	<u>B. vulgatus</u> (7)		2		4				1	
Cefoxitin	<u>B. fragilis</u> (57)		15	22	10	7	3			
	<u>B. ovatus</u> (18)				2	14		2		
	<u>B. distasonis</u> (8)			5	1	2				
	<u>B. vulgatus</u> (7)		2	2	2	1				

Table 8. The Range of MIC, MIC₅₀ and MIC₉₀ of Bacteroides fragilis Group to β -Lactam Antibiotics*

Antibiotics	Species	MIC ($\mu\text{g./ml.}$)		
		Range	MIC ₅₀	MIC ₉₀
Ampicillin	<u>B. fragilis</u>	4 - >256	16	64
	<u>B. ovatus</u>	16 - >256	32	>256
	<u>B. distasonis</u>	4 - >256	32	>256
	<u>B. vulgatus</u>	4 - >256	16	>256
Penicillin G	<u>B. fragilis</u>	16 - >256	16	128
	<u>B. ovatus</u>	16 - >256	32	>256
	<u>B. distasonis</u>	16 - >256	16	>256
	<u>B. vulgatus</u>	2 - 256	8	128
Cefoxitin	<u>B. fragilis</u>	4 - 64	8	32
	<u>B. ovatus</u>	16 - 128	32	64
	<u>B. distasonis</u>	8 - 32	8	32
	<u>B. vulgatus</u>	4 - 32	8	16

* The break point for ampicillin and penicillin G are 16 $\mu\text{g./ml.}$, cefoxitin is 32 $\mu\text{g./ml.}$ Breakpoints are the concentration above which an organism is considered resistant and at or below which an organism is classified as susceptible. [114]

Table 9. Number of Bacteroides Strains with Indicated β -Lactamase Activity and MIC to β -Lactam Antibiotics.

Antibiotics	β -Lactamase Activity*	No. of strains with indicated MIC ($\mu\text{g./ml.}$)								
		2	4	8	16	32	64	128	256	>256
Ampicillin	+++	0	2	1	2	0	1	0	0	6
	++	0	3	1	14	14	8	1	0	2
	+	0	0	1	5	16	8	0	0	0
	-	0	1	0	2	1	1	0	0	0
Penicillin G	+++	0	0	0	1	4	1	0	0	6
	++	0	0	0	20	6	1	1	2	3
	+	1	0	0	8	10	6	4	0	1
	-	1	0	0	2	2	0	0	0	0
Cefoxitin	+++	0	1	1	1	4	3	2	0	0
	++	0	9	14	8	12	0	0	0	0
	+	0	6	12	4	8	0	0	0	0
	-	0	2	2	1	0	0	0	0	0

* +++, strongly β -lactamase detection

++, moderately β -lactamase detection

+, slightly β -lactamase detection

-, no β -lactamase detection

Table 10. Correlation of β -Lactamase Activity and MIC Range, MIC₅₀ MIC₉₀ of B. fragilis Group

Antibiotics	β -Lactamase Activity	No. of Strains	MIC Range ($\mu\text{g.}/\text{ml.}$)	MIC ₅₀ ($\mu\text{g.}/\text{ml.}$)	MIC ₉₀ ($\mu\text{g.}/\text{ml.}$)
Ampicillin	+++	12	4 - >256	64	>256
	++	43	4 - >256	16	64
	+	30	8 - 64	32	64
	-	5	4 - 64	16	64
Penicillin G	+++	12	16 - >256	64	>256
	++	43	16 - >256	16	256
	+	30	2 - >256	32	128
	-	5	2 - 32	16	32
Cefoxitin	+++	12	4 - 256	32	64
	++	43	4 - 32	8	32
	+	30	4 - 32	8	32
	-	5	4 - 16	4	16

Table 11. Transferability study of Ampicillin Resistance from Bacteroides fragilis Group to E. coli and B. vulgatus.

Part	Donors	No.	Recipients	Method	Transferability
I	<u>B. fragilis</u>	7	1. <u>E. coli</u> K12 J53	Broth-mating	Negative
	<u>B. ovatus</u>	5	2. <u>E. coli</u> C600 Nali _{R*}	1. BHI B	Negative
			3. <u>E. coli</u> C600 Rif _{R**}	2. Chopped meat	Negative
II	<u>B. fragilis</u>	9	<u>E. coli</u> K12 J53	filter mating	Negative
	<u>B. ovatus</u>	1			Negative
	<u>B. distasonis</u>	1			Negative
III	Enhanced plasmid copies c 64 ug./ml. ampicillin				
	<u>B. fragilis</u>	4	<u>E. coli</u> K12 J53	filter mating	Negative
	<u>B. ovatus</u>	2			Negative
	<u>B. distasonis</u>	1			Negative
IV	<u>B. fragilis</u>	11	<u>B. vulgatus</u> Rif _{R**}	filter mating	Negative
	<u>B. ovatus</u>	7			Negative
	<u>B. ovatus</u>	3			Positive
	<u>B. distasonis</u>	3			Negative

* Nalidixic acid resistance

** Rifampicin resistance

Table 12. Minimum Inhibitory Concentration and β -lactamase Properties of Donors, Recipient, and Transconjugant.

Strains		MIC ($\mu\text{g./ml.}$)			β -lactamase	
		Amp	pen G	Fox*	Activity	pI value
Donor	: <u>B. ovatus</u> No.30	128	>256	32	++	4.3
Recipient	: <u>B. vulgatus</u> No.48	4	2	8	-	-
Transconjugant	: T. No.30	128	>256	32	++	4.3
Donor	: <u>B. ovatus</u> No.32	>256	>256	128	+++	4.3, 4.6
Recipient	: <u>B. vulgatus</u> No.48	4	2	8	-	-
Transconjugant	: T. No.32	>256	>256	32	+++	4.3, 4.6
Donor	: <u>B. ovatus</u> No.51	>256	>256	128	+++	4.3, 4.6
Recipient	: <u>B. vulgatus</u> No.48	4	2	8	-	-
Transconjugant	: T. No.51	>256	>256	32	+++	4.3, 4.6

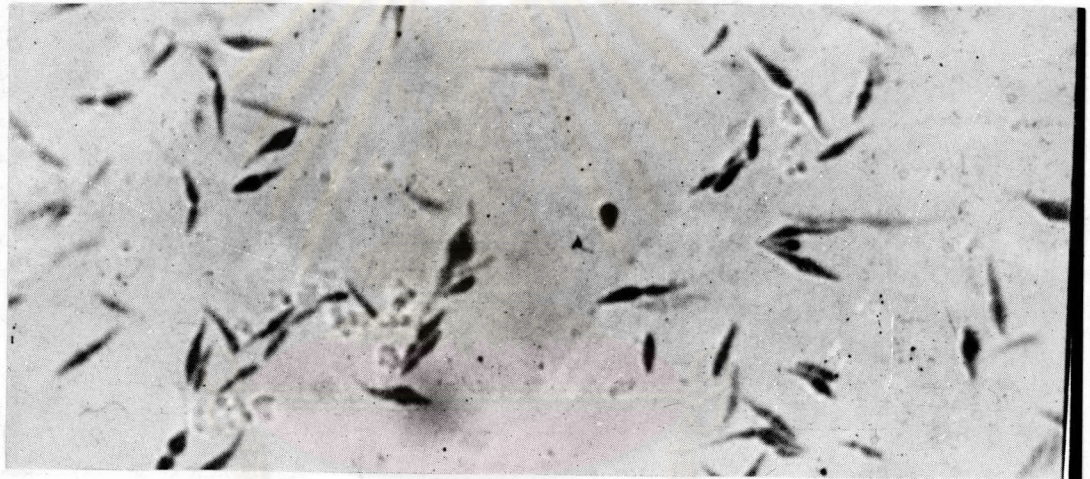
* Amp, Ampicillin

Pen G, Penicillin G

Fox, Cefoxitin

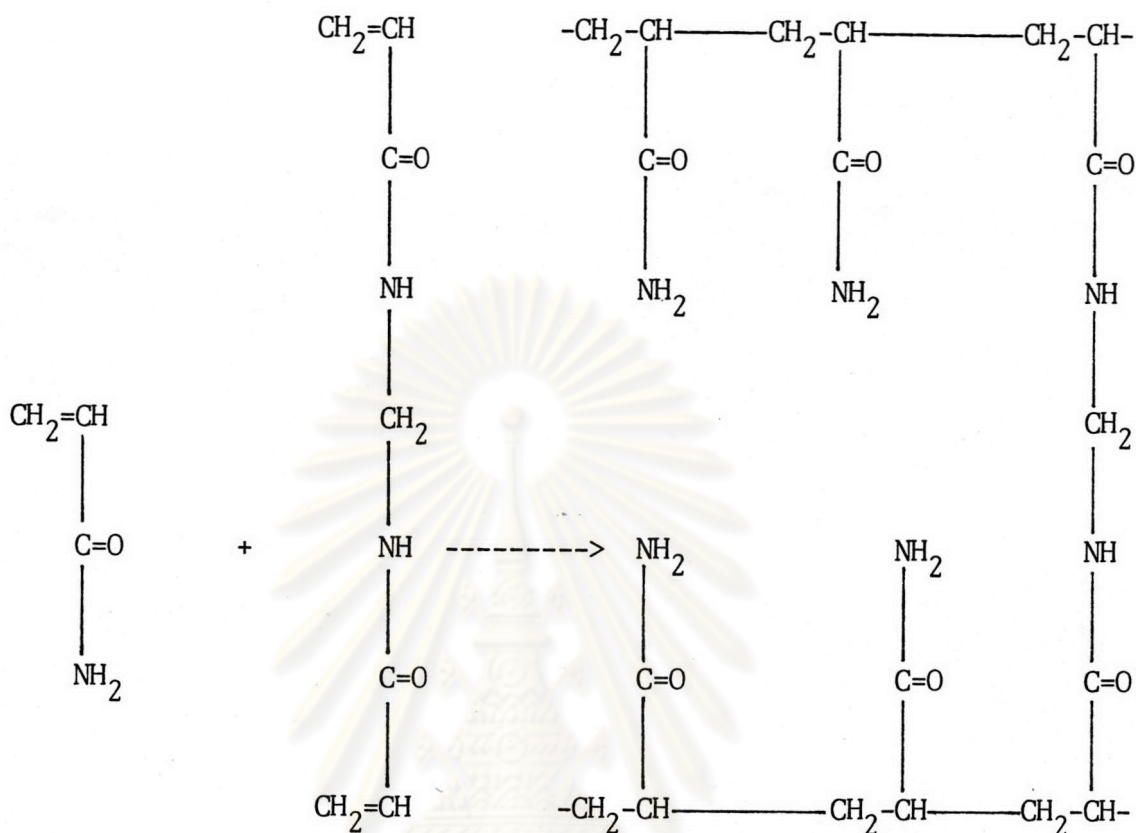
Table 13. The Isoelectric Point of β -Lactamases from B. fragilis Group.

Species	No. of Strains	No. of Strains at β -lactamase activity			pI Value from this study	pI Value from Other Investigators
		+++	++	+		
<u>B. fragilis</u>	30	8	22	0	4.9	4.9 [82, 83, 85]
	5	1	4	0	4.9, 5.1	4.9, 5.1 [9]
	19	0	0	19	non-detectable	
<u>B. ovatus</u>	9	0	7	2	4.3	4.3, 4.6, 4.8, 7.1 [82]
	5	2	1	2	4.3, 4.6	
	4	0	0	4	non-detectable	
<u>B. distasonis</u>	2	1	1	0	4.8	4.8 [12]
	3	0	3	0	4.8, 4.9	
	3	0	1	2	non-detectable	
<u>B. vulgatus</u>	2	0	2	0	4.5	4.5 [9]
	2	0	1	1	non-detectable	



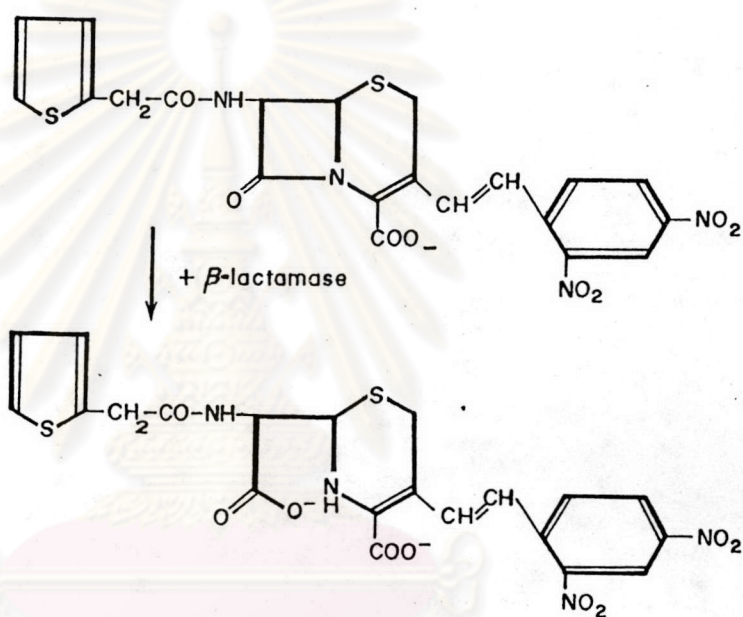
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Figure 1. Microscopic Morphology of B. fragilis (1,000 x)



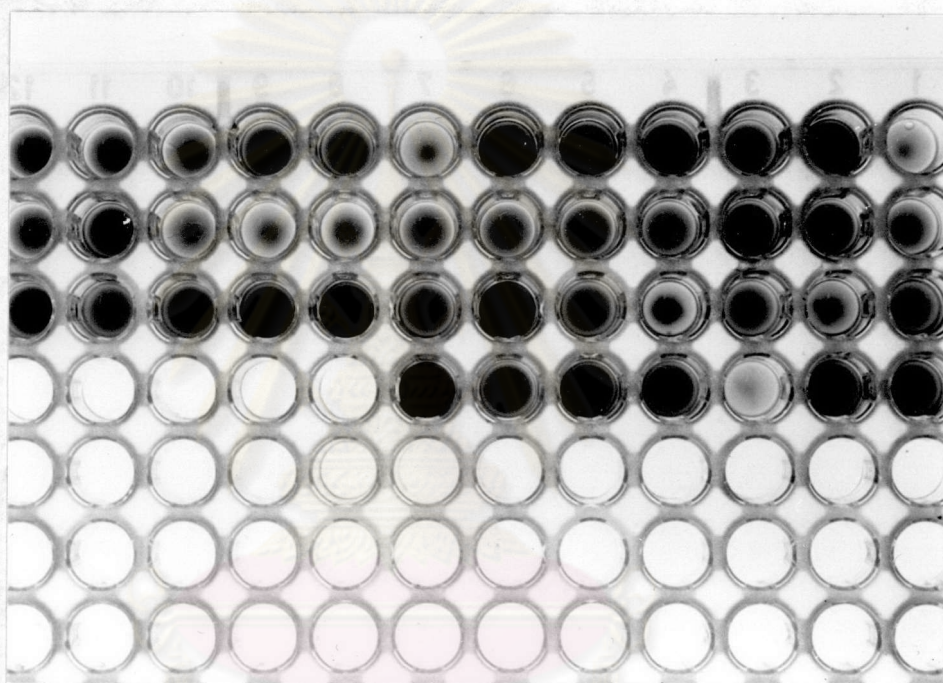
Acrylamide + N,N' -methylene- -----> Polyacrylamide
bisacrylamide

Figure 2. The polymerization reaction of polyacrylamide. The structure of acrylamide, and the crosslinking, N,N' -methylene-bisacrylamide is shown. A section of the crosslinked polyacrylamide is also shown.



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Figure 3. Structure of chromogenic cephalosporin before and after enzymatic hydrolysis. The absorption peak of the solution will change from 386 nm. to 482 nm.



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Figure 4. The color of nitrocefin after incubated with B. fragilis, the result showed both positive (red) and negative (yellow).

Donor : Bacteroides with MIC of Ampicillin ≥ 64 $\mu\text{g./ml.}$

Recipient : B. vulgatus Amp^S Rif^R

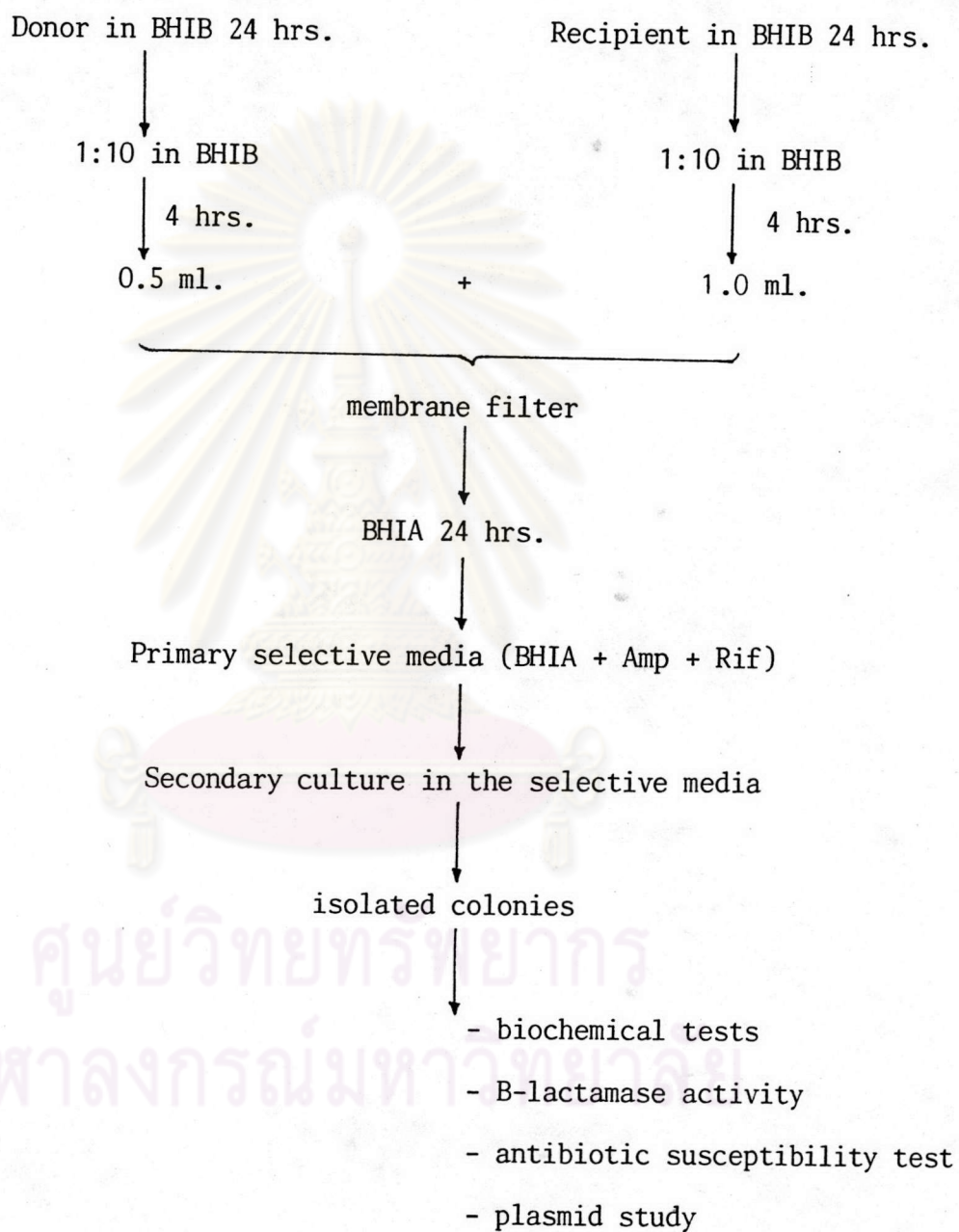


Figure 5. The method of bacterial conjugation.

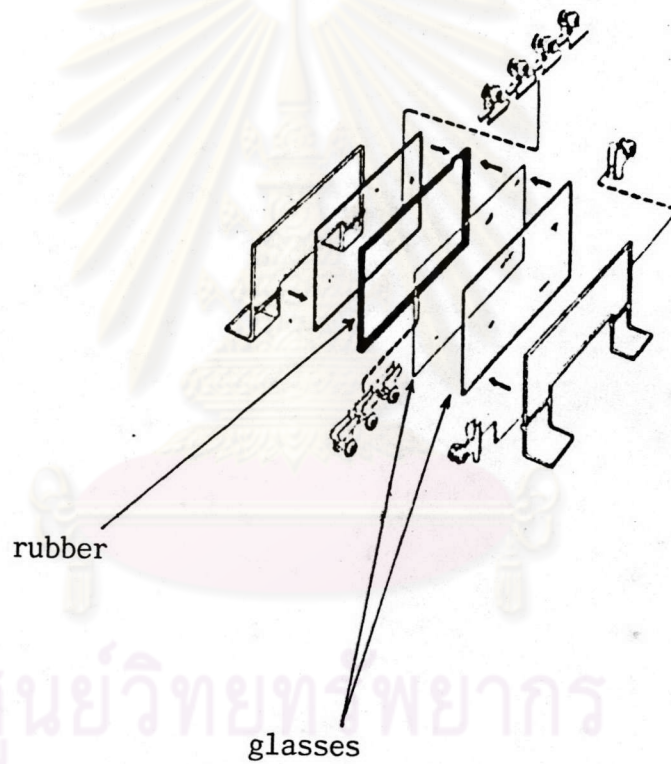


Figure 6. Diagram of the cassette for casting polyacrylamide gels.

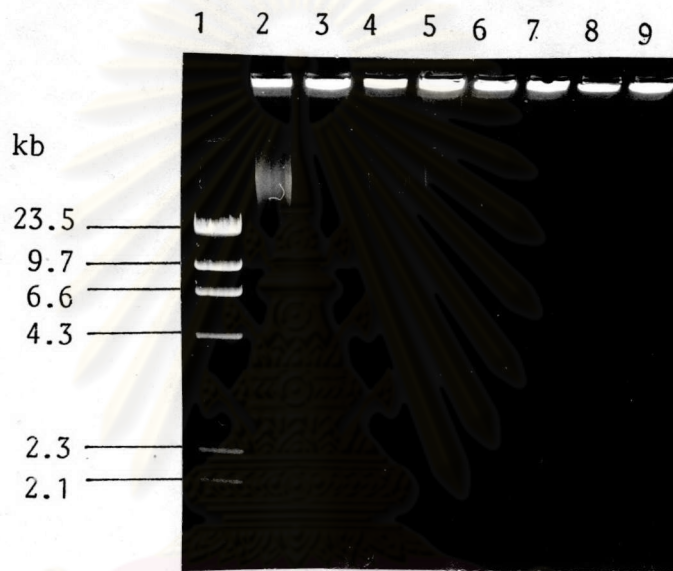


Figure 7. Agarose gel electrophoresis of plasmid DNA of donors, transconjugants, and recipient.

Lane 1 contained reference fragments resulting from Hind III digestion of bacteriophage Lambda.

Lane 2,4,7 were donors No. 30, 32, and 51 respectively.

Lane 3,5,6,8 were transconjugants No. 30, 32, 32, 51 respectively.

Lane 9 was recipient.

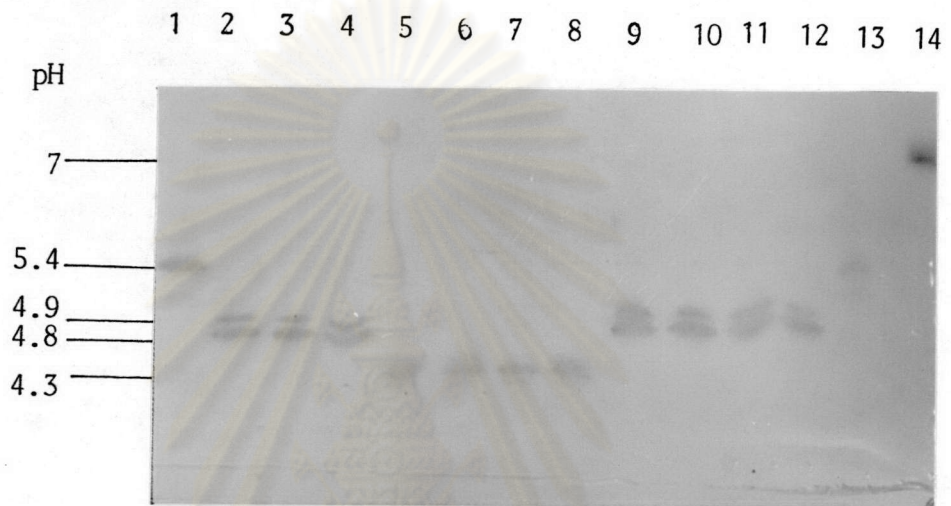


Figure 8. Isoelectric focusing of B-lactamases from various strains of B. fragilis group (ampholine pH 3.5-10)

Lane 1, 13 = TEM-1

Lane 2,3,4 = B-lactamases from various strains of

B. distasonis

Lane 5,6,7,8 = B-lactamases from various strains of B. ovatus

Lane 9,10,11,12 = B-lactamases from various strains of

B. fragilis

Lane 14 = Haemoglobin