ATIM POMPALARINA BAĞLI

ANTİMİKROBİYAL DİRENÇ

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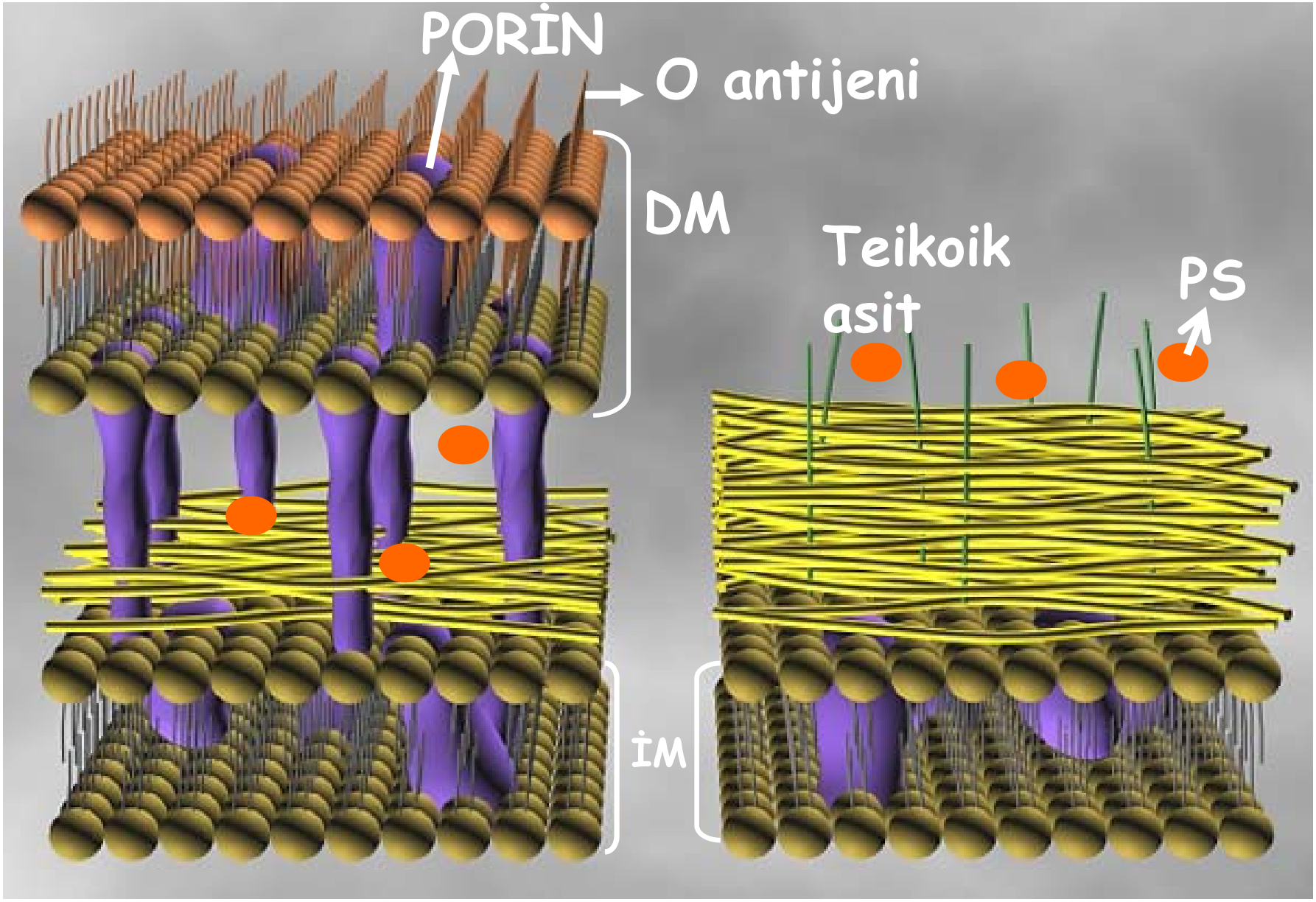
AKG 2012 İstanbul

Bakterilerde Antibiyotik Direnci

- Hedefte değişiklik
- Enzimatik modifikasyon veya inaktivasyon
- **Aktif Pompa**
- **Geçirgenlikte azalma**

Bakterilerde Hücre Yüzeyi

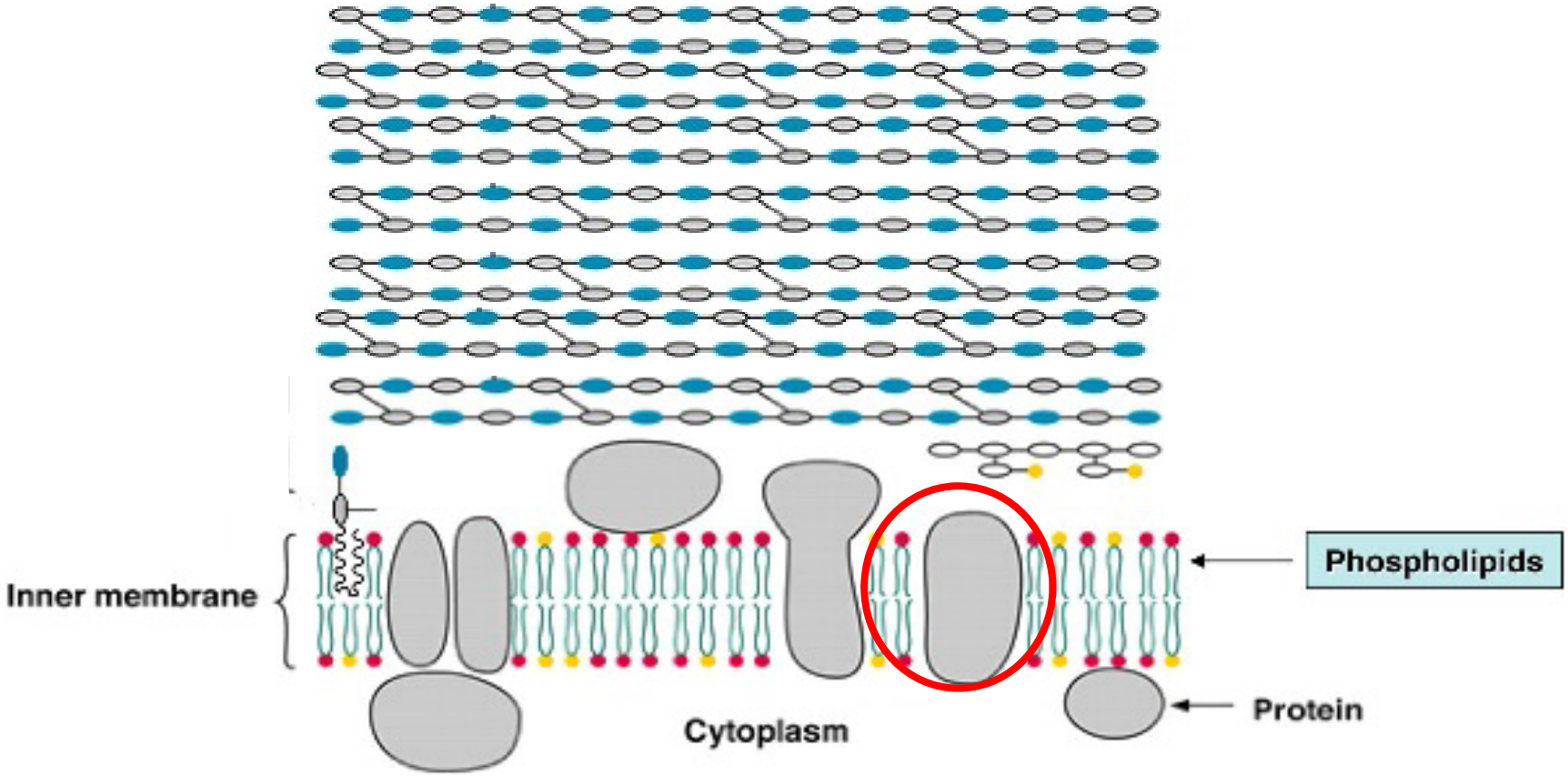
Gram-negatif	Gram-pozitif
• Sitoplazmik membran	• Sitoplazmik membran
• Peptidoglikan tabaka	• Peptidoglikan tabaka
• **Dış membran**	• **Teikoik asit**
• Biyofilm Lipooligosakkarit	• Biyofilm Polisakkarit-teikoik asit



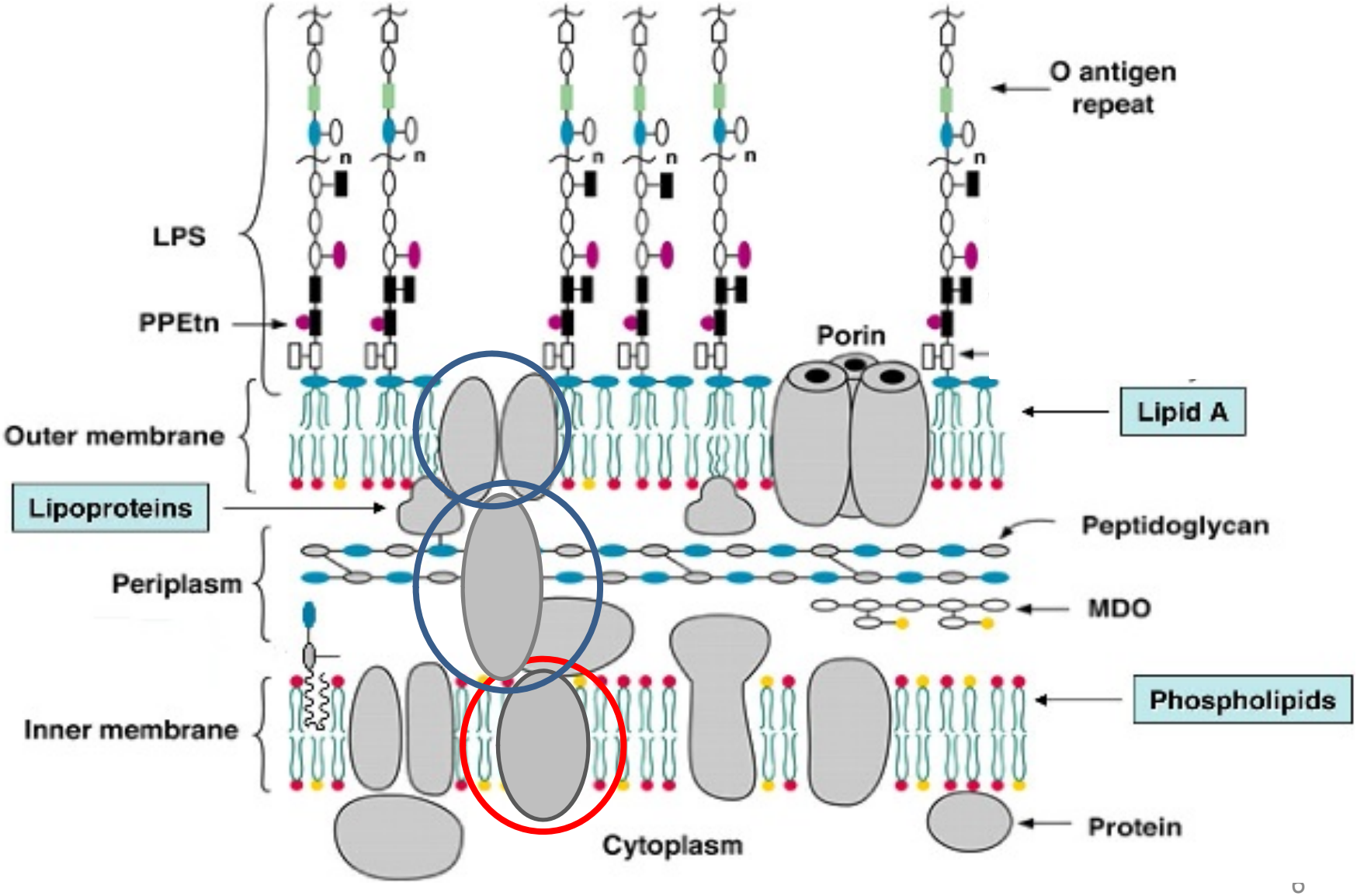
Gram Negatif

Gram Pozitif

Gram-Pozitif Pompa Proteinleri

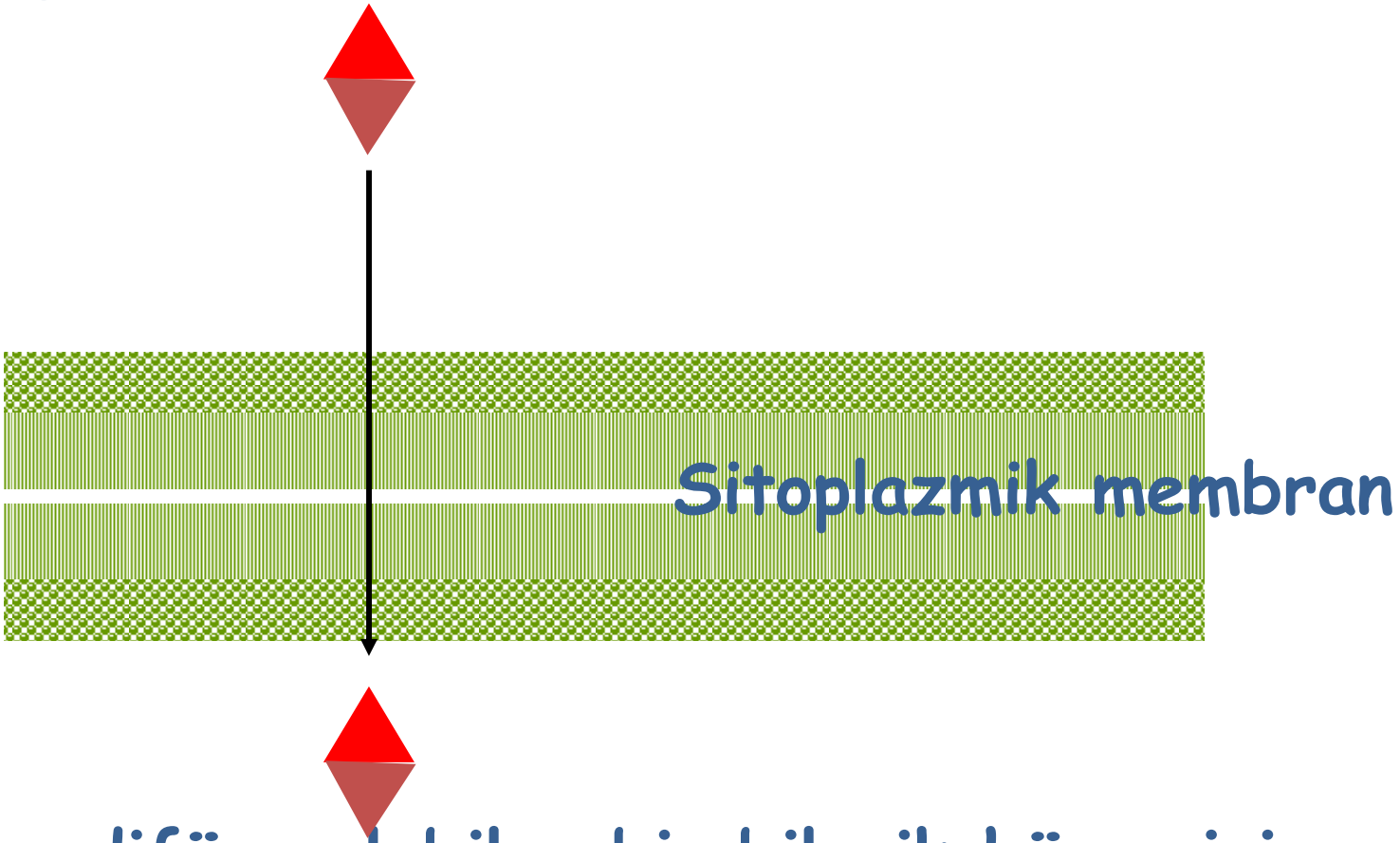


Gram-Negatif Pompa Proteinleri



Membran Transportu

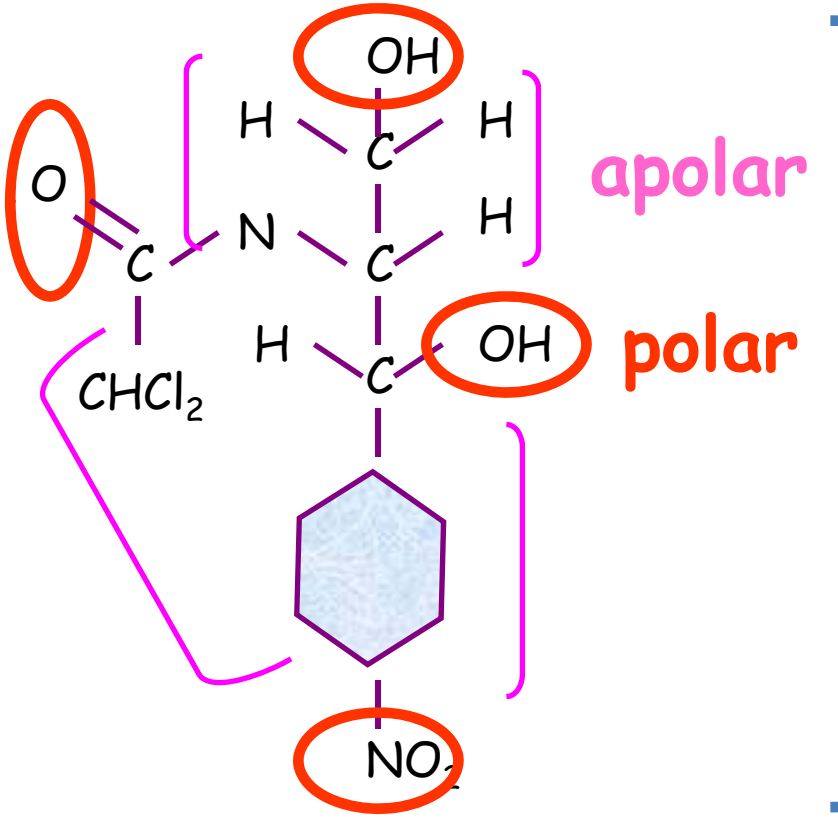
- Polar ve apolar gruplar içeren bileşikler membranı kolay geçer



Kolay difüze olabilen bir bileşik hücre için zaralı olabilir

Aktif Pompa Proteinleri

Kolay difüze olan moleküllere karşı doğal korunma



apolar

polar

Ampifilik Ab.

Aktif Pompa Protein Aileleri: Enerji

Adenozin trifosfat

1-ATP Binding Casette – ABC

Proton Motivasyon Kuvveti

2-Major Facilitator Superfamily (MFS)

3-Multidrug and Toxic Compound Extrusion (MATE)

4-Small Multidrug Resistance (SMR)

5-Resistance-Nodulation-CellDivision (RND)

Tet Pompaları

Gram-negatif bakteriler

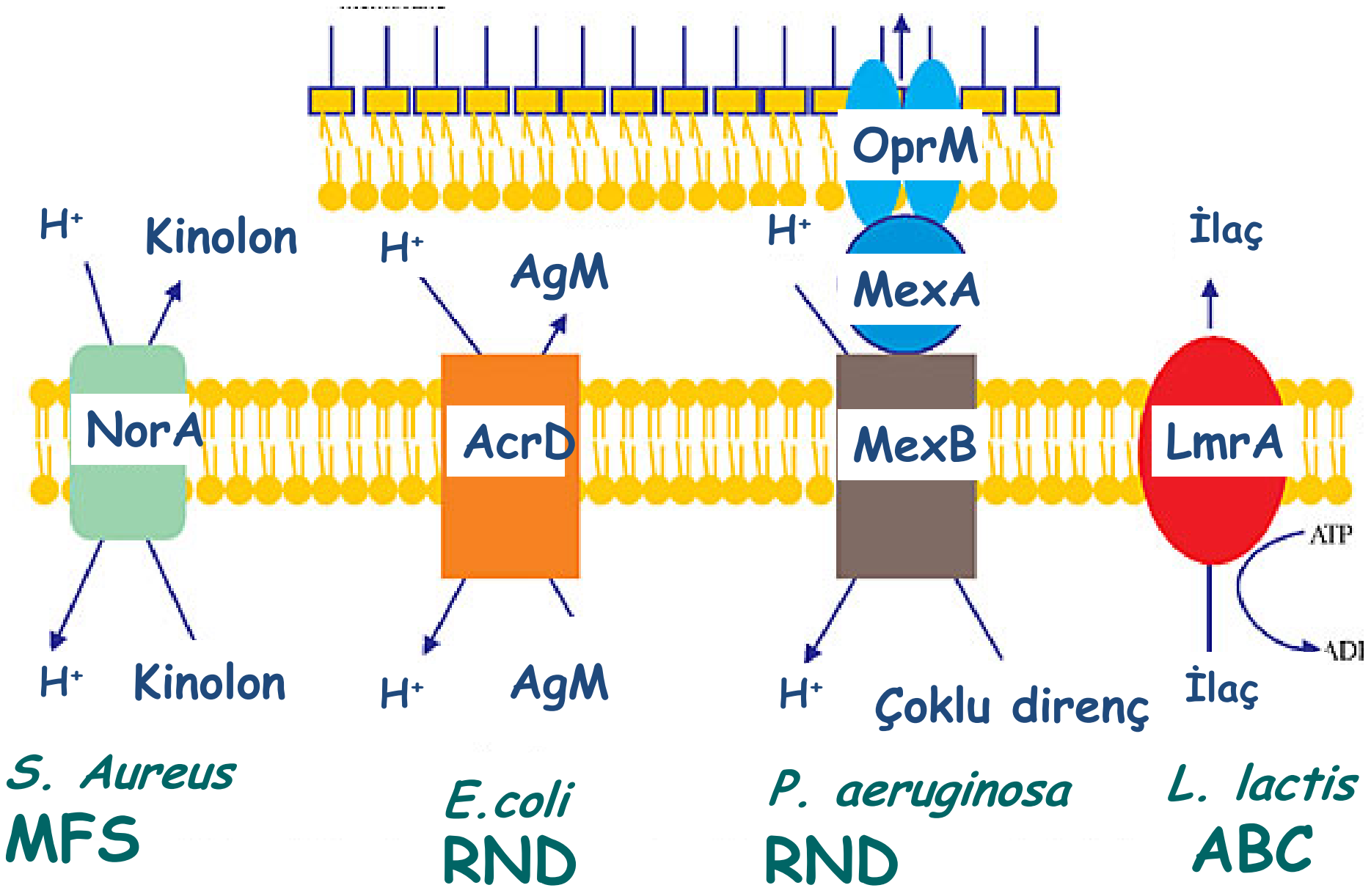
Kromozomal

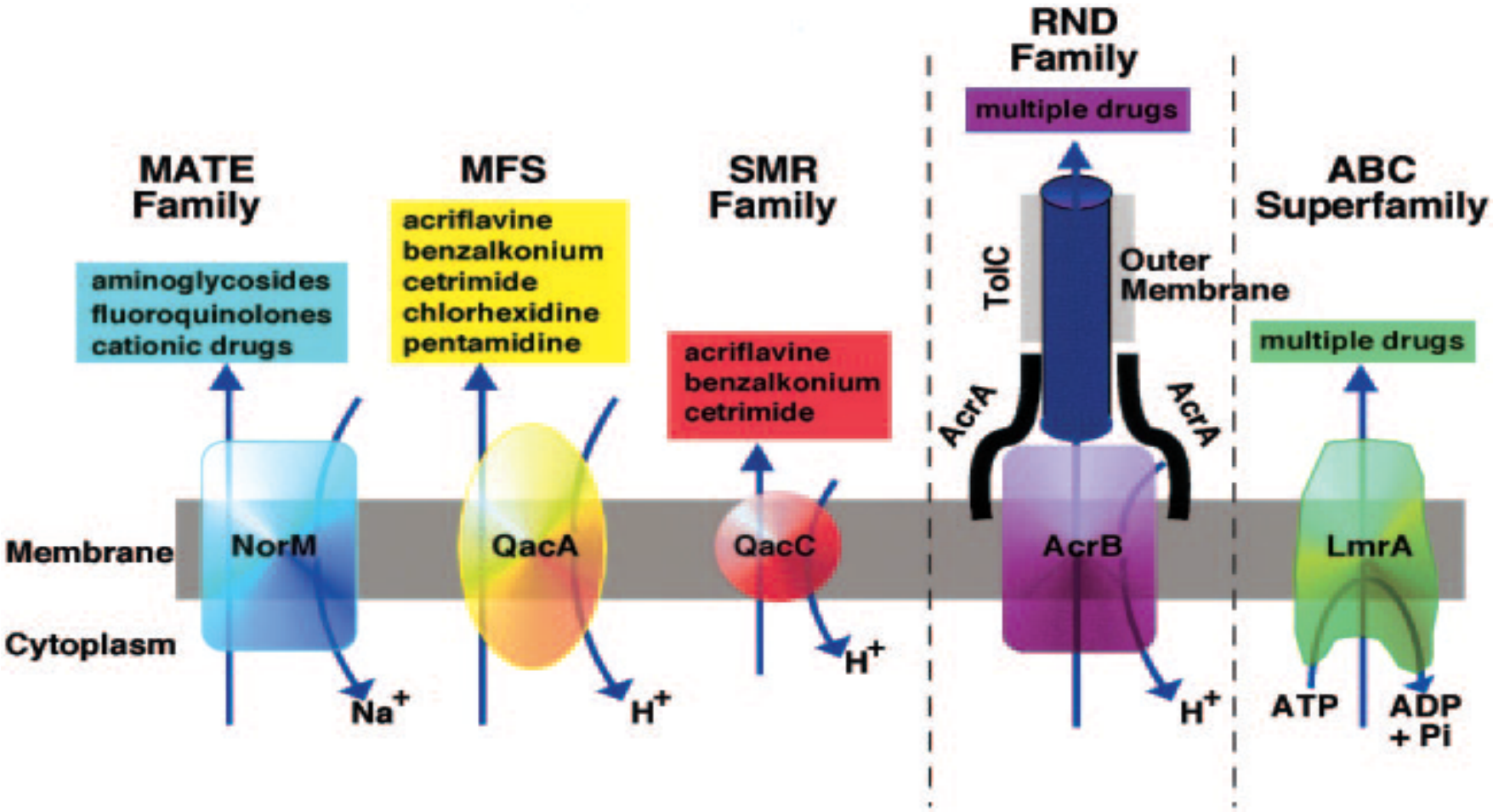
Tet(A), Tet(B), Tet(C), Tet(D)

Gram-pozitif bakteriler

Plazmid

Tet(K)	*S. aureus*
tet(L)	*Bacillus spp.*
	Staphylococcus spp.
	Streptococcus spp.


OprM
MexA
İlaç
H+
Kinolon
H+
AgM
H+
NorA
AcrD
MexB
LmrA
ATP
ADP
H+
Kinolon
H+
AgM
H+
Çoklu direnç
İlaç
S. Aureus
MFS
E.coli
RND
P. aeruginosa
RND
L. lactis
ABC


MATE Family
aminoglycosides
fluoroquinolones
cationic drugs
MFS
acriflavine
benzalkonium
cetrimide
chlorhexidine
pentamidine
SMR Family
acriflavine
benzalkonium
cetrimide
RND Family
multiple drugs
TolC
Outer Membrane
AcrA
AcrA
ABC Superfamily
multiple drugs
Membrane
Cytoplasm
NorM
QacA
QacC
AcrB
LmrA
Na+
H+
H+
H+
ATP
ADP + Pi

Aktif Pompa: Klinik Önem Gram Negatif Bakteriler

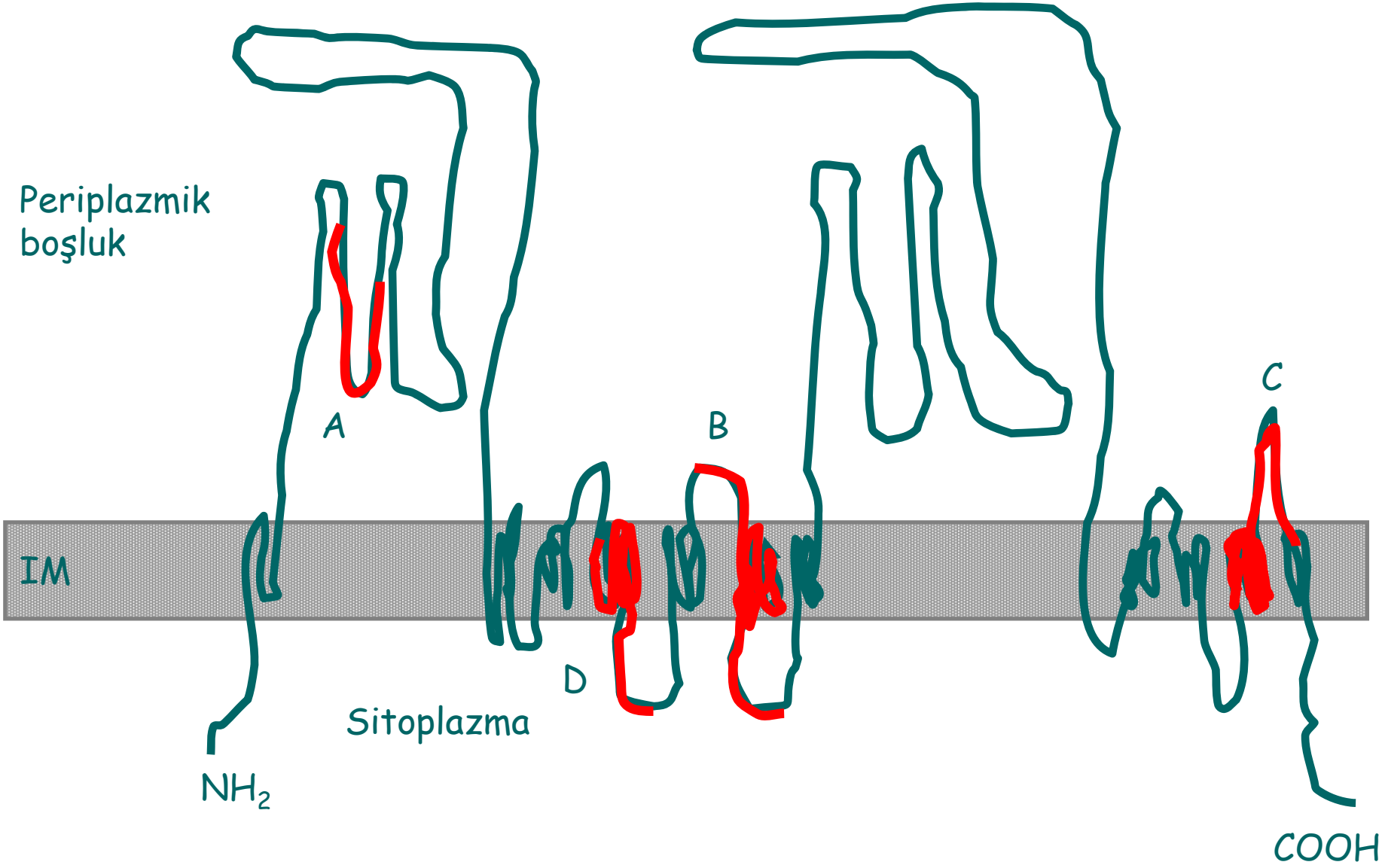
Gram-Negatif Bakteriler

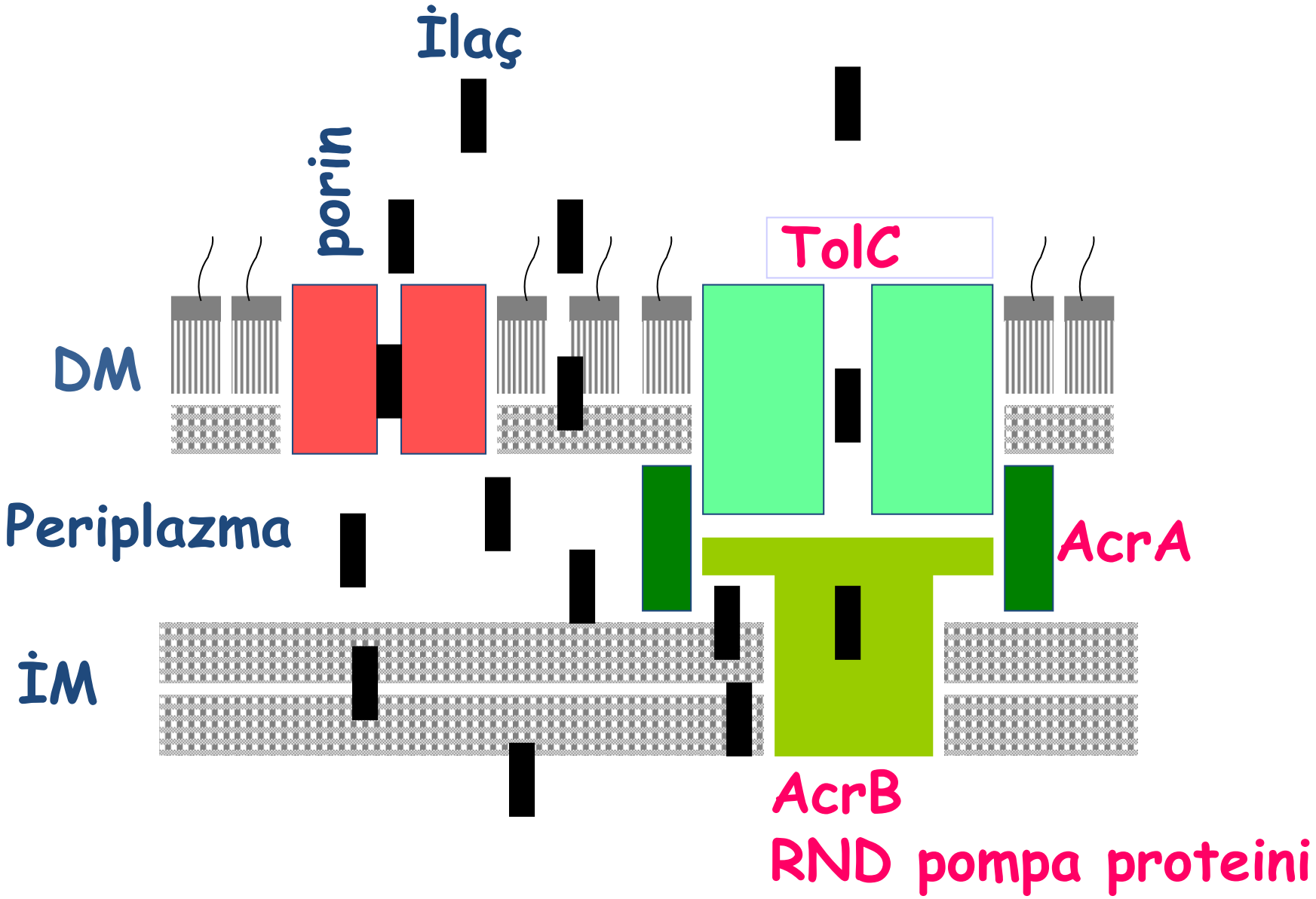
ABC, MFS, MATE, SMR, RND

Çoklu İlaç Direncinde
RND ailesi aktif pompa proteinleri

- Kromozomal
- Doğal ve kazanılmış direnç
- Membran Füzyon Proteini ve Dış Membran Proteini ile kompleks

RND Pompa Proteini: İki büyük eksternal çıkıntı




İlaç
porin
TolC
DM
Periplazma
AcrA
İM
AcrB
RND pompa proteini

RND: Enterobacteriaceae

Escherichia coli

- AcrAB-TolC
- AcrAD-TolC
- AcrEF-TolC
- MdtABC-TolC
- YhiUV-TolC

Enterobacter aerogenes

- AcrAB-TolC

Salmonella enterica serovaryant typhimurium

- AcrAB-TolC

RND: Enterobacteriaceae

Proteus vulgaris

- AcrAB-?

Klebsiella pneumoniae

- AcrAB-?

Serratia marcesecens

AcrAB-?

RND: Diğer Gram negatifler

Haemphilus influenzae

- AcrAB-TolC

Neisseria gonorrhoeae

- MtrCDE (*N. Meningitidis*'te homolog)

Campylobacter jejuni

- CmeABC
- CmeDEF

Vibrio cholerae

- ??-TolC

Substratlar
AcrAB-TolC

- Lipofilik Beta-laktamlar
- Kloramfenikol
- Florokinolon
- Makrolid
- Novobiyosin
- Fusidik asit
- Sodyum dodesil sulfat
- Trimetoprim/sulfa.
- Safra tuzları
- Organik çözücüler
- Benzalkonyum
- Metotreksat
- Etidyum bromid
- Akriflavin
- Kristal viyole
- Triklozan
- Triton-X100

CmeABC

- Florokinolon
- Ampisilin
- Kloramfenikol
- Nalidiksik asit
- Sefotaksim
- Gentamisin
- Rifampisin
- Etidyum bromid
- Akridin
- Protamin
- Sodyum dodesil sulfat
- Deoksikolat

MtrCDE

- Florokinolon
- Azitromisin
- Makrolid
- Rifampisin
- Etidyum bromid
- Kristal viyole
- Yağ asitleri
- Triton-X-100

E. coli	CIP	CHL	TET	CO-TRIM
CLSI	4	32	16	4/16
BSAC	8	16	2	4
WT(W3110)	0.015	8	1	>32
tolC::	0.0025	1	1	1
acrAB::	0.0025	1	0.12	16
acrD::	0.015	8	0.12	>32
acrEF::	0.0025	1	1	>32

RND: S. enterica serovar Typhimurium

S. enterica serovar T	NA	CIP	TET	C	TRIC
CLSI	32	4 ?	16	32	
BSAC	16	2 ?	2	16	
WT(SL1344)	4	0.06	4	4	
acrB:	1	0.015	2	0.5	
acrB +++	32	0.5	16	16	0.5
acrD::	4	0.03	4	4	0.06
acrF::	4	0.06	4	1	0.12
TolC::	1	0.015	0.5	1	0.015

RND: *Campylobacter jejuni*

C. jejuni	CIP	E	AMP	TET	C	TRIC
CLSI	4	8	32	16	32	
BSAC	1		16	2	8	
WT	0.25	0.5	4	0.5	1	4
cmeB::	0.12	0.25	2	0.25	0.5	1
cmeB +++	8	4	32	16	16	32
cmeF::	0.25	0.5	0.5	0.25	1	1

RND: *Neisseria gonorrhoeae*

.	PEN	NAF	CIP	TET
CLSI	2		1	2
BSAC	4		4	16
WT (FA19)	0.015	0.25	0.025	0.25
mtrCDE +++	0.03	1	0.025	0.25
mtrD::	0.015	0.03	0.025	0.25

RND: *Pseudomonas aeruginosa*

MexAB-OprM
MexCD-OprJ
Mex-EF-OprN
MexXY-OprM
MexJK-OprM
MexGHI-OpmD

P. aeruginosa: MexAB-OprM

- İntrinsik direnç

  Tetrasiklin, makrolid, kloramfenikol, norfloksasin, β-laktam

- **Yüksek düzey direnç**

  β-laktamlar (inhibitörler dahil), meropenem, kinolonlar, makrolidler, tetrasiklin, kloramfenikol, novobiyosin, sulfonamidler, trimetoprim, boyalar, deterjanlar

- İmipeneme etkisiz
- N-açil homoserin (elastaz, piyosiyanin) laktonların efluksu

RND: *Pseudomonas aeruginosa*

Yüksek düzey ekspresyon

- **MexCD-OprJ**

  Kinolon, tetrasiklin, makrolid, trimetoprim, kloramfenikol, sefalosporin.

- **MexEF-OprN**

  Kinolon, kloramfenikol, trimetoprim, imipenem (OprD azalması)

- **MexXY-OprM**

  Aminoglikozid direnci

RND : *Pseudomonas aeruginosa*

P. aeruginosa	CIP	E	AMP	TET
CLSI	512	4	32	16
BSAC	256	8	8	2
WT (PAO1)	64	0.12	64	16
mexAB-M::	0.5	0.03	2	1
MexAB-M +++	256	0.25	256	64
MexAB-M +++	128	1	256	
ΔmexCD-OpRJ		0.002	1	0.12
ΔmexXY-OpRM		0.25	512	64
mexXY-OprM +++		0.5	8	32

RND: Acinetobacter baumannii

- **AdeABC**

  Aminoglikozid, β-laktam, florokinolonlar, tetrasiklin, tigesiklin, eritromisin, kloramfenikol, trimetoprim

- **AdeIJK**

- **AdeFGH**

RND: Acinetobacter baumannii

A. baumannii	GEN	NET	OFX	CTX	MEM	TET
CLSI	16	32	8	64	16	16
BSAC	2		4	16	4	2
BM4454	8		64	16		64
BM adeB::	<0.25		4	4		8
U10247		2	8		0.5	2
U11177		16	64		2	32

RND Pompalar

- ***Stenotrophomonas maltophilia***

SmeAB-C	Florokinolon
	Beta-laktam
	AgM
SmeDE-F	Florokinolon
	Eritromisin
	Tetrasiklin
	Organik çözücüler

- **Burkholderia cepacia**

CeoAB-OprM	Kloramfenikol
	Florokinolon
	Trimetoprim

Diğer Çoklu Direnç Pompaları

E. coli	ABC	MacAB-TolC	ML
	MFS	EmrAB-TolC	NA, CCCP, EB,TL
	MFS	MdfA/Cmr/CmlA	C, PU, RF, T, EB, RD,TPP, IPTG
	MFS	Tet	TS
	MATE	YdhE	FK, AK, TTP
	SMR	EmrE	AK, EB, DAB, MV
V. chole..	MFS	VceAB	NA, DK, PKP, CCCP, PMA
	MATE	VcmA	FK, C, S, AK, DA, DO, EB
V. phaemo..	MATE	NorM	FK, C, S, EB
P.aerugin..	SMR	EmrE	AG, AK, EB
B.cepa...	MFS	BcrA	T, NA
B. theta..	MATE	BexA	FK, EB
B. fragilis	MFS	NorA	NOR, EB, PM

Aktif Pompa: Klinik Önem
Gram Pozitif Bakteriler

Gram Pozitif: Aktif Pompa

S. aureus	ABC	**MsrA**	Makrolid, Streptogramin B
	MFS	**NorA**	Florokinolon
	MFS	**QacA**	C, AK, KV, EB, DD, DAB
	MATE	**MepA**	FK, biyosit
S. pneumoniae	MFS	**PmrA**	Florokinolon
	MFS	**MefE**	Makrolid
S. pyogenes	MFS	**MefA**	Makrolid
S. agalactiae	MFS	**MreA**	C, Makrolid
E. faecalis	MFS	**EmeA**	EB, Norfloksasin

Staphylococcus aureus NorA

S. aureus	NOR	CIP	NAF	VAN
CLSI	16	4	8	32
BSAC		2		8
SA1199	0.5	0.5	0.5	0.5
SA1199B	64	16	0.5	0.5
SA1199-3	16	4	0.5	0.5

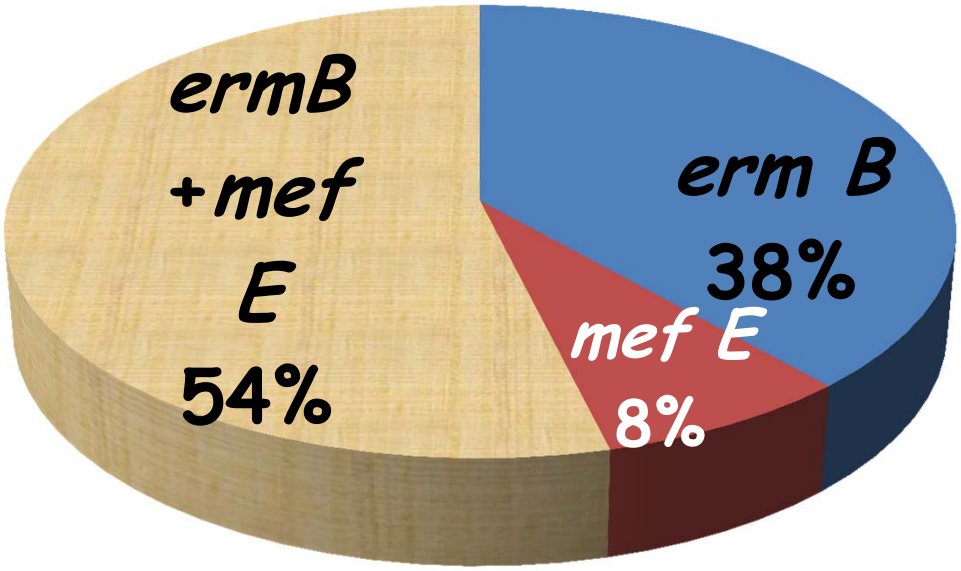
Gram Pozitif: Aktif Pompa

B. subtilis	MFS	**Bmr**	FK, DO, AKr, EB, RD, SD, TTP
E. faecalis	ABC	**ABC7**	OFX, DO, DA, EB
	ABC	**ABC11**	PT, KH
	ABC	**ABC16**	E, AZ, CLA
	ABC	**ABC23**	QD, VM
	ABC	**Lsa**	CL, QD
	MFS	**EmeA**	C, E, FK, CL, NO, AK, EB
L. lactis	ABC	**LmrA**	DA, DO, EB, OL, RD, VB, VK
L. monocytogenes	MFS	**MdrL**	S, ML, EB

S. pneumoniae / Makrolid direnci: *mefE*

Merkezimize ait sonuçlar

erm(B) tek başına	39 %
mef(E) tek başına	22.1 %
	(Düşük düzey direnç)
erm(B) + *mef*(E)	39 %

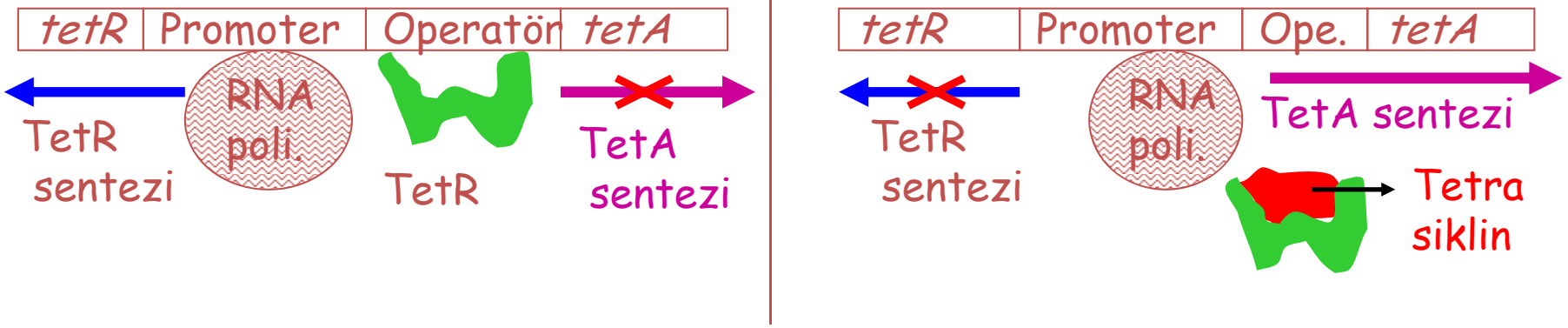

ermB +mef E 54%
erm B 38%
mef E 8%

Mycobacterium spp.

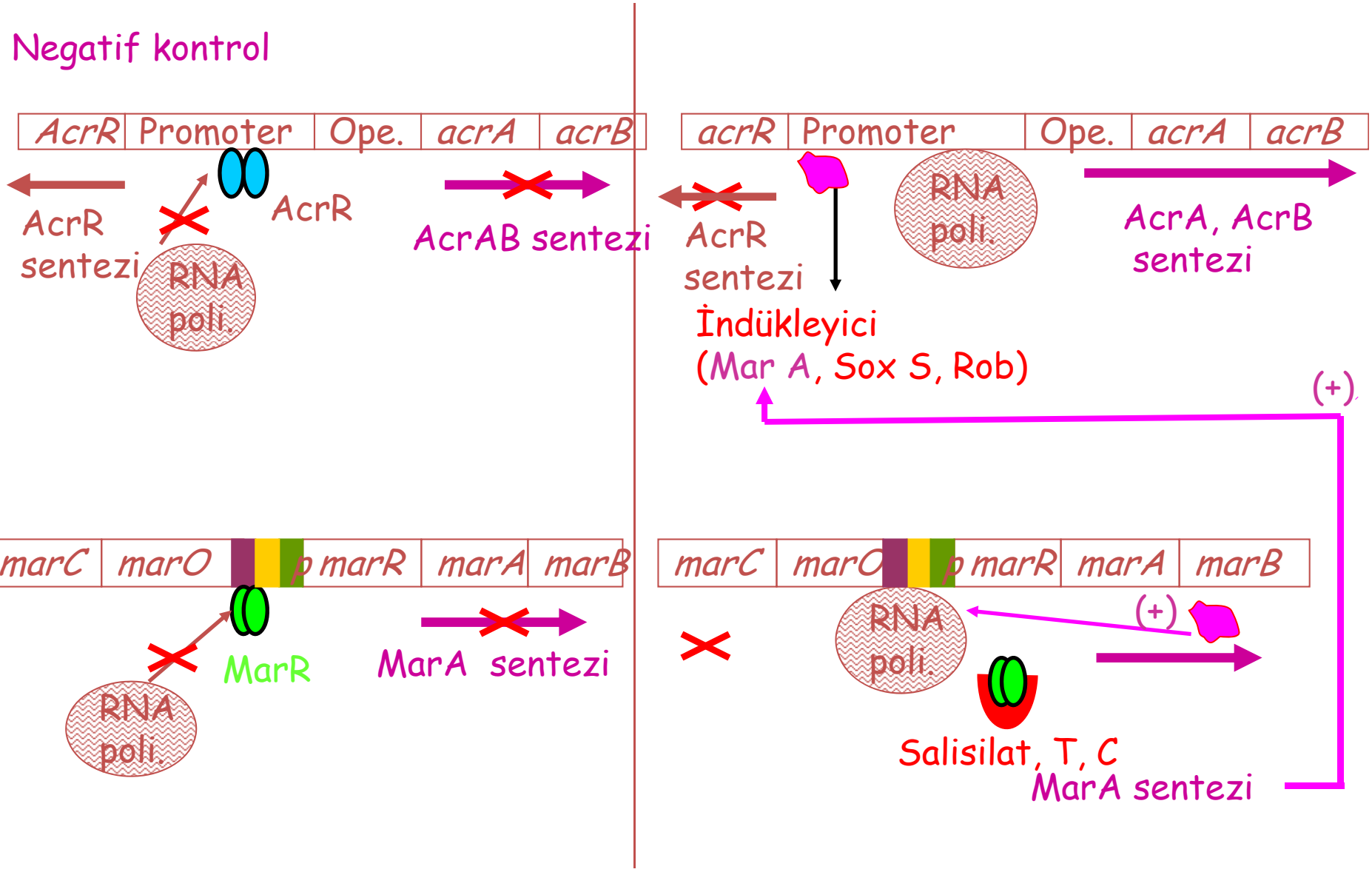
M. tuberculosis	ABC	**DrrAB**	DA, DO, EB
	MFS	**EfpA**	İzoniyazid
	MFS	**P55**	AgM, T
	MFS	**Tap**	T
	SMR	**Mmr**	E, AK, EB, TPP
M. smegmatis	MFS	**LfrA**	FK, EB
M. fortiutum	MFS	**Tap**	AgM, T

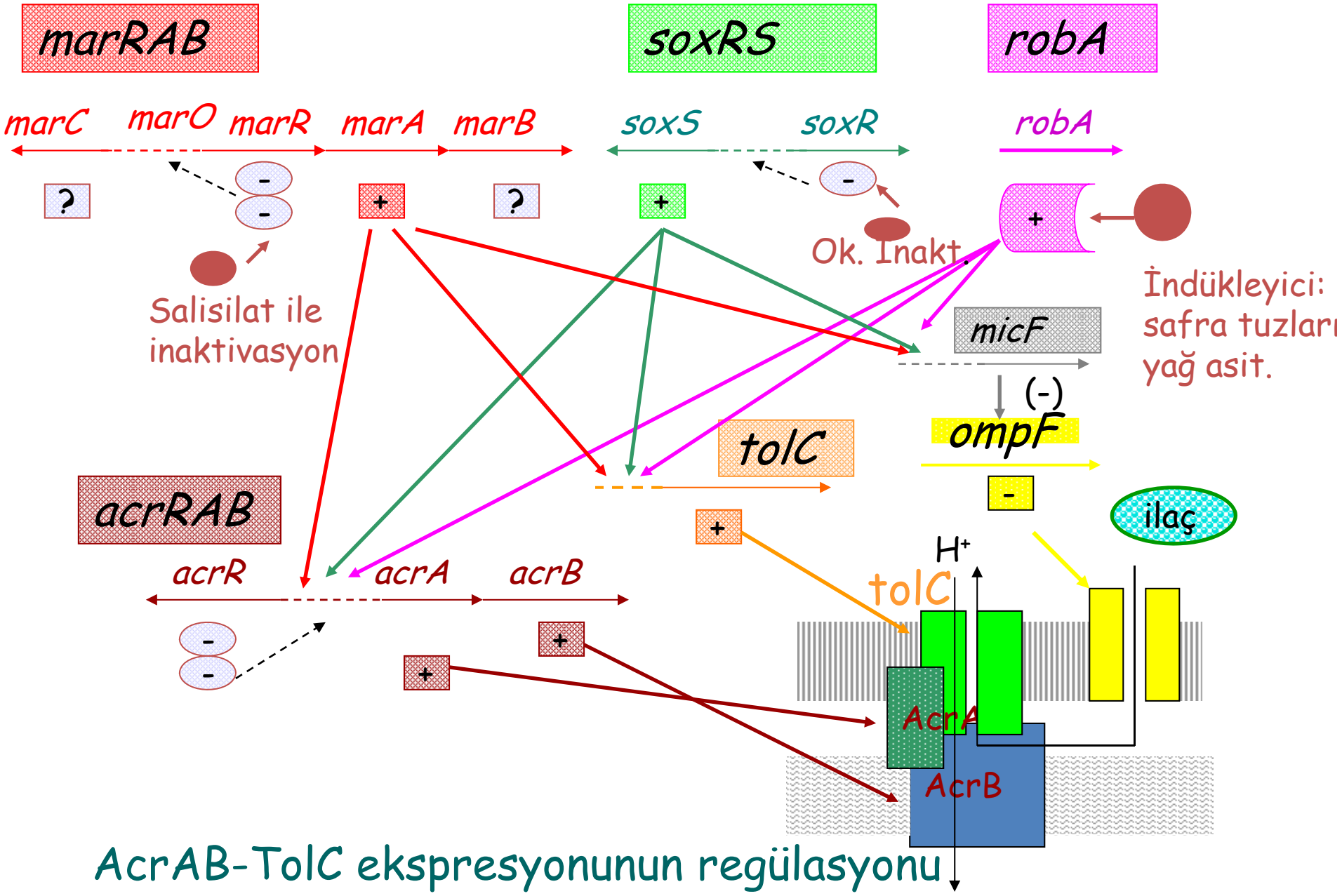
Aktif İlaç Atım Pompa Proteinlerinin Ekspresyonunun Transkripsiyonel Kontrolü

Negatif kontrol



İLAÇ ATIM POMPALARININ TRANSKRİPSİYONEL KONTROLÜ





AcrAB-TolC ekspresyonunun regülasyonu

Aktif Pompa Denetimindeki Direnç Nasıl Önlenebilir

- Aktif Pompa İnhibitorleri (EPIs)
- Atım pompalarından etkilenmeyen antimikrobiyallerin geliştirlmesi

İlaca Özgü Atım Pompası İnhibitörleri

Tet atım pompası inhibisyonu

- 4 üyeli naftalin yapısı gerekli
- *13-cyclopentylthio-5-OH tetracycline*
- *Ginsenosides*
- İndol deriveleri

Çoklu İlaç Atım Pompası İnhibitörleri

Verapamil:

- Kalsiyum kanal antagonisti
- Memeli hücrelerinde, *P. falciparum*'da ilaç atım inhibitörü

Rezerpin

- Bir bitki indol alkaloidi
- Memeli hücrelerinde ve bakterilerde (NorA, bmr) ilaç atım inhibitörü

RND Atım Pompası İnhibitörleri

- Phenylalanyl-arginyl-β-naphtylamide
- 1-(1-naphthylmethyl)-piperazine
- Peptidomimetikler
- Benastatin

adeB RE	EPI	CIP	ERY	CHL	TMP	TET
0,93	**PABN NMP**	16 2 4	4 2 4	32 8 16	32 8 16	512 128 512
1,08		16 4 8	16 4 8	512 128 256	8 2 8	16 4 8
1,06		32 4 16	16 2 8	64 32 32	16 4 8	16 8 16
0,75		32 4 16	32 16 32	64 32 32	32 8 16	16 8 16
1,1		32 4 16	32 4 8	256 64 128	32 4 16	128 32 64

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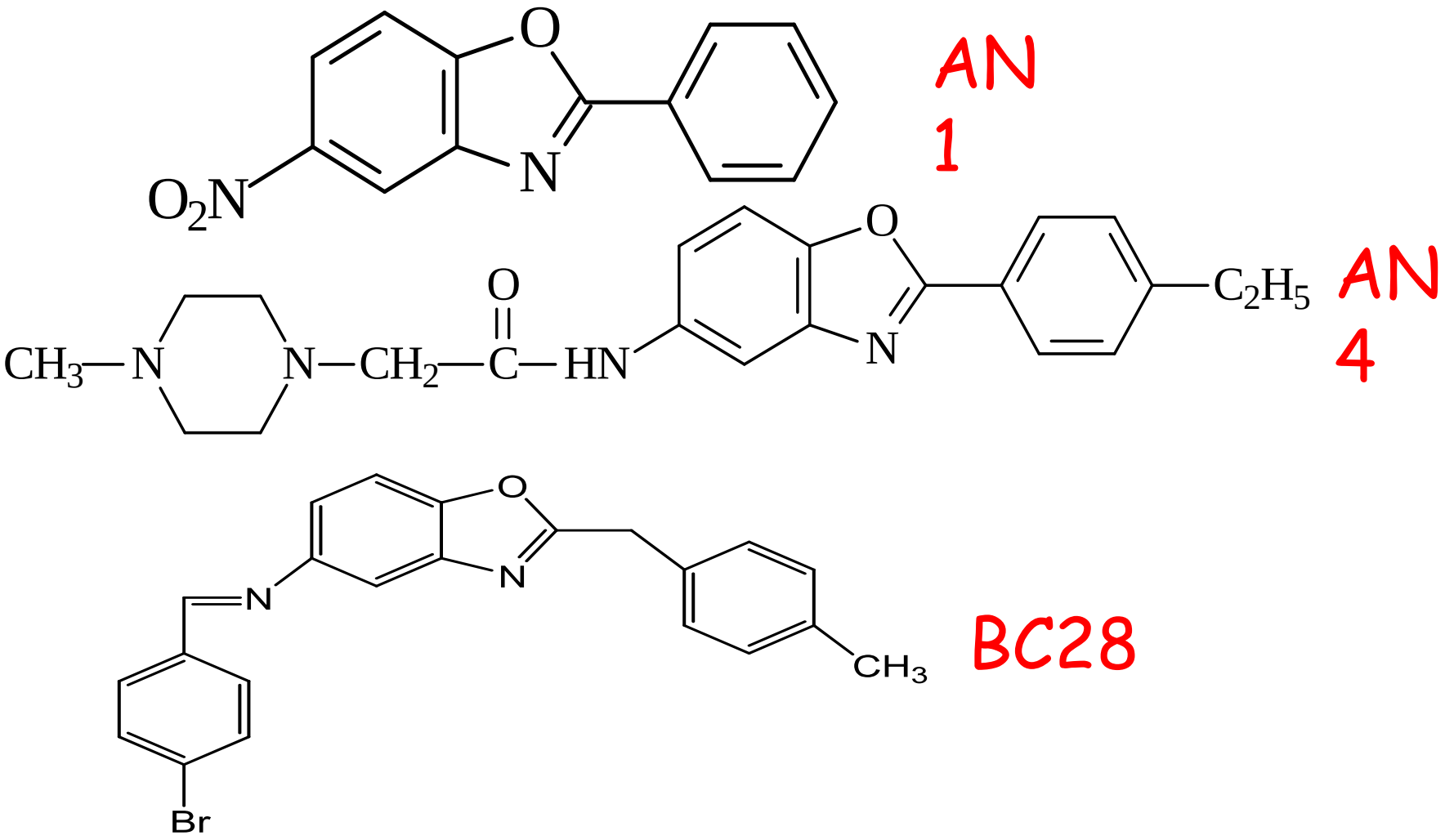


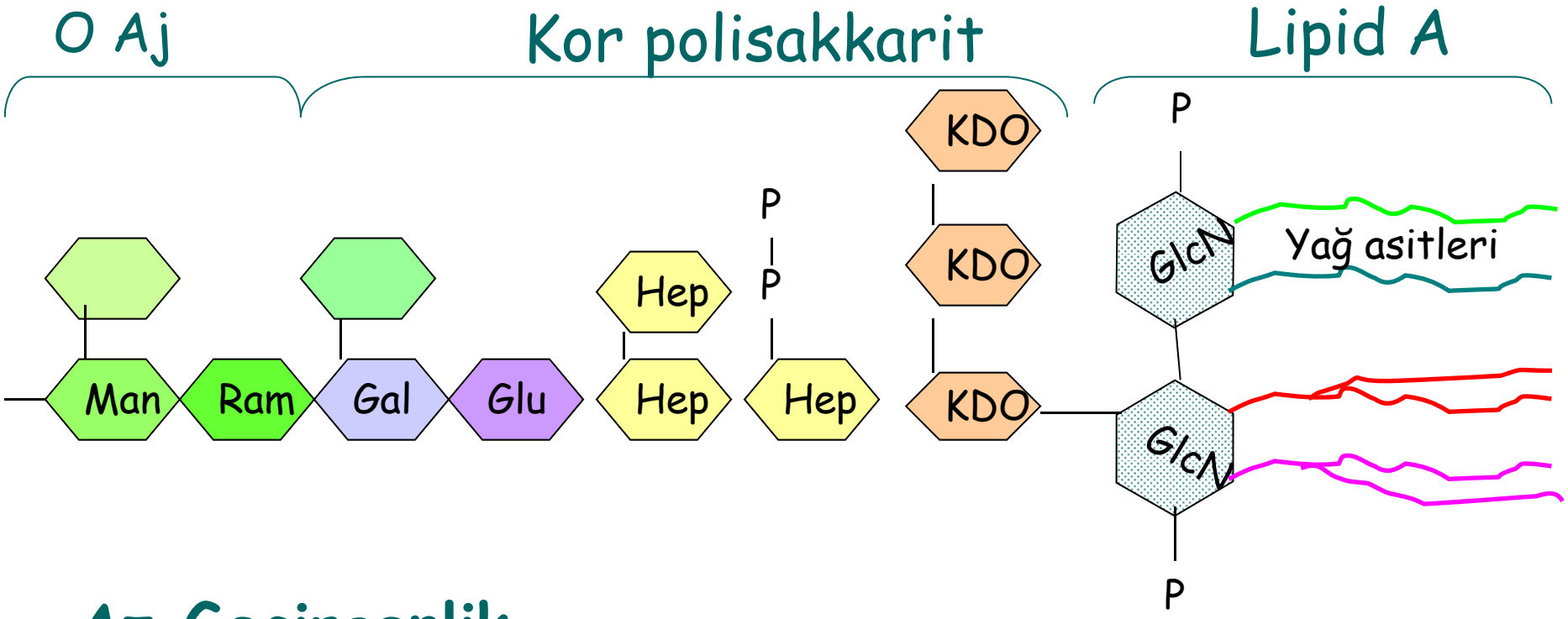
	MIC (ug/mL	
	CIP	CHL
SBMox2	128	256
BC-15	2	128
BC-16	2	128
BC-17	16	256
BC-22	2	128
BC-23	2	128
BC-26	2	128
BC-27	4	256
BC-28	1	32
BC29	64	16
AN1	2	16
AN4	2	64
AN28	0,5	32
XT5B	4	16
XT5BR	0,5	128
XT2B	4	64

	MIC (ug/mL)	
	CIP	CHL
SBMox2	128	256
SY3	0,5	8
SY4	<0,5	<0,5
SY6	128	256
SY16	2	2
SY17	2	128
SY18	<0,5	
SY21	2	128
TD5	4	256
TD2B	2	
TD3B	32	4
TD10B	16	128
MHA-PIP	32	256
MHA-MEP	128	256

	MIC (ug/mL)	
	CIP	CHL
AG102	0.125	64
BC-15	<0.5	64
BC-16	0.125	64
BC-17	0.125	32
BC-22	<0.5	64
BC-23	<0.5	64
BC-26	<0.5	64
BC-27	<0.5	64
BC-28		<0.5
BC29	0.125	32
AN1	<0.5	16
AN4	0.03	32
AN28	0.125	<0.5
XT5B	0.125	<0.5
XT5BR	0.125	32
XT2B	0.125	32

	MIC (ug/mL)	
	CIP	CHL
AG102	0.125	64
SY3	0.125	16
SY4	0.125	8
SY6	<0.5	64
SY16	0.125	8
SY17	<0.5	64
SY18	0.125	
SY21	<0.5	64
TD5	<0.5	64
TD2B		<0.5
TD3B	0.125	32
TD10B		64
MHA-PİP	0.125	128
MHA-MEP	0.125	64

DIŞ MEMBRAN



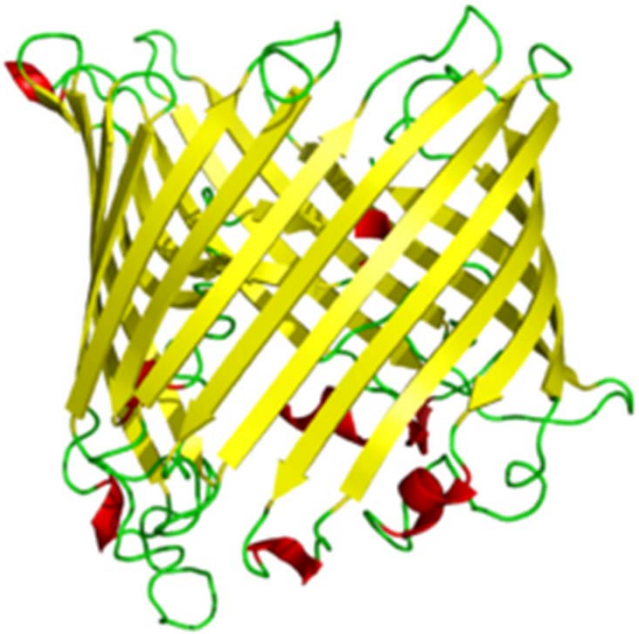
Az Geçirgenlik

- Anyonik lipopolisakkaritler
- LPS meoleküller arasındaki kuvvetli yan bağlar
- Doymamış yağ asiti eksikliği

Porin Kaybı veya mutasyonu

Porin kaybı ve Direnç

- *OprD porin kaybı*
  - *P. aeruginosa* 'da İMİPENEM direnci
- CarO kaybı
  - A. baumanii KARBAPENEM direnci



	MIC µg/ml			
Antibiotic	Isolate 2	Isolate 9	Isolate 13	Isolate 14
Imipenem	8	8	64	32
Meropenem	4	4	8	8
Cloxacillin	>512	>512	>512	>512

A. baumannii
CarO kaybı veya mutasyonu

	Isolate 2	Isolate 9	Isolate 13	Isolate 14
Oxa-23	N	P	N	N
Oxa-24	N	N	P	P
Oxa-58	N	N	P	P
Imp-1	N	N	N	N
Vim-2	N	N	N	N