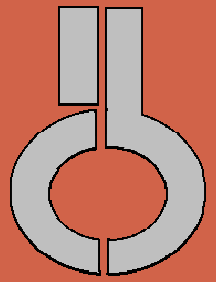
DEPARTAMENTO DE
MICROBIOLOGIA
UNIVERSIDADE DE SÃO PAULO



BMM 5753 – Bacteriologia de Anaeróbios

BOMBAS DE EFLUXO

MSc. Aline Ignacio

2016

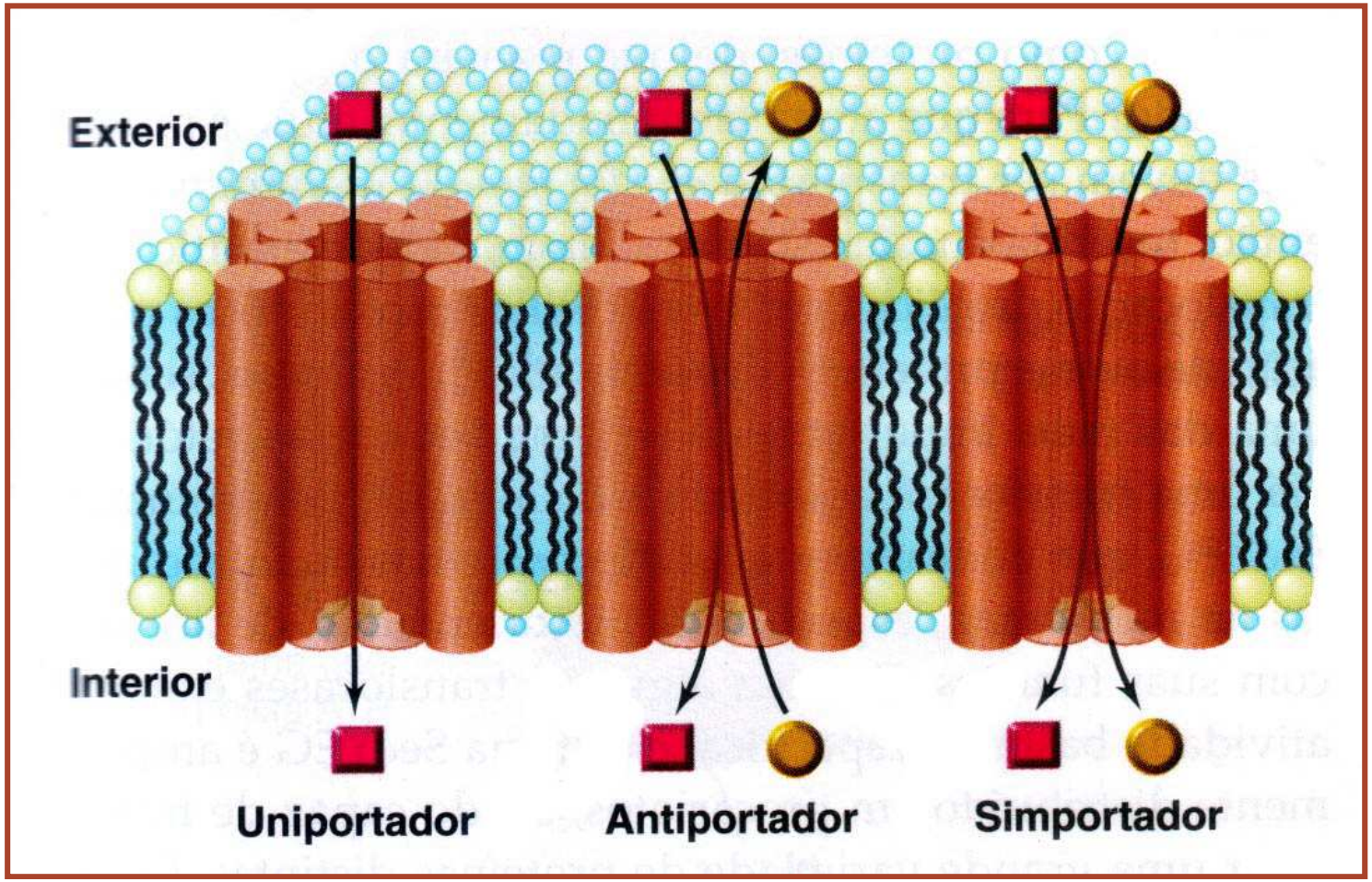
INTRODUÇÃO

- Proteínas transmembrânicas que atuam como transportadores – Procariotos e Eucariotos

- Afinidade por um único substrato ou vários

- Energia:
  - hidrólise do ATP
  - gradiente (H^+, Na^+)

Transportadores transmembrânicos e tipos de transporte



- Influxo de substratos

Aminoácidos

Vitaminas

Açúcares

- Efluxo de substratos

Solventes

Bile e Sais biliares

Cátions tóxicos

Metabólitos

Detergentes

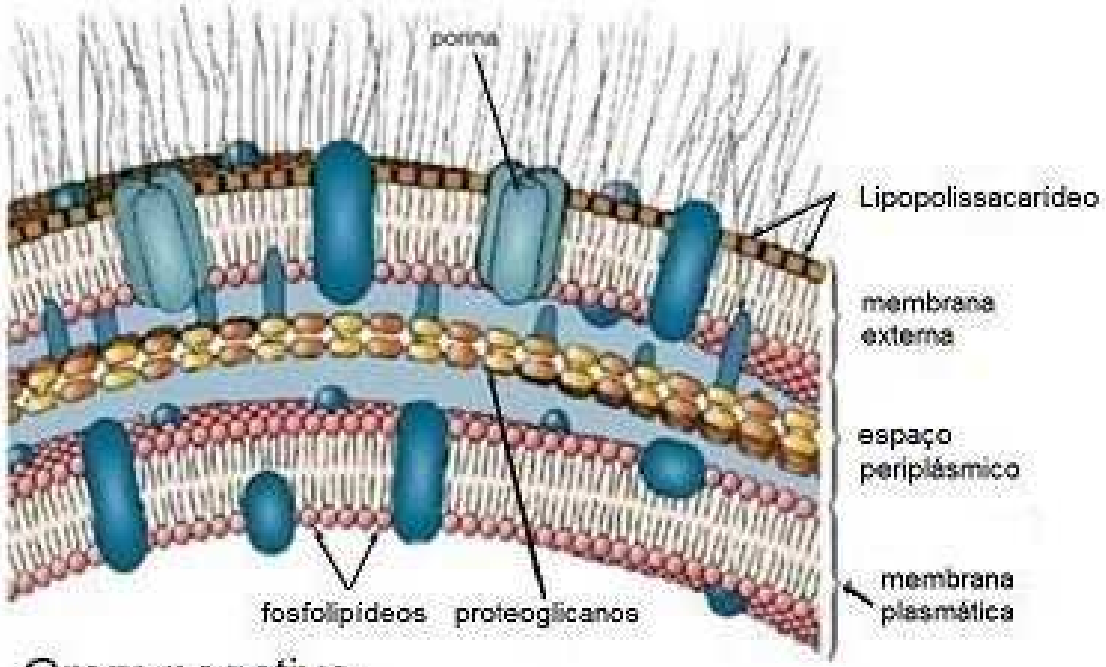
Corantes

Importância

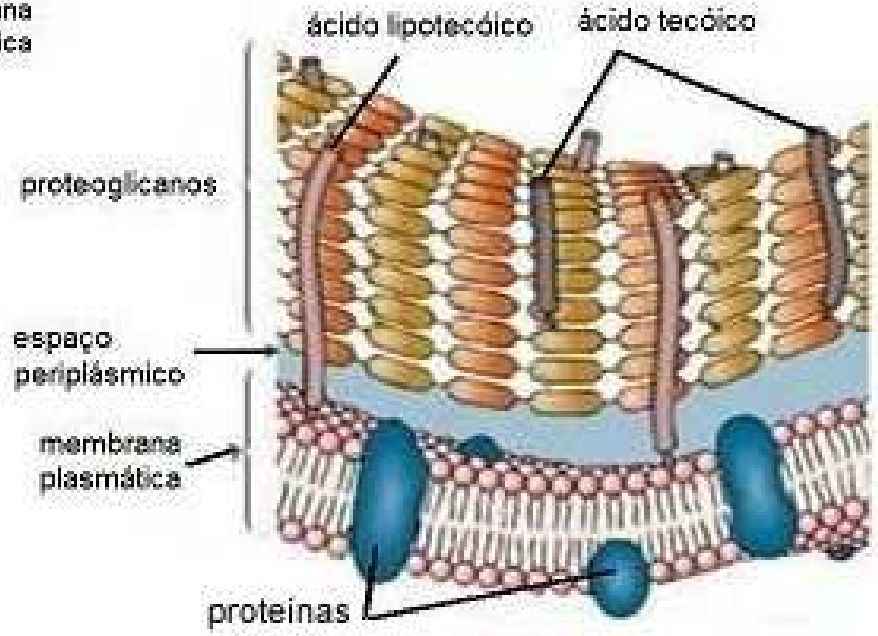
- Homeostase
- Detoxificação
- Comunicação celular – Quorum sensing
- Virulência (?)

Resistência múltipla aos antibióticos

(Multidrug efflux – MDR)

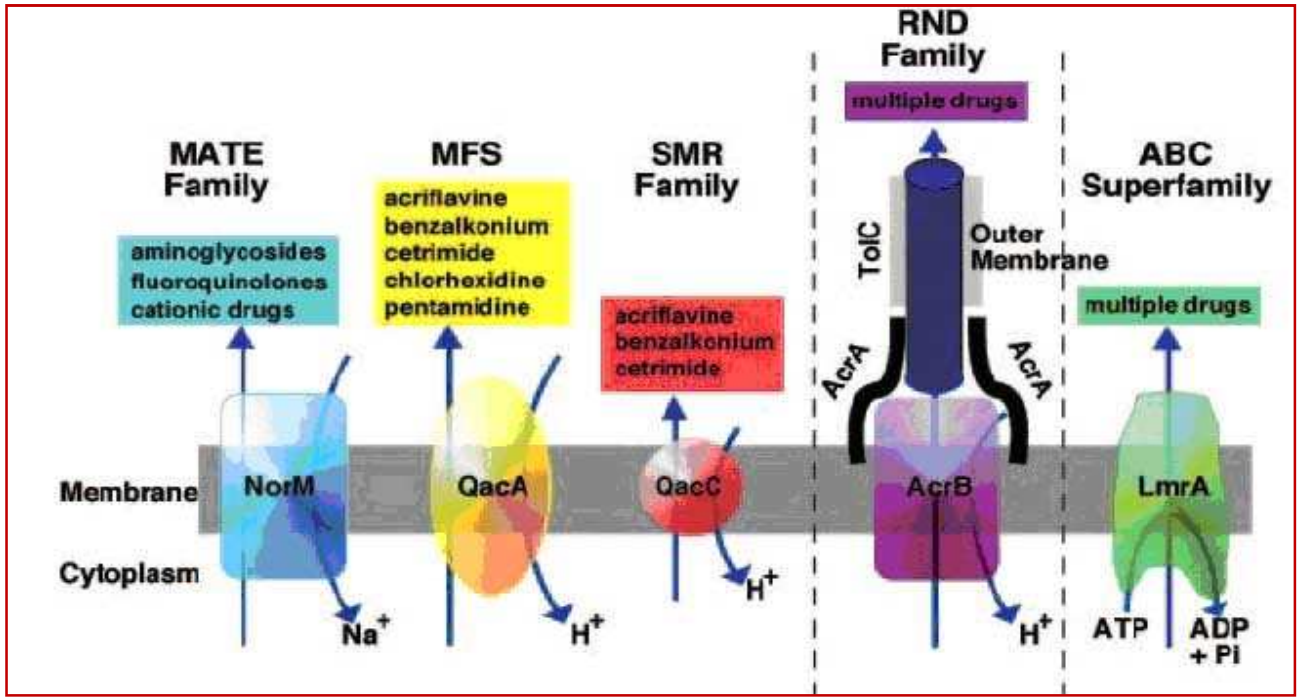


Gram negativo



Gram positivo

CARACTERIZAÇÃO



MFS: Major Facilitator Superfamily

ABC: ATP Binding Cassette

SMR*: Small Multidrug Resistance

RND*: Resistance Nodulation Division

MATE*: Multidrug and Toxic Compound Extrusion

* Exclusiva em procariotos

(Piddock, 2006)

Família MFS

Major Facilitator Superfamily

Procariotos e Eucariotos

gram-positivas

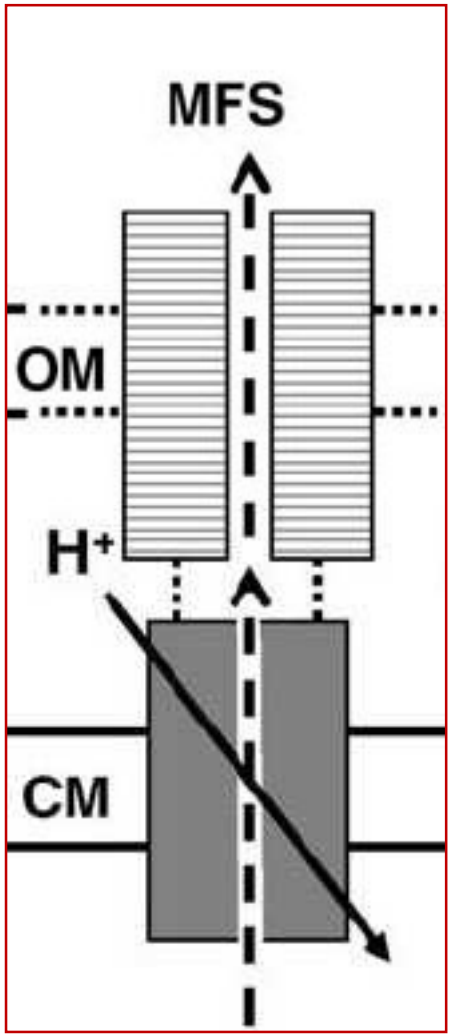
gram-negativas (MFS+Canal ME)

Força próton motiva (H^+)

Açúcares, metabólitos intermediários e drogas (tetraciclinas, cloranfenicol e fluoroquinolonas)

S. aureus (NorB) e *B. subtilis* (LmrB)

E. coli (EmrAB-TolC/EmrKY-TolC)



(Lynch, 2006)

Superfamília ABC

ATP Binding Cassette

Procariotos e Eucariotos

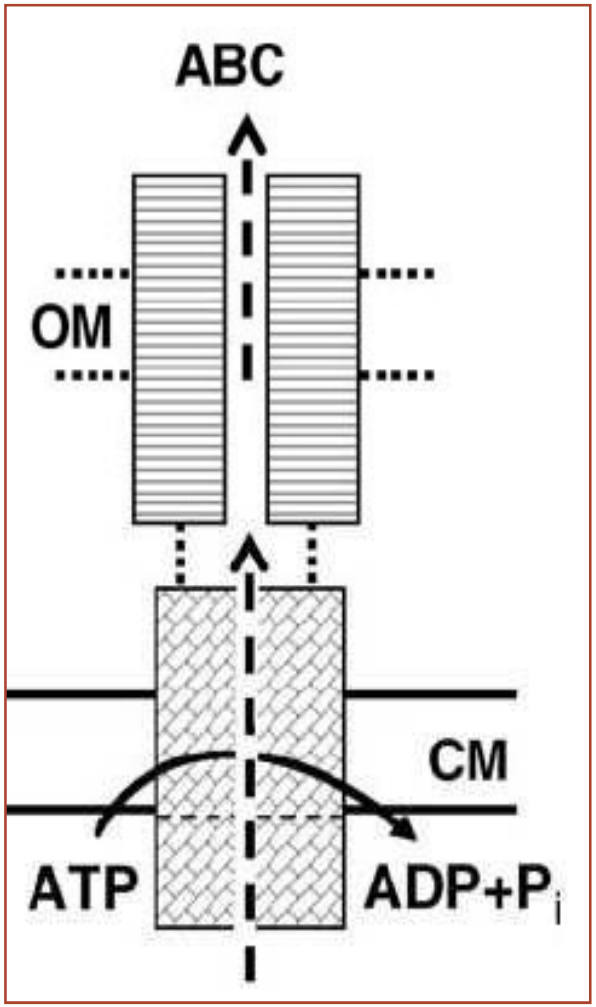
Humanos – agentes tumorais

Hidrólise do ATP

Açúcares, aa, cátions e drogas (macrolídeos, cloranfenicol, tetraciclinas e fluoroquinolonas)

L. lactis (LmrCD) e *S. aureus* (AbcA)

E. coli (MacAB-TolC)



(Lynch, 2006)

Família SMR

Small Multidrug Resistance

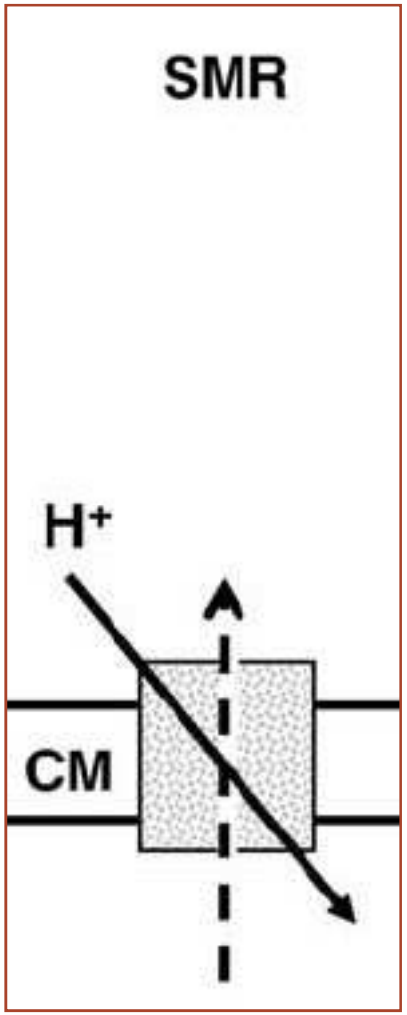
Menor sistema de efluxo

Transporta drogas catiônicas

(macrolídeos e tetraciclinas)

Efluxo realizado por força próton motiva

S. aureus (SepA)



(Lynch, 2006)

Família MATE

Multidrug and Toxic Compound Extrusion

Topologia similar MFS

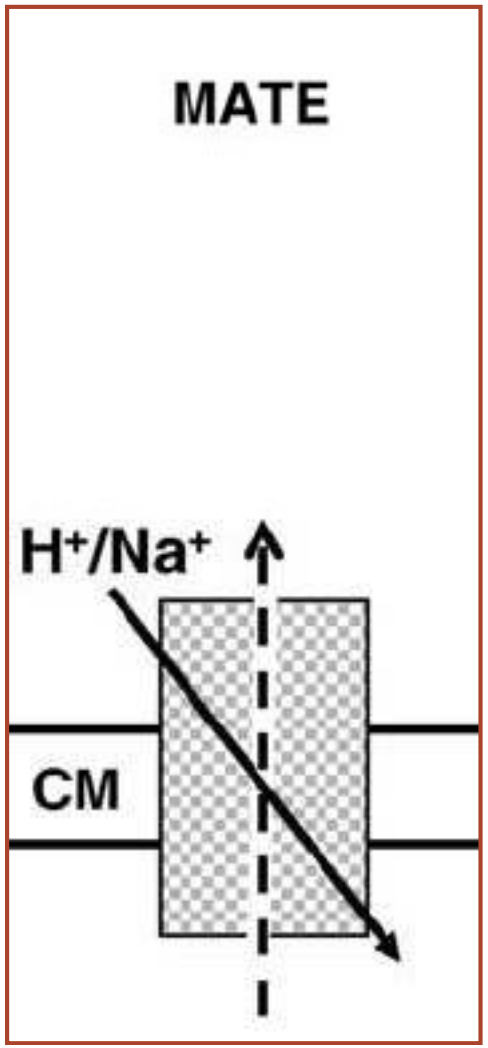
Efluxo realizado por força próton

motiva e gradiente de íons sódio

Transporta detergentes e drogas

(aminoglicosídeos e fluoroquinolonas)

S. aureus (MepA) e *H. influenza* (HmrM)



(Lynch, 2006)

Família RND

Resistance Nodulation Division

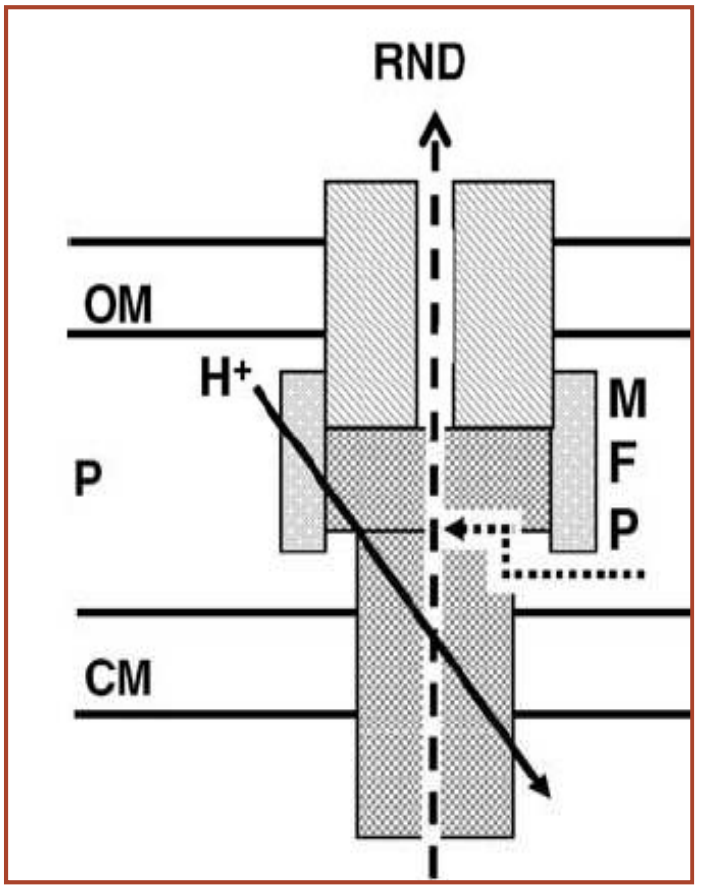
Gradiente de prótons

Transporta moléculas lipofílicas e anfifílicas

Responsável pela tolerância de bactérias a solventes

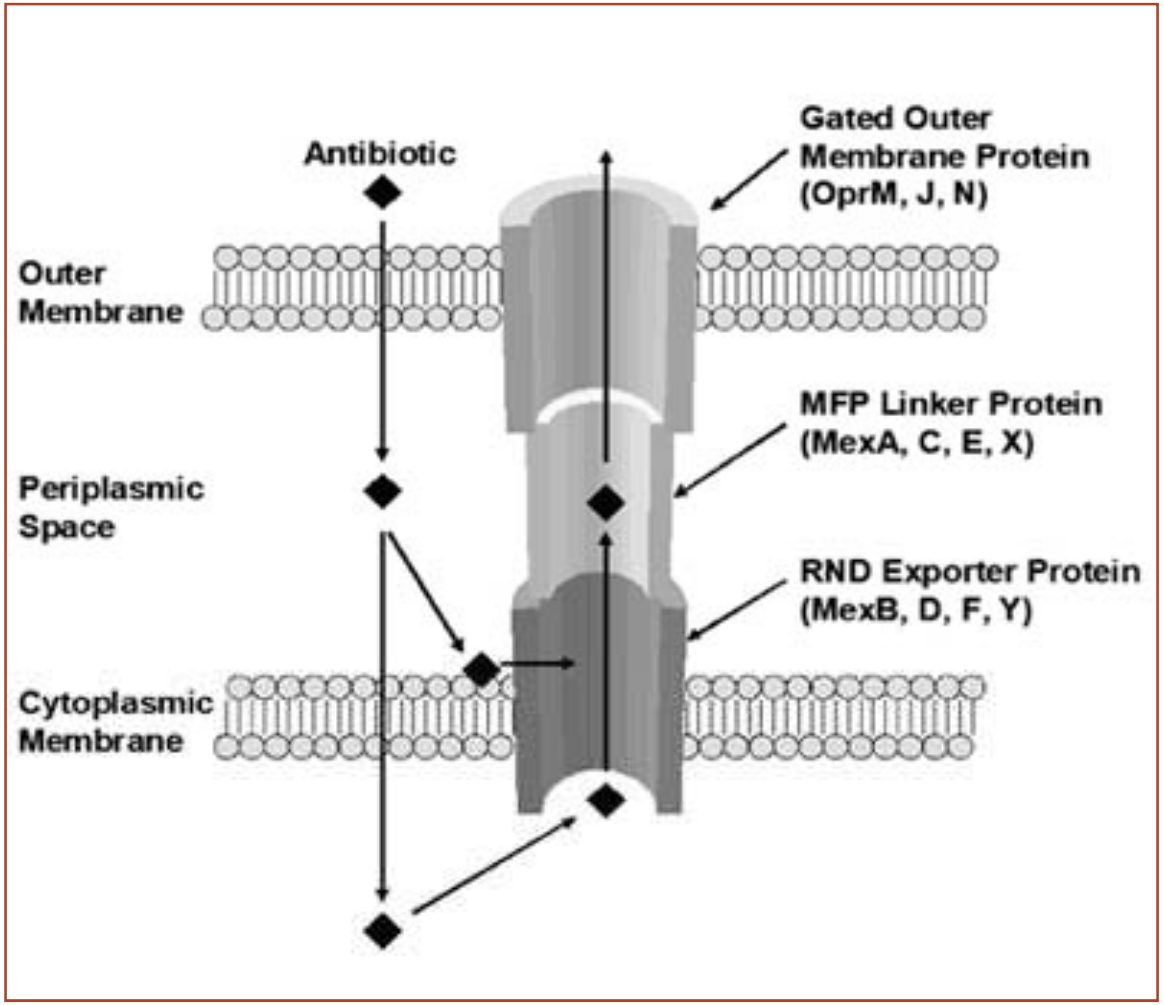
Aminoglicosídeos, fluoroquinolonas, macrolídeos, tetraciclinas e β-lactâmicos

A bomba está divida em três partes



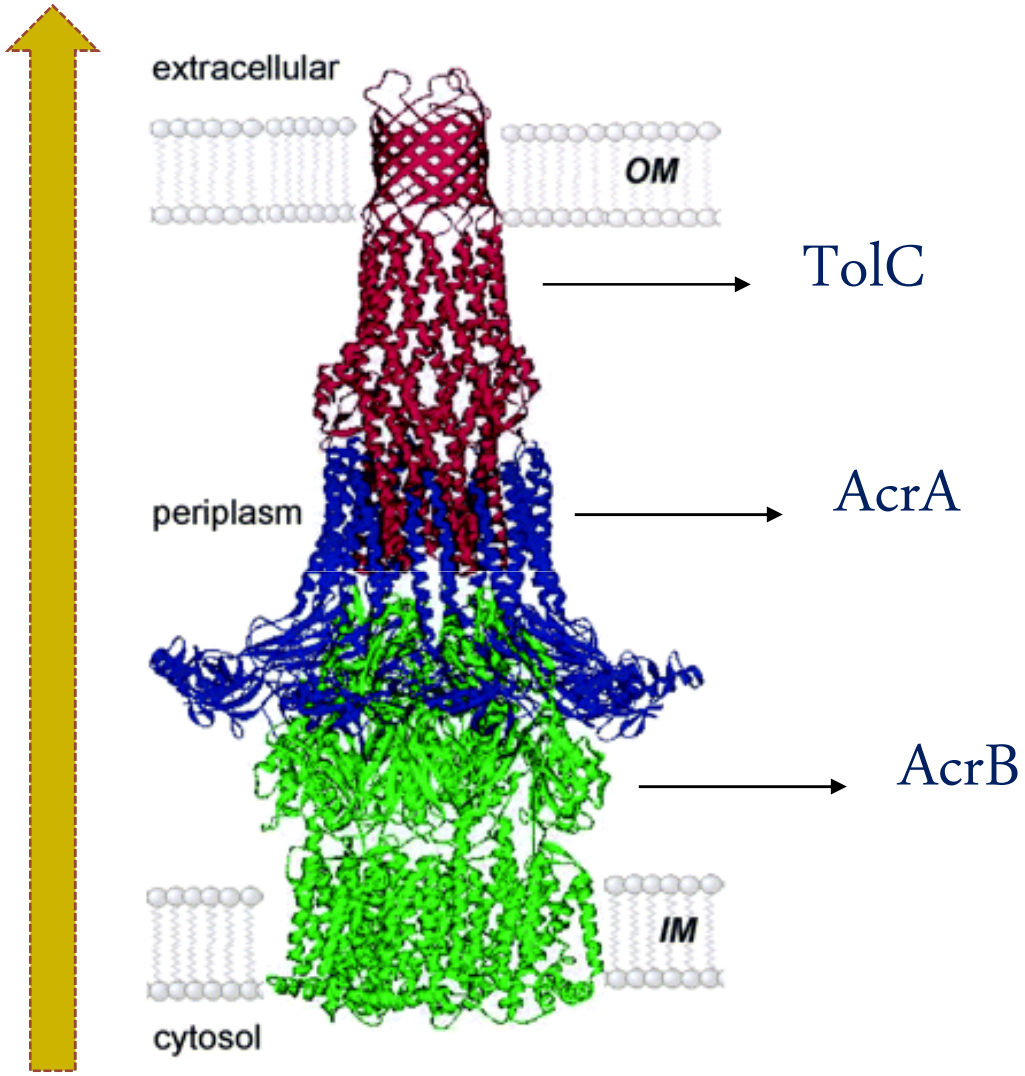
(Lynch, 2006)

P. aeruginosa

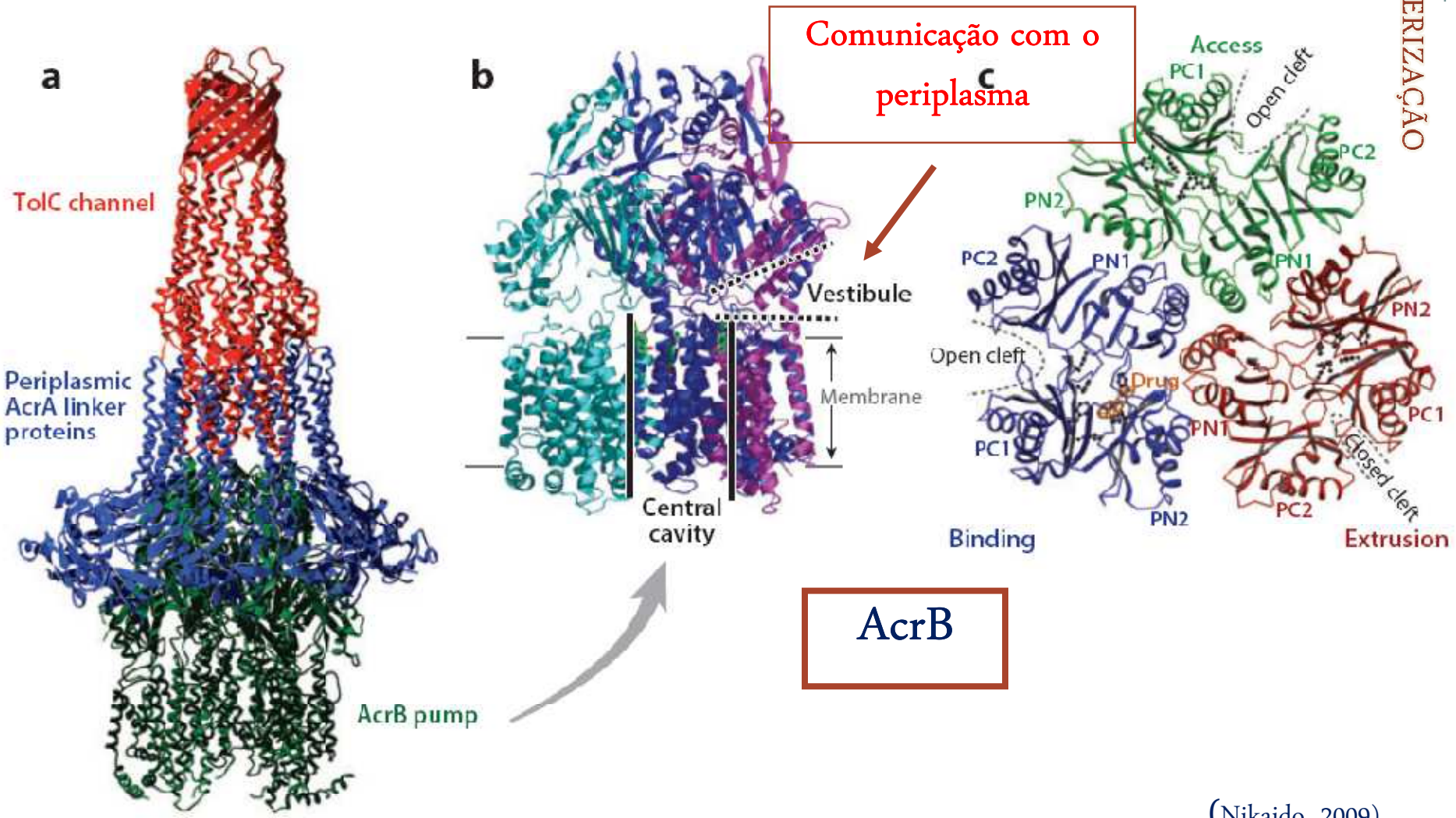


Cada um dos três componentes é essencial para o efluxo da droga e a ausência de um deles pode comprometer todo o funcionamento do complexo.

E. coli



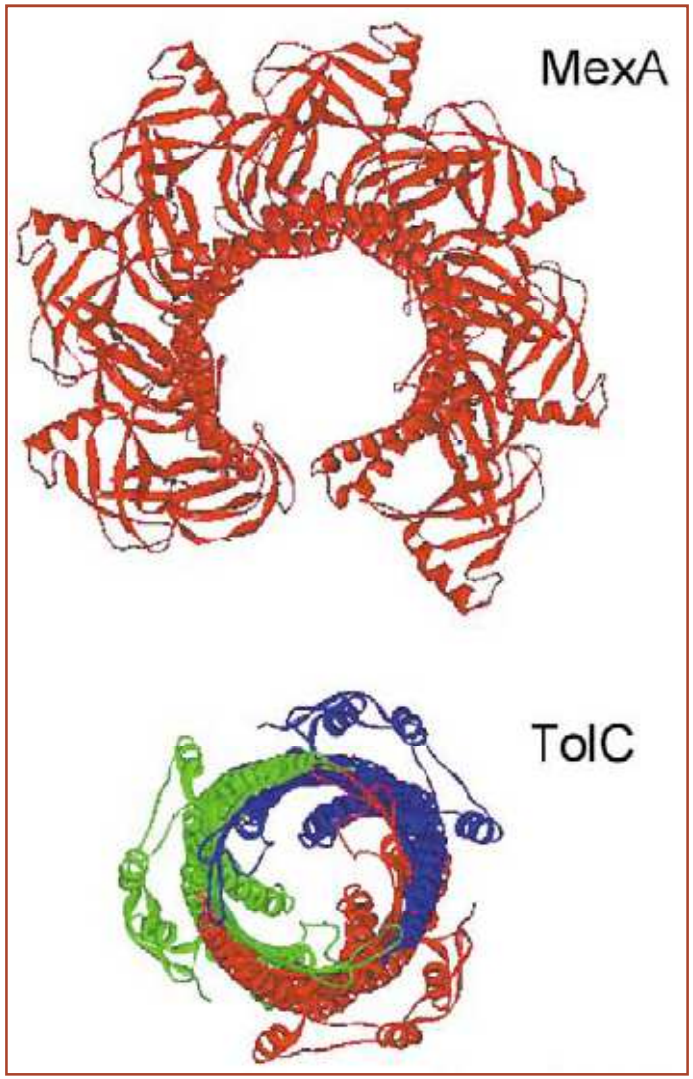
PIDDOCK (2006)

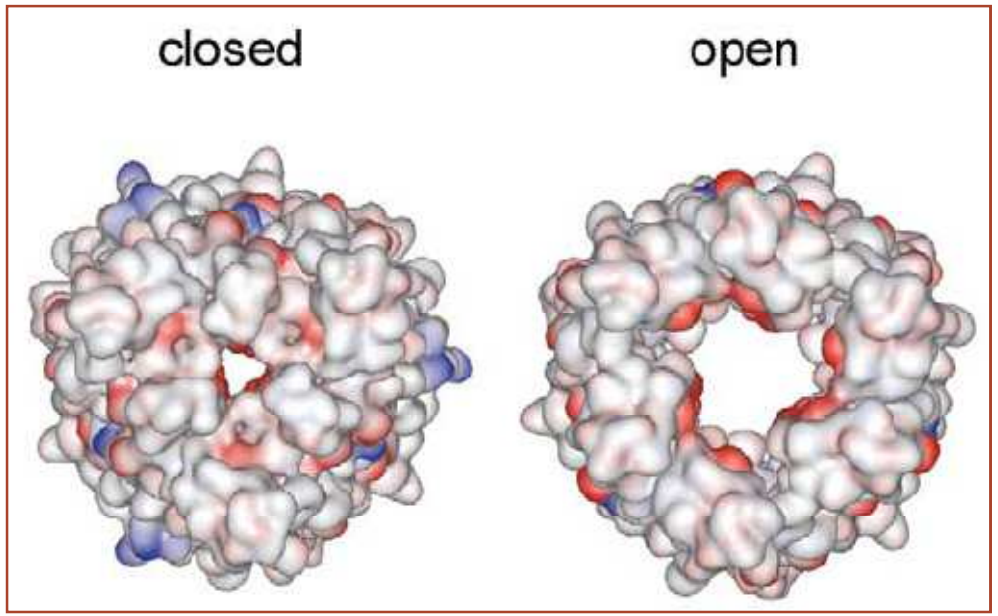


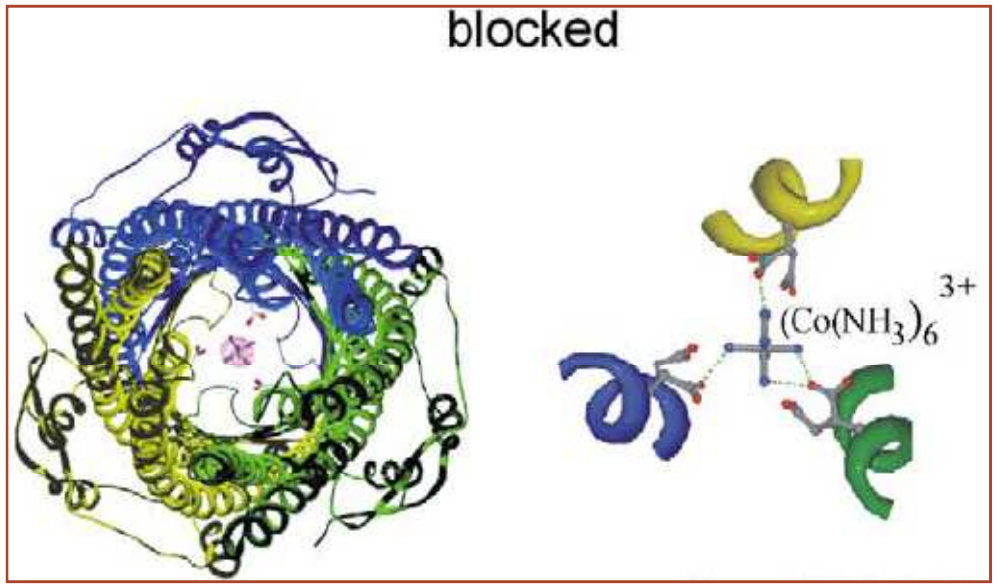
Comunicação com o periplasma

AcrB

(Nikaido, 2009)







(Eswaran et al., 2004)

K. pneumoniae

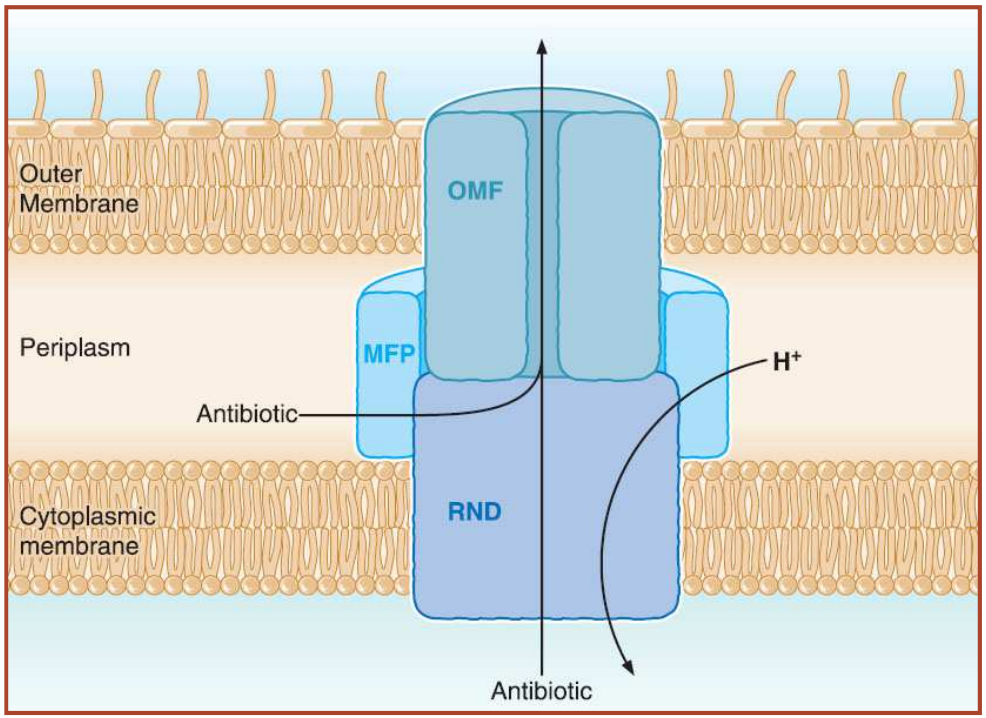
(AcrABR)

P. aeruginosa

(MexAB-OprM)

E. coli

(AcrAB-TolC)



A. baumannii

(AdeIJK)

P. aeruginosa

(MexXY-OprM)

P. aeruginosa

(MexCD-OprJ)

N. gonorrhoeae

(FarAB-MtrE)

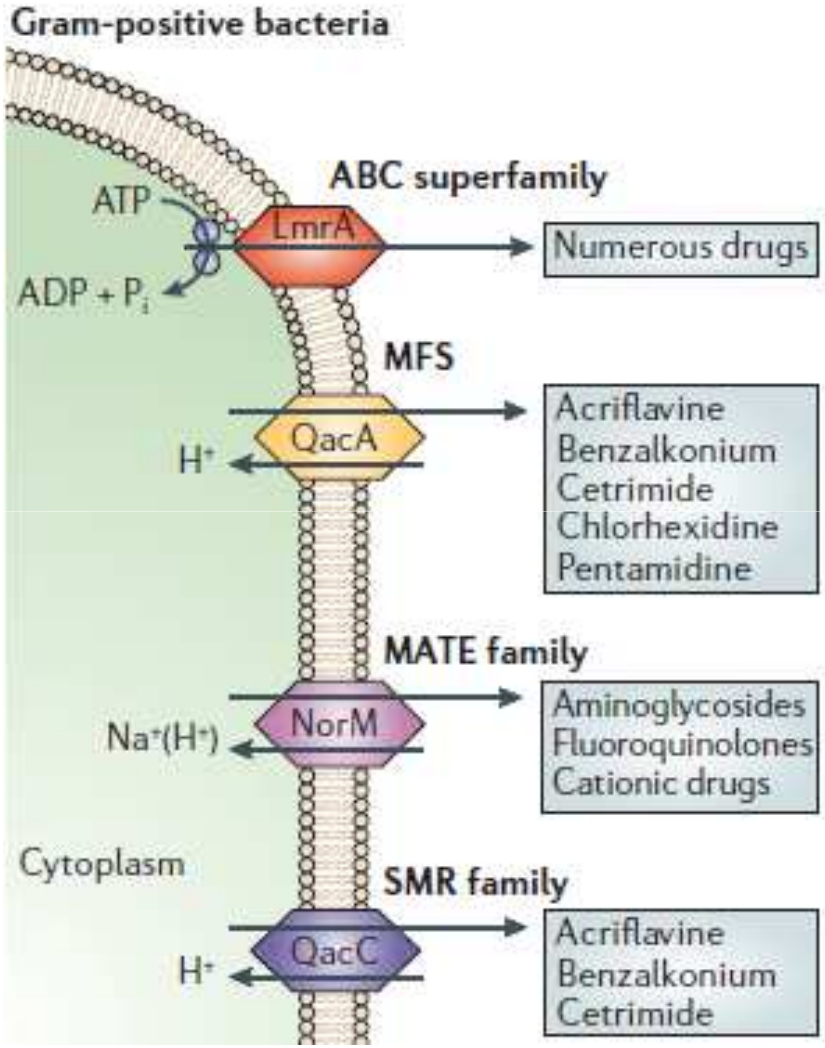
S. enterica sorovar Typhimurium

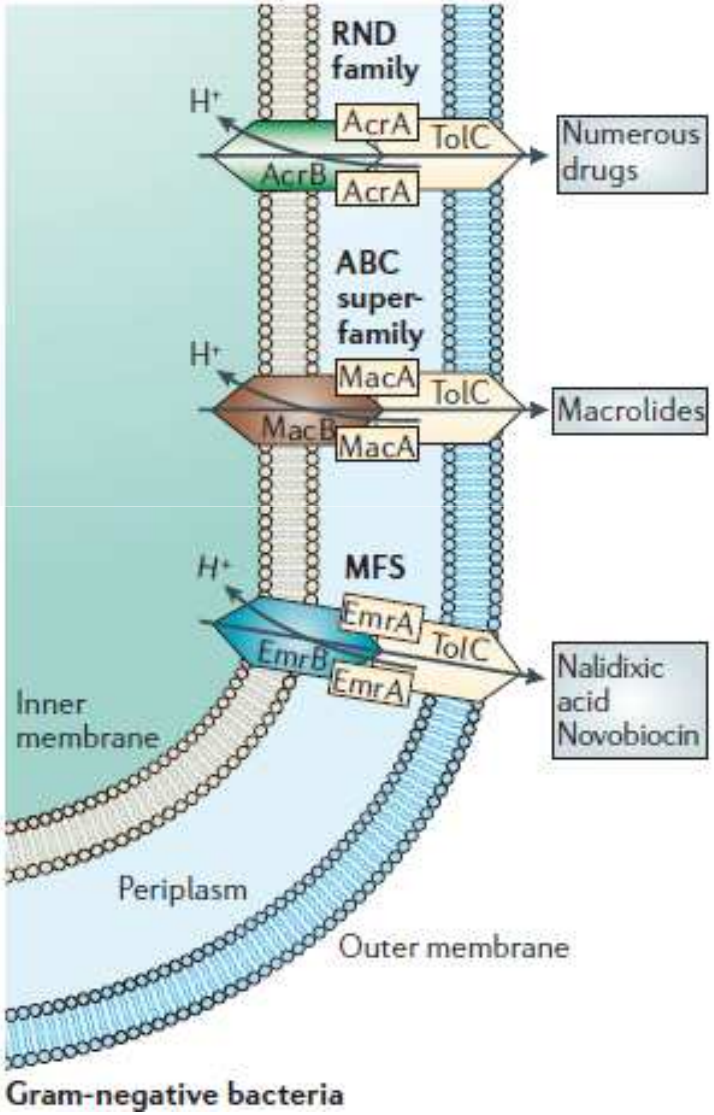
(AcrAB-TolC)

P. aeruginosa

(MexEF-OprN)

Resistência intrínseca



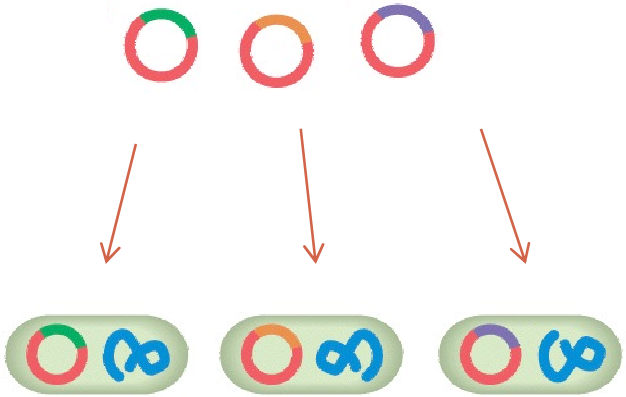


Como podemos estudar

resistência associada às bombas de efluxo?

Como estudar resistência associada às bombas de efluxo?

- ❑ Genômica e Microarray
- ❑ Clonagem e expressão
- ❑ Construção de cepas mutantes (mutação pontual ou deleção)
- ❑ Complementação do mutante (deleção)
- ❑ Perfil de substratos



Journal of Antimicrobial Chemotherapy (2003) **52**, 176–179
DOI: 10.1093/jac/dkg308
Advance Access publication 1 July 2003

JAC

An RND-type multidrug efflux pump SdeXY from *Serratia marcescens*

Jing Chen, Teruo Kuroda, Md Nazmul Huda, Tohru Mizushima and Tomofusa Tsuchiya*

Department of Microbiology, Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700-8530, Japan

pSTV28 – plasmídio/vetor

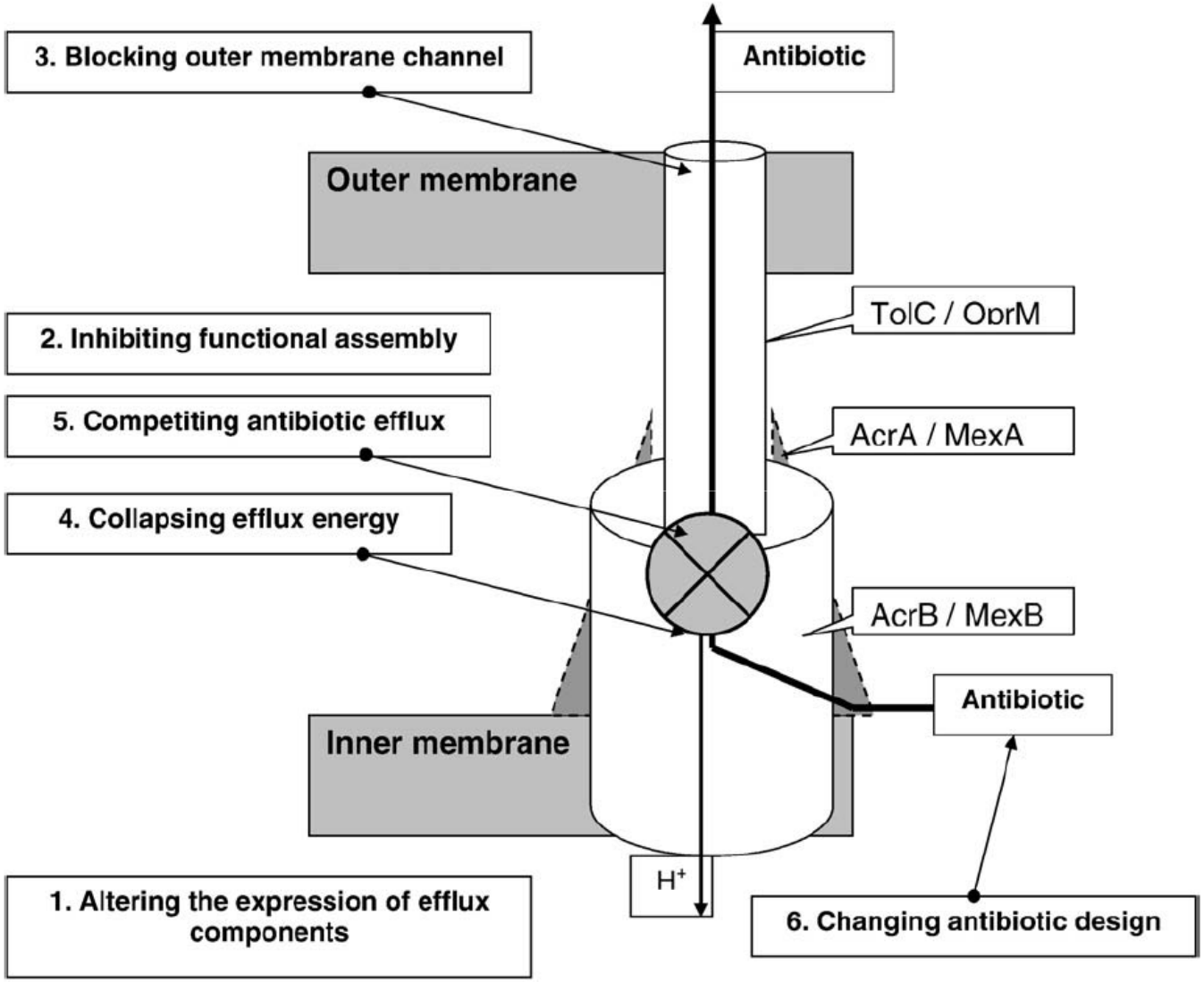
pSEC38: sedXY

Transformaram: *E. coli* KAM 32 (Δ acrAB)

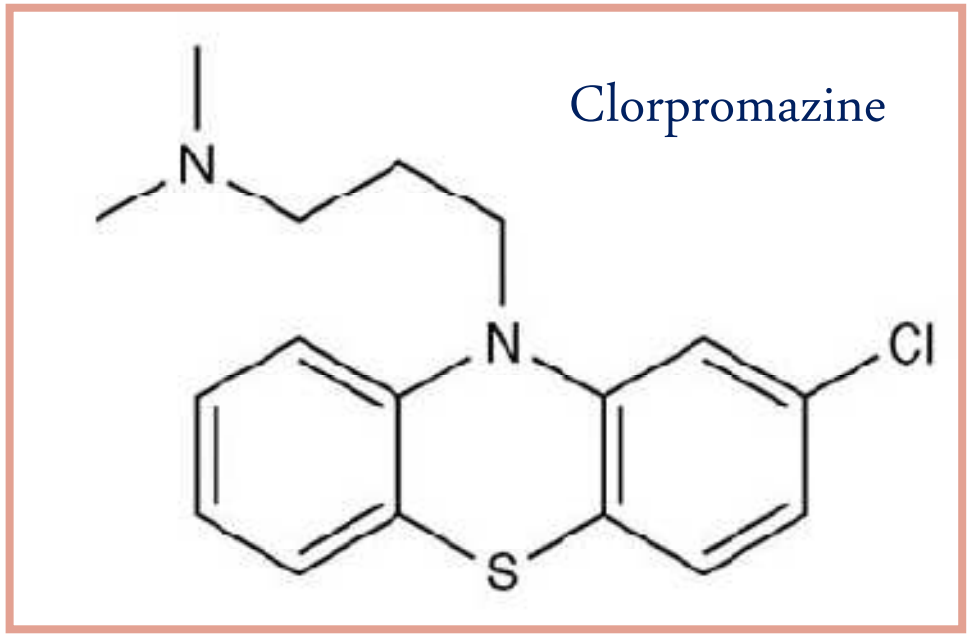
Table 2. MICs of various antimicrobial agents for *E. coli* KAM32 harbouring pSTV28 (control) or pSEC38 (carrying *sdeXY*)

Drug	MIC (mg/L)	
	KAM32/pSTV28	KAM32/pSEC38
Erythromycin	4	64
Tetracycline	0.5	2
Norfloxacin	0.016	0.06
Ampicillin	8	16
Streptomycin	2	2
Benzalkonium chloride	2.5	40
Chlorhexidine gluconate	1	2.5
Triclosan	1	4
Acriflavine	2	64
Ethidium bromide	4	>128
Rhodamine 6G	4	128
Hoechst 33342	0.5	16

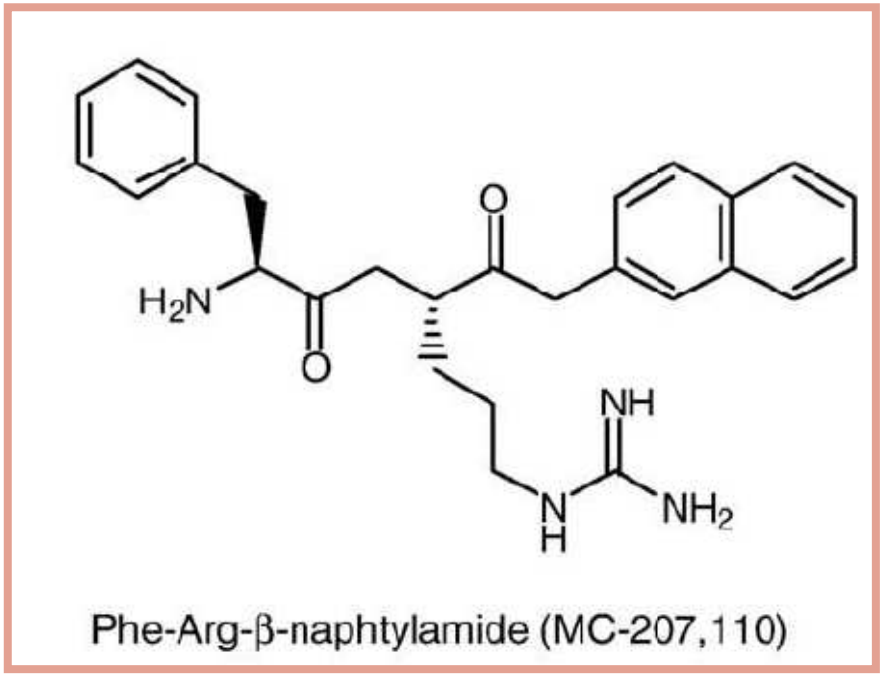
Uso de inibidores



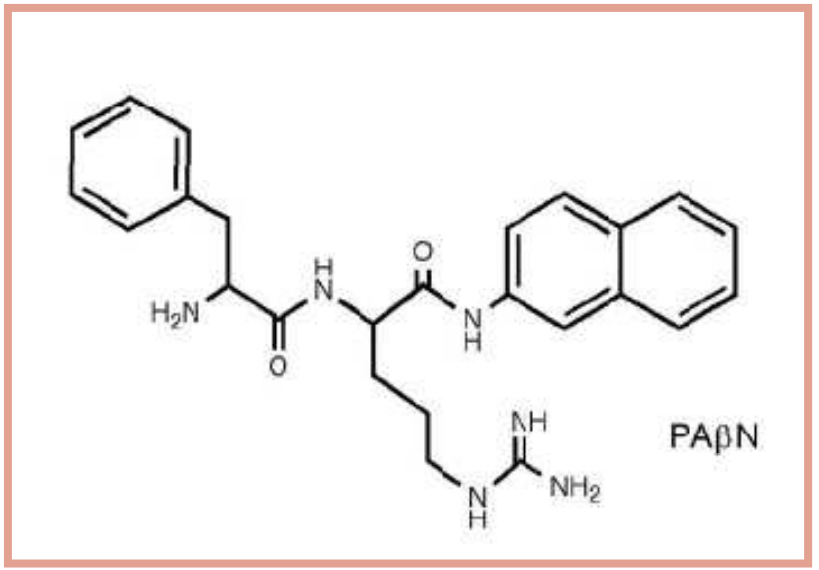
Pagés & Amaral (2009)



Redução da expressão de componentes da bomba

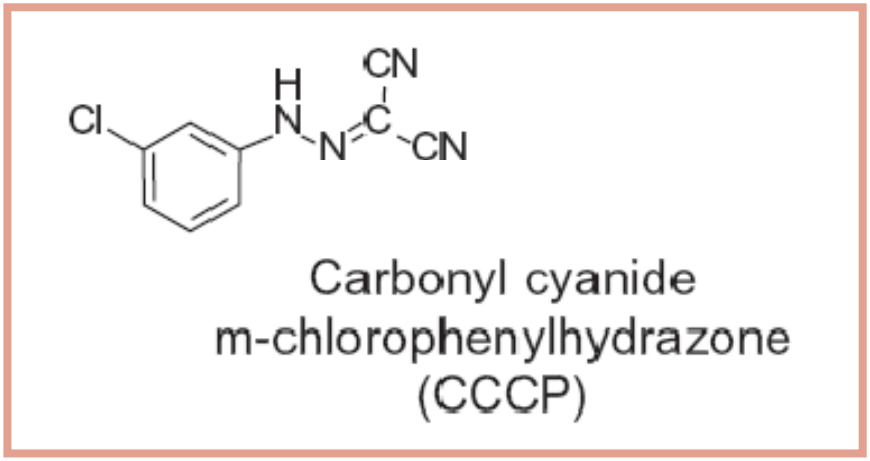


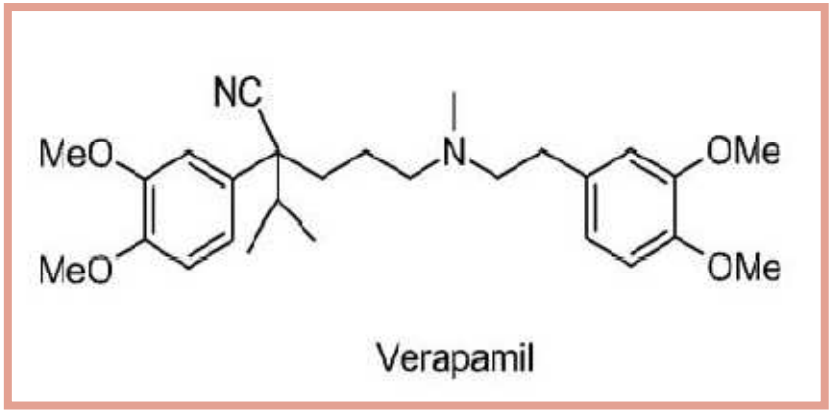
Phe-Arg-β-naphtylamide (MC-207,110)

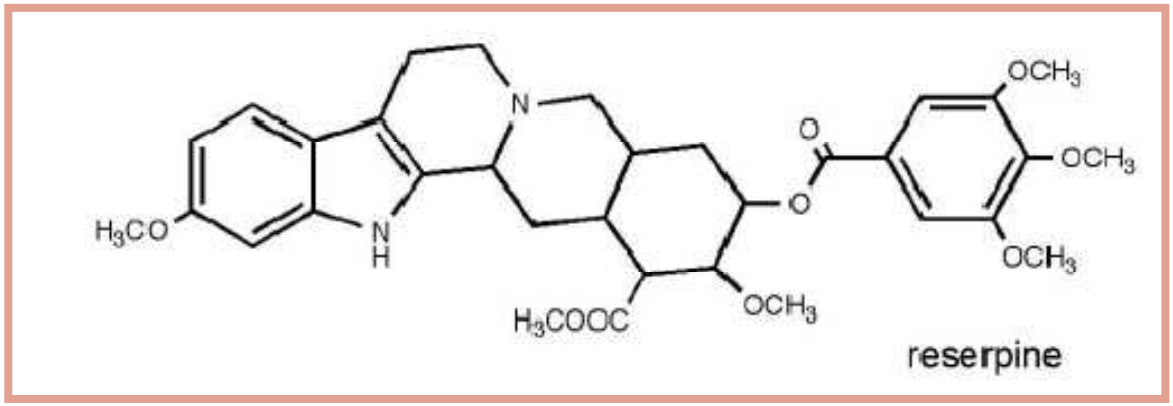


PAβN

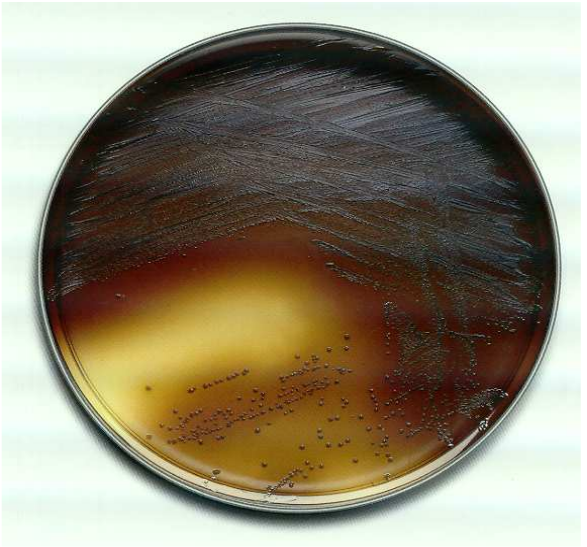
Competem com o antibiótico pelo sítio de ligação na bomba de efluxo



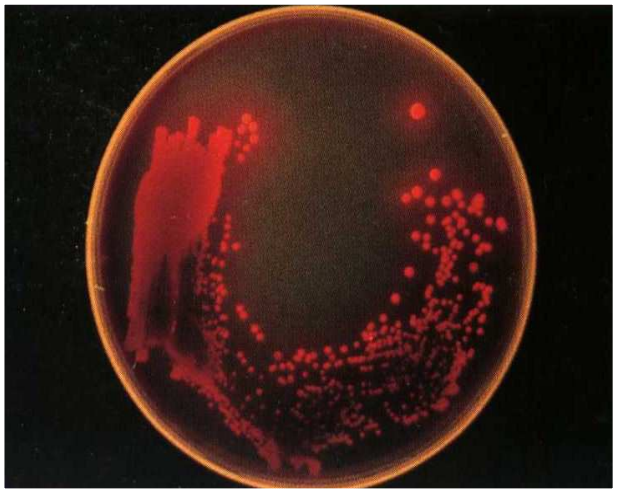




Causam colapso no sistema de energia da bomba



ANAERÓBIOS



Bombas MATE foram encontradas em *Bacteroides thetaiotaomicron* (Miyamae et al., 2001)

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2001, p. 3341–3346 Vol. 45, No. 12
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.12.3341–3346.2001

A MATE Family Multidrug Efflux Transporter Pumps out Fluoroquinolones in *Bacteroides thetaiotaomicron*

SHIN MIYAMAE,[1,2,3] OHMI UEDA,[2] FUMINOBU YOSHIMURA,[1,2] JAIWEON HWANG,[1]†
YOSHINOBU TANAKA,[3] AND HIROSHI NIKAIDO[1]*

Department of Molecular and Cell Biology, University of California, Berkeley, California,[1] and Department of Microbiology[2] and the First Department of Prosthodontics,[3] School of Dentistry, Aichi-Gakuin University, Nagoya, Japan

5 – 10 Kb

E. coli sensível

LB norfloxacina (50 ng)

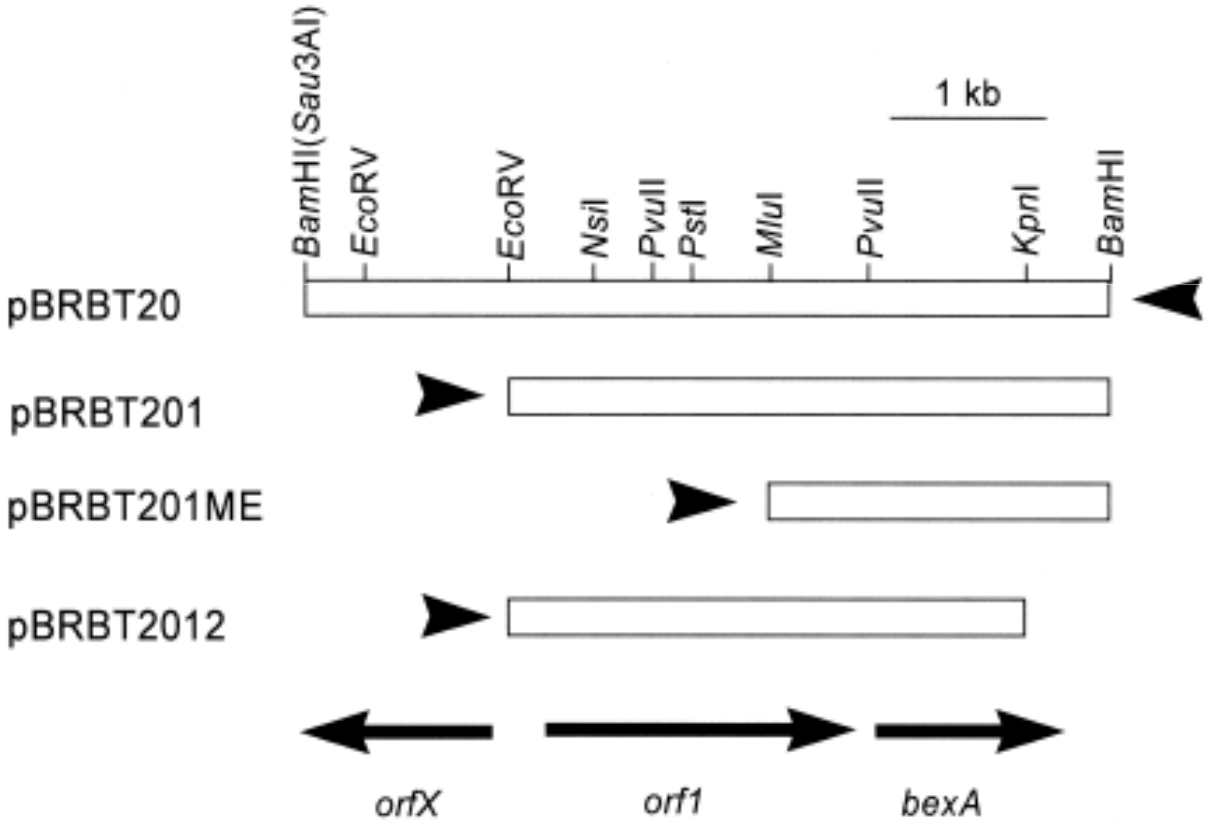


TABLE 1. Antibiotic susceptibilities of various strains[a]

Strain	MIC (μg/ml)				
	Norfloxacin	Ciprofloxacin	Sparfloxacin	Ethidium bromide	Chloramphenicol
E. coli					
AG102AX[b]	0.03	0.004	0.001	8	1
AG102AX/pBRBT20	0.25	0.016	0.001	64	1
AG102AX/pBRBT201	0.25	0.016	0.001	64	1
AG102AX/pBRBT201ME	0.25	0.016	0.001	64	1
AG102AX/pBRBT2012	0.03	0.004	0.001	8	1
AG102AX/pYEB	0.03	0.004	0.001	8	1
AG102AX/pNEB	0.25	0.016	0.001	64	1
AG102AX/pNBE	0.03	0.004	0.001	8	1
DH5α[b]	0.25	0.06	0.03	64	4
DH5α/pUC18BT201	1.0	0.25	0.03	512	4
B. thetaiotaomicron					
ATCC 29741	128	16	2	128	14
OUT4 (*bexA* mutant)	32	8	2	32	14

Bombas MATE foram encontradas em *Clostridium difficile* (Dridi et al., 2004)

MICROBIAL DRUG RESISTANCE
Volume 10, Number 3, 2004

CdeA of *Clostridium difficile*, a New Multidrug Efflux Transporter of the MATE Family

L. DRIDI, J. TANKOVIC, and J.-C. PETIT

2– 10 Kb

Isolado resistente

E. coli sensível

DH10B

Ticarcilina, norfloxacina e

brometo de etídio

TABLE 2. ANTIBIOTIC SUSCEPTIBILITIES OF *E. COLI* AG100A AND *C. PERFRINGENS* 13R

	MICs (mg/L)					
	E. coli				C. perfringens	
Drugs	*AG100A/pCDE6*	*AG100A/pUC18*	*DH10B/pCDE11*	*DH10B/pBK-CMV*	*13R/pCDE3*	*13R/pKNT19*
Norfloxacin	0.03	0.03	0.25	0.03	2	2
Ciprofloxacin	0.006	0.006	0.03	0.003	0.5	0.5
Chloramphenicol	4	4	ND	ND	ND	ND
Tetracyclin	8	8	ND	ND	ND	ND
Erythromycin	16	16	ND	ND	ND	ND
Gentamicin	2	2	ND	ND	ND	ND
Ethidium bromide	128	8	512	8	64	4
Acriflavin	64	4	ND	ND	128	2

Primeira bomba RND foi em *Porphyromonas gingivalis*

XepCAB (Ikeda e Yoshimura, 2002)

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2002, p. 3257–3260 Vol. 46, No. 10
0066-4804/02/$04.00+0 DOI: 10.1128/AAC.46.10.3257–3260.2002

A Resistance-Nodulation-Cell Division Family Xenobiotic Efflux Pump in an Obligate Anaerobe, *Porphyromonas gingivalis*

Takeshi Ikeda[1,2]* and Fuminobu Yoshimura[1]

Department of Microbiology[1] and Research Institute of Advanced Oral Science,[2] School of Dentistry, Aichi-Gakuin University, Nagoya 464-8650, Japan

AcrB

AcrA

TolC

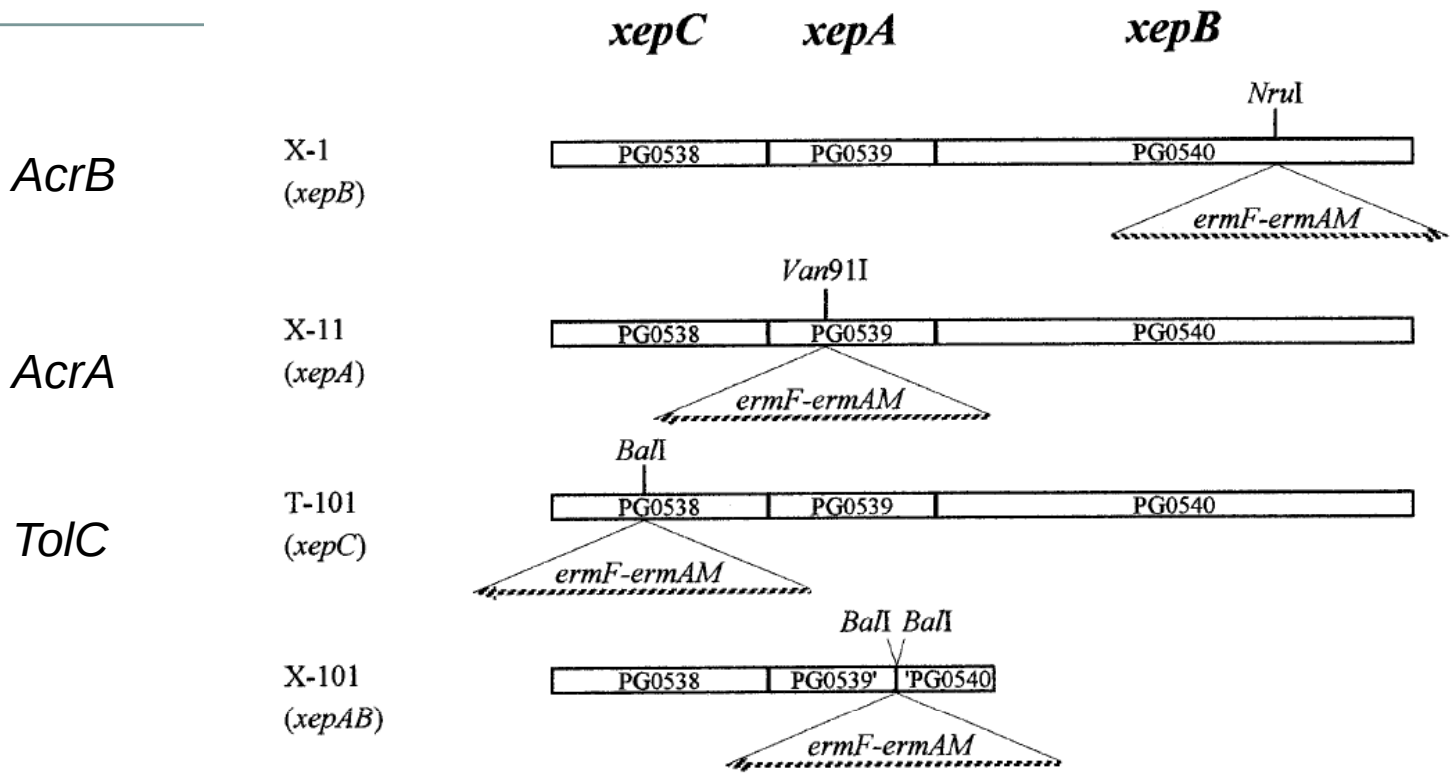


TABLE 2. MICs of various agents for *P. gingivalis* strains

Strain	MIC (μg/ml)[a]											
	Ethidium bromide	Puro-mycin	Rifam-pin	Nor-floxacin	Oflox-acin	Cipro-floxacin	Spar-floxacin	Tetra-cycline	Mino-cycline	Ber-berine	Acri-flavine	SDS
ATCC 33277 (parent)[b]	5	12.5	0.012	4	0.5	1	0.4	0.12	0.05	32	2.5	500
X-1 (*xepB*)	0.6	3.1	0.003	2	0.25	0.5	0.4	0.06	0.025	16	1.25	125
X-11 (*xepA*)[c]	0.6	3.1	0.003	2	0.25	0.5	0.4	0.06	0.025	16	1.25	125
T-101 (*xepC*)	0.6	3.1	0.003	2	0.5	1	0.2	0.12	0.05	16	1.25	125

Primeiro relato de bombas RND em *B. fragilis* foi em 2005

(Ueda et al.)

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 2005, p. 2807–2815 Vol. 49, No. 7
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.7.2807–2815.2005

Sixteen Homologs of the Mex-Type Multidrug Resistance Efflux Pump in *Bacteroides fragilis*

Ohmi Ueda,[1]* Hannah M. Wexler,[2] Kaname Hirai,[1] Yukinaga Shibata,[3] Fuminobu Yoshimura,[4] and Setsuo Fujimura[1]

Department of Oral Microbiology, Matsumoto Dental University, Shiojiri, Japan[1]; Department of Wadsworth Anaerobe Laboratory, GLAVAHCS, and Department of Medicine, UCLA School of Medicine, Los Angeles, California[2]; Department of Institute for Oral Science, Matsumoto Dental University, Shiojiri, Japan[3]; and Department of Microbiology, School of Dentistry, Aichi-Gakuin University, Nagoya, Japan[4]

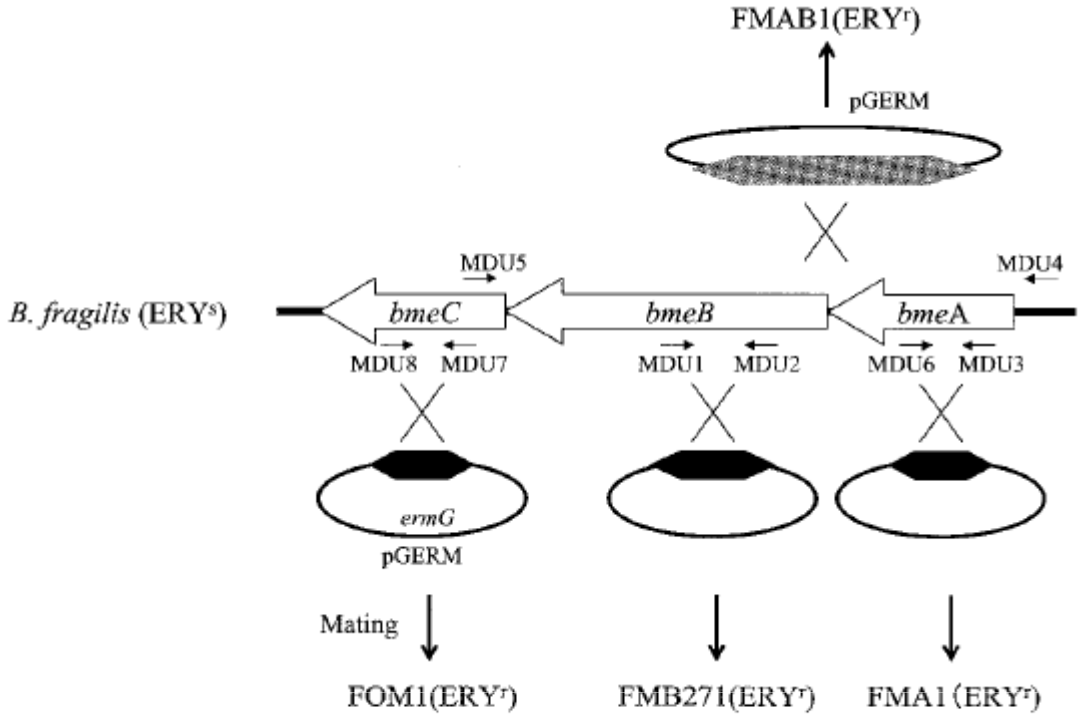
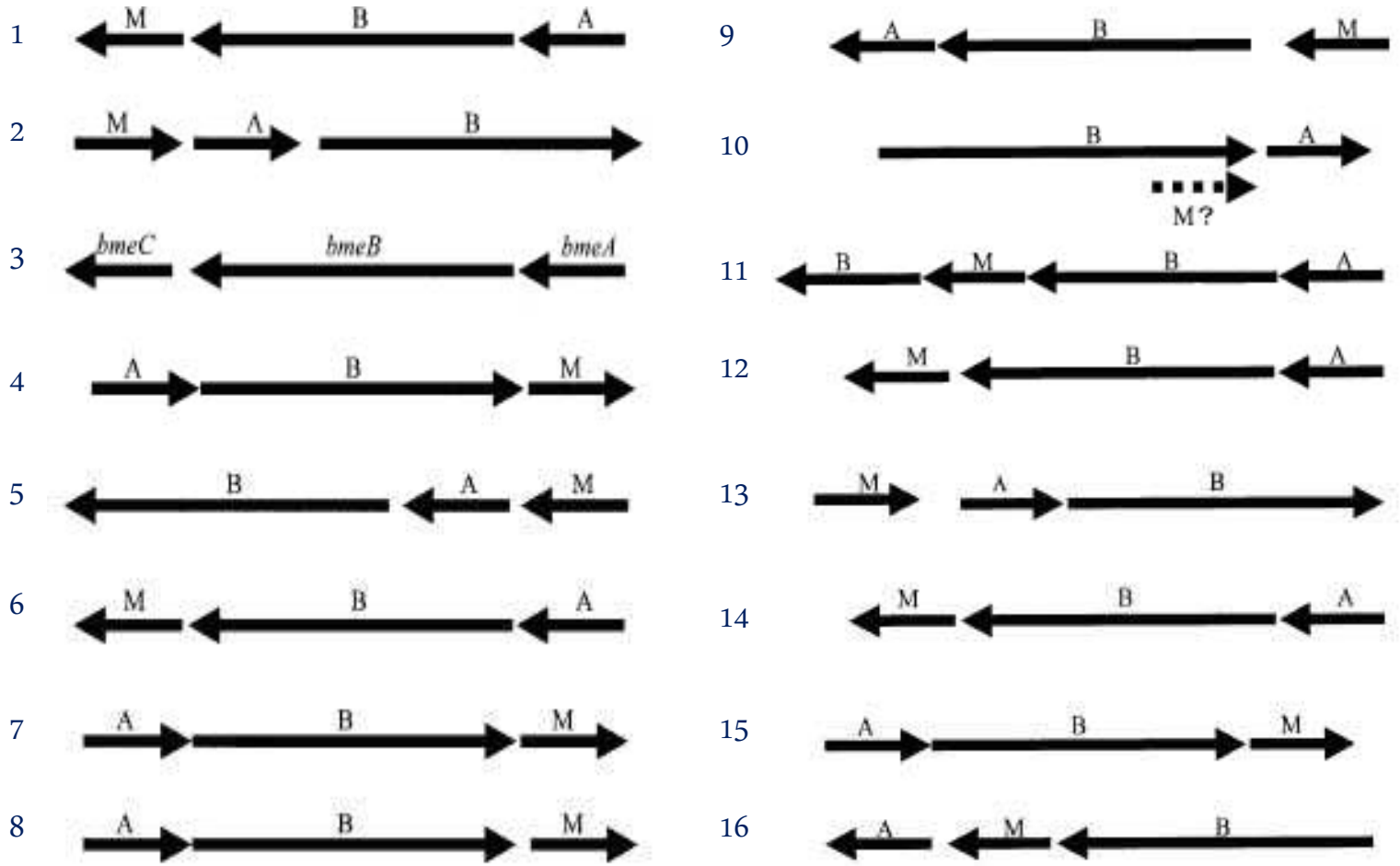


TABLE 4. Antibiotic susceptibilities of various strains[a]

	MIC (μg/ml)[b]										
	CEX[c]	CFZ	ZOX	LEX	CLS	PLB	BCT	VAN	FDA	NVB	PUR
B. fragilis ATCC 25285	128	256	64	256	512	256	2,048	48	2	128	32
FMB271 (Ω*bmeB*::pGERM)	64	128	16	64	256	64	512	16	1	64	8
FMAB1 (Ω*bmeAB*::pGERM)	64	128	32	64	256	64	1,024	32	1	96	24
FMA1 (Ω*bmeA*::pGERM)	64	128	16	64	256	64	512	16	1	64	8
FOM1 (Ω*bmeC*::pGERM)	128	256	64	256	512	256	2,048	48	2	128	32
E. coli KAM3	8	1	0.0625	16	0.5	2	>2,048	512	4	4	4
KAM3-pTPOUT25	16	2	0.125	32	1	4	>2,048	512	4	4	16
KAM3-pTPIN5	8	1	0.0625	16	0.5	2	>2,048	512	4	4	4

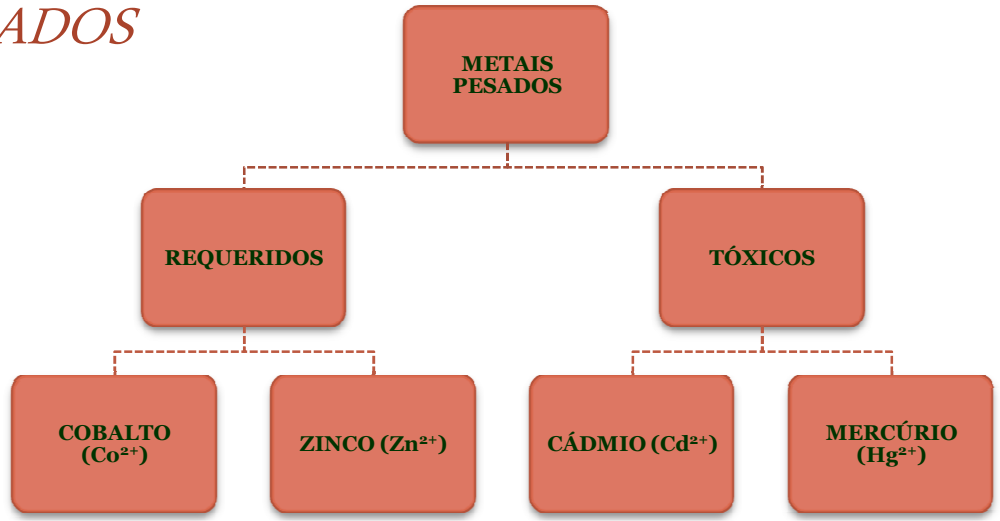
Bacteroides fragilis multidrug efflux (bme)



16 bombas RND (BmeABC)

(Ueda et al., 2005)

METAIS PESADOS



VITAMINA B12

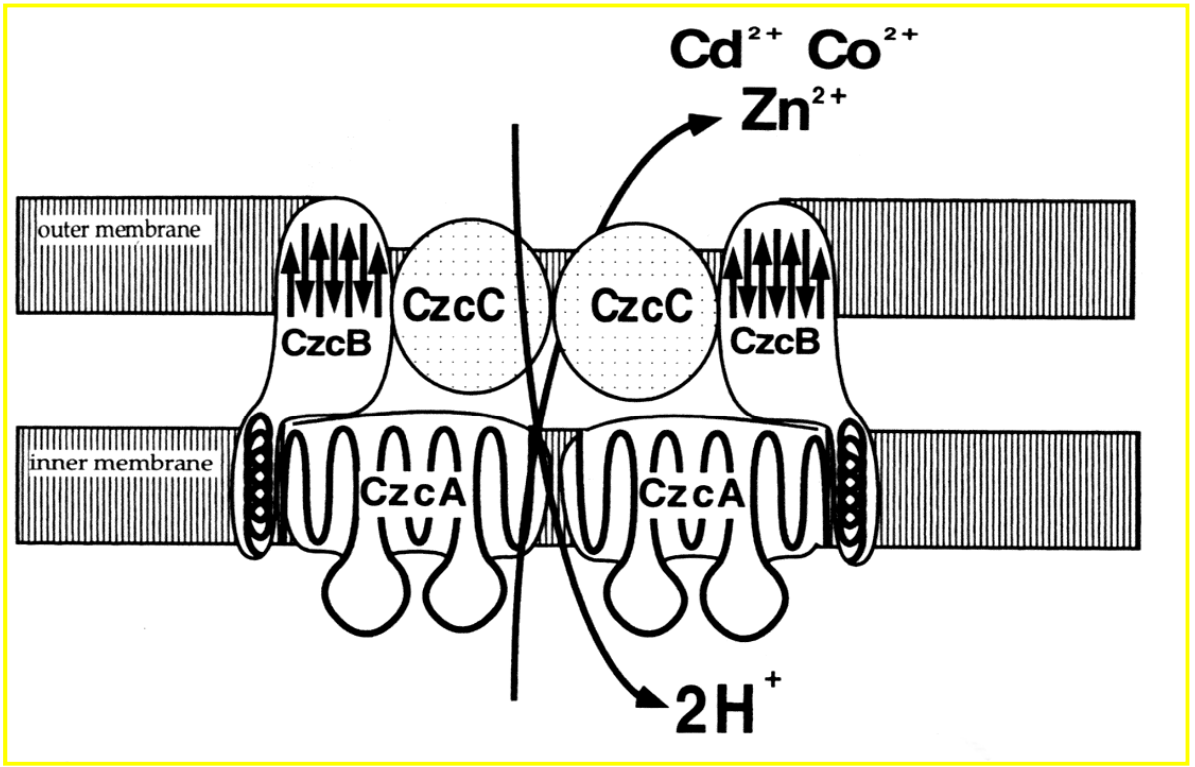
ESTABILIZA ENZIMAS

LIGAÇÃO AO GRUPO TIOL; QUELANTE DE Ca^{2+}

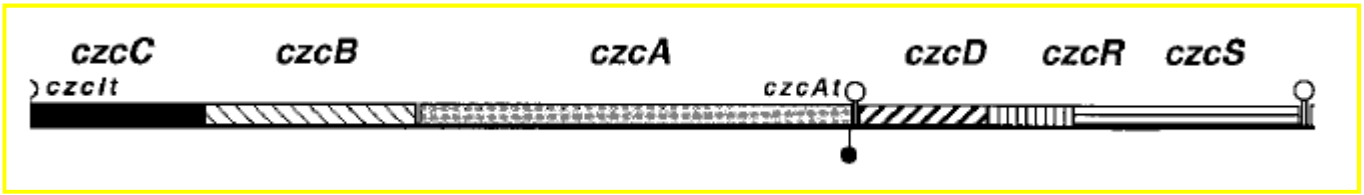
LIGAÇÃO AOS GRUPOS SULFIDRIL E TIOL

CÁDMIO (Cd^{2+}), COBALTO (Co^{2+}) E ZINCO (Zn^{2+})

Alcaligenes eutrophus CH34: pMOL30 (AE128)



Silver (1996)



Groβe et al. (1999)

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Original Research

ScopeMed

Intestinal *Bacteroides vulgatus* showing resistance to metals

Aline Ignacio, Viviane Nakano, Mario Julio Avila-Campos

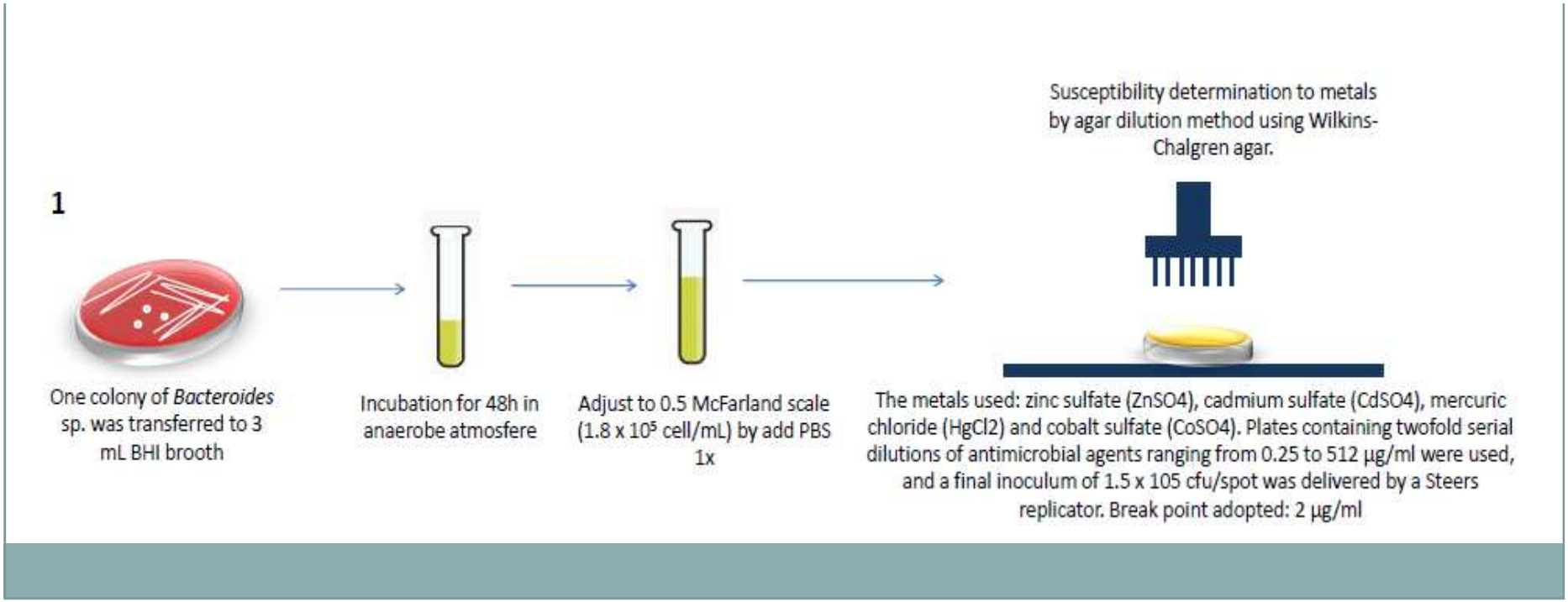
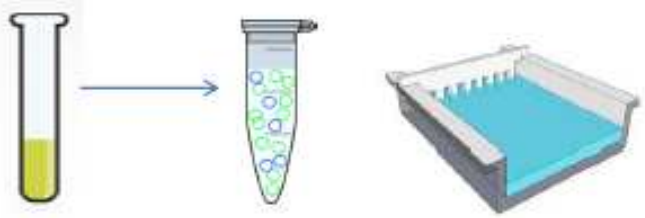


Table 2: Susceptibility to four metal ions of *Bacteroidales* species

Metals	MIC (μg/mL)			Resistance
	Range	50%	90%	Percentage
Human strains (22)				
$HgCl_2$	1-8	2	4	91
$ZnSO_4$	2-≥512	64	≥512	93
$CoSO_4$	1-≥512	256	≥512	95.5
$3CdSO_4.8H_2O$	1-32	8	16	95.5
Animal strains (22)				
$HgCl_2$	1-8	4	8	77
$ZnSO_4$	1-≥ 512	128	256	95.5
$CoSO_4$	2-≥ 512	64	≥512	100
$3CdSO_4.8H_2O$	0.5-64	32	64	91

Breakpoint for all metals was 2 μg/mL. $HgCl_2$: Mercuric chloride, $ZnSO_4$: Zinc sulfate, $CoSO_4$: Cobalt sulfate, $3CdSO_4.8H_2O$: Cadmium sulfate

2

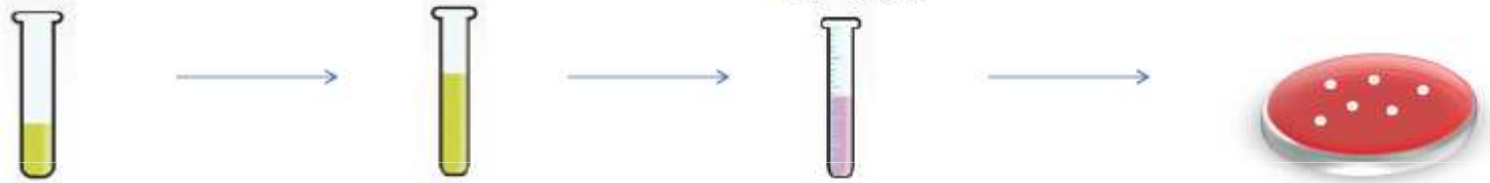


Resistant isolates were cultivated in 3mL BHI broth

Plasmid verification was performed by Miniprep Plasmid kit and gel eletrophoresis (agarose gel 0.8%)

3

Plasmid cure



Plasmid-positive strains were grown in 3 mL BHI brotoh for 48h.

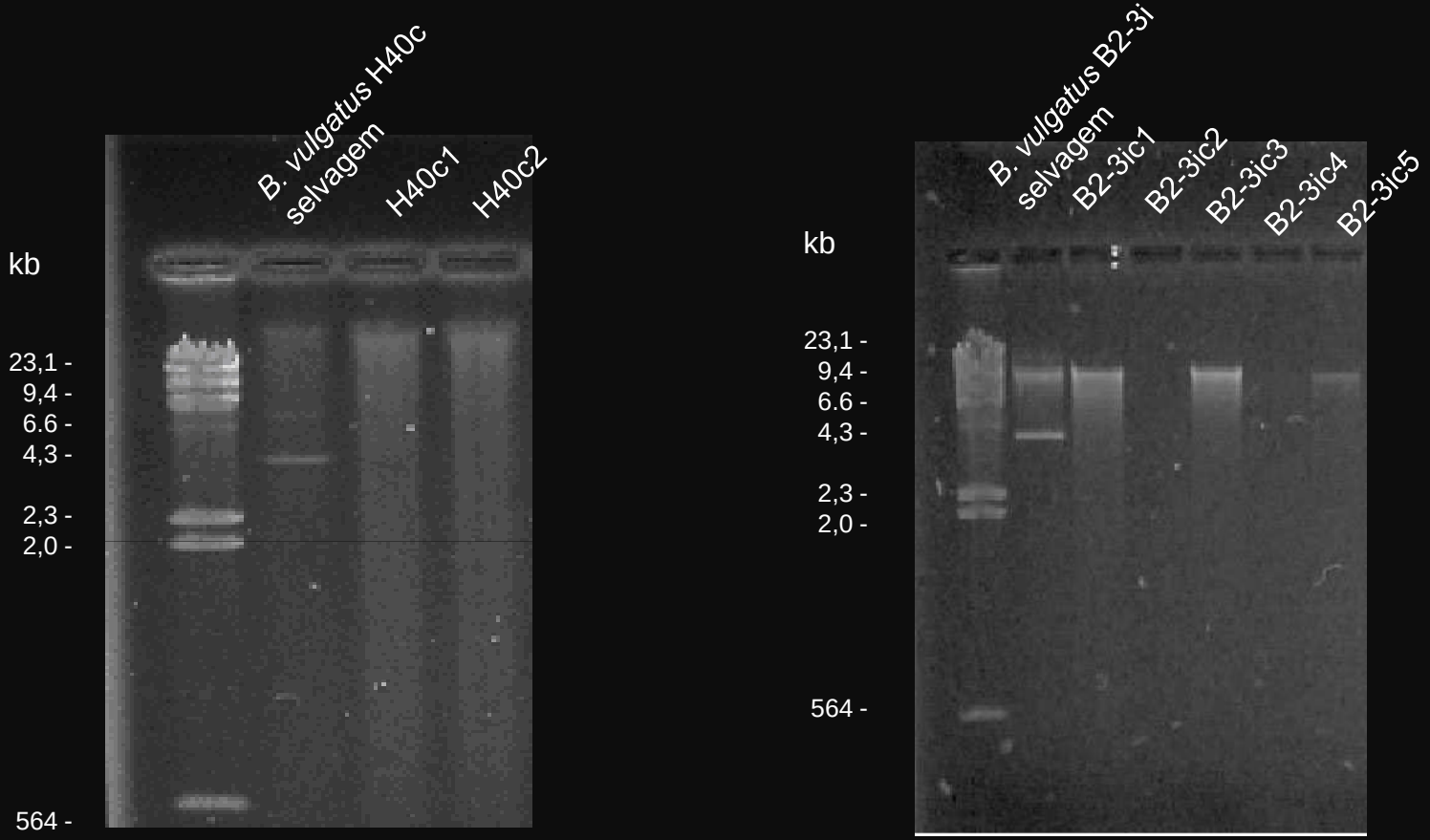
Adjust to 0.5 McFarland scale (1.8 x 10^5 cell/mL) by add PBS 1x

0.1 mL were grown in BHI broth with different ethidium bromide (EtBr) concentrations (from 1 to 512 µg/ml) for 48h.

From the first tube wihtout growth 0.1 mL were plated into blood agar

The susceptibility to metals was also evaluated to verify the resistance values

Five colonies were randomly select and plasmid DNA was extracted and analyzed by agarose gel electrophoresis in order to verify the plasmid loss



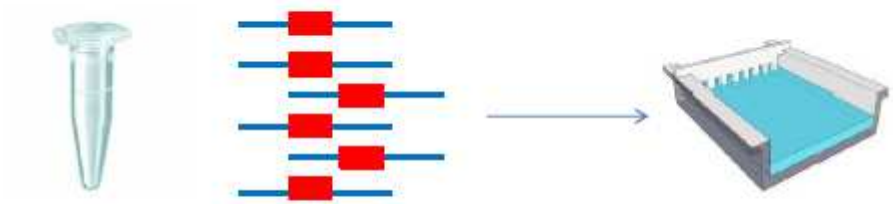
peso molecular λ*Hind III* (Invitrogen)

Table 3: Antimicrobial susceptibility and plasmid profile in *B. vulgatus* (wild strains and respective clones) from human and animal origin

Strain/clones	$HgCl_2$ (μg/mL)	$ZnSO_4$ (μg/mL)	$CoSO_4$ (μg/mL)	$3CdSO_4.8H_2O$ (μg/mL)	Plasmid No. (kb)
Human					
B. vulgatus H40c[a]	4	256	≥512	8	2 (3.0; 5.0)
B. vulgatus H40c1[b]	2	256	≥512	8	-
B. vulgatus H40c2[b]	2	128	≥512	8	-
Animal					
B. vulgatus B2-3i[a]	2	64	64	32	1 (4.0)
B. vulgatus B2-3i1[b]	0,5	64	32	8	-
B. vulgatus B2-3i2[b]	1	64	16	8	-
B. vulgatus B2-3i3[b]	1	64	32	4	-
B. vulgatus B2-3i4[b]	2	32	32	8	-
B. vulgatus B2-3i5[b]	1	64	64	8	-

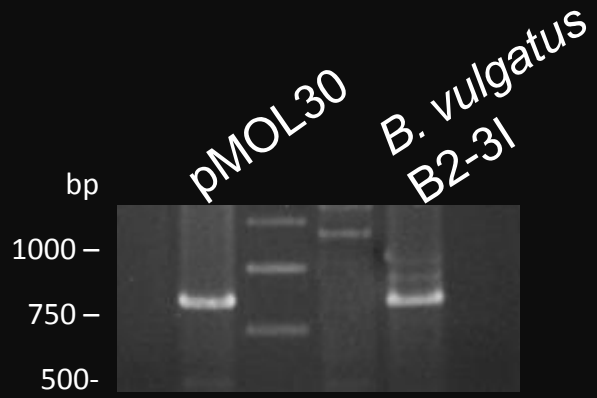
Breakpoint for all metals was 2 μg/mL. [a]Wild strain, [b]Clones from wild strain. *B. vulgatus: Bacteroides vulgatus*

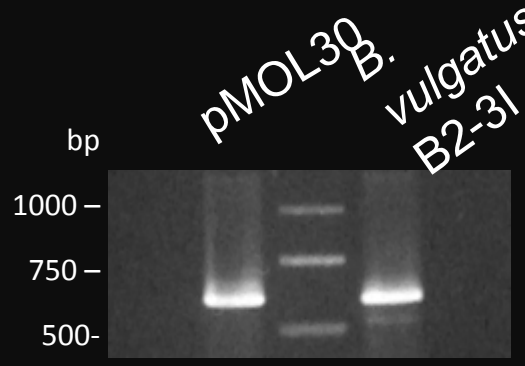
4



Detection of resistance genes were performed by conventional PCR

PCR products were analyzed by 1% agarose gel electrophoresis, stained with ethidium bromide and photographed under UV light





czcA peso molecular 1kb (Fermentans) ***czcD***

Perspectivas

By-passing a atividade do efluxo → Novas drogas

Inibidores da proteína de membrana externa (TolC)

Entender o papel dos sistemas de efluxo na virulência

Obrigada!