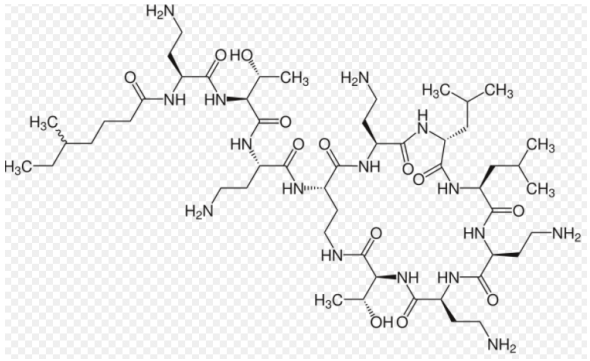
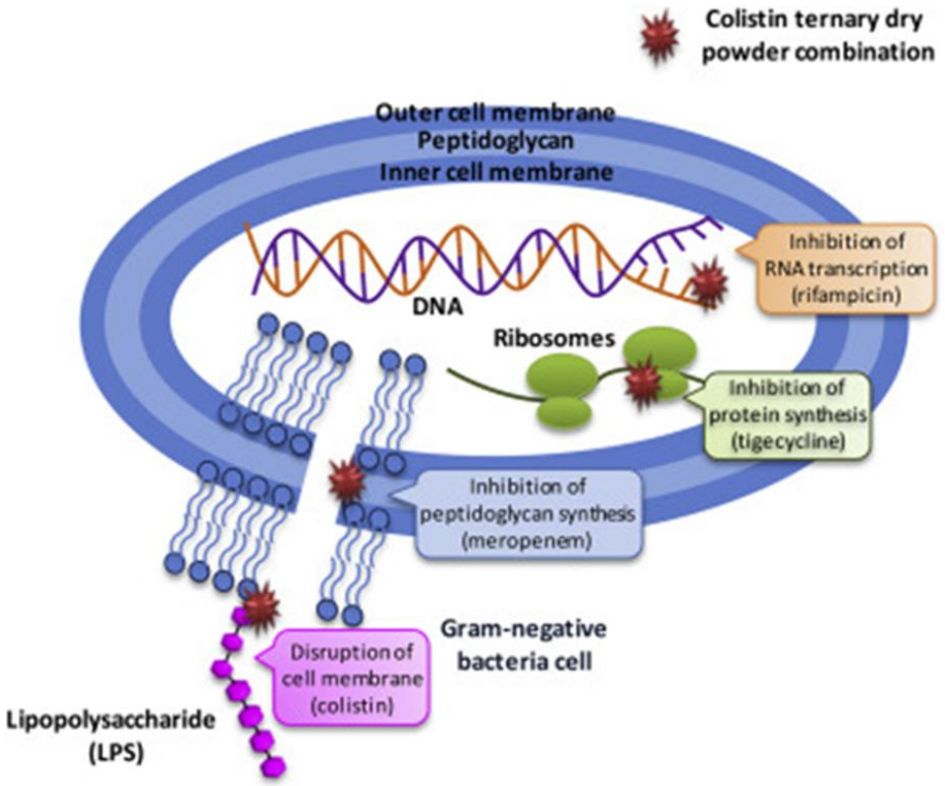


# KOLİSTİN

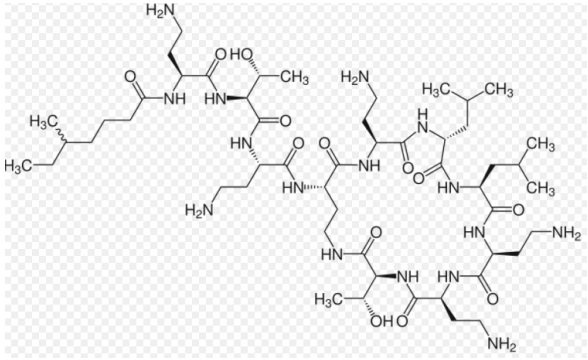


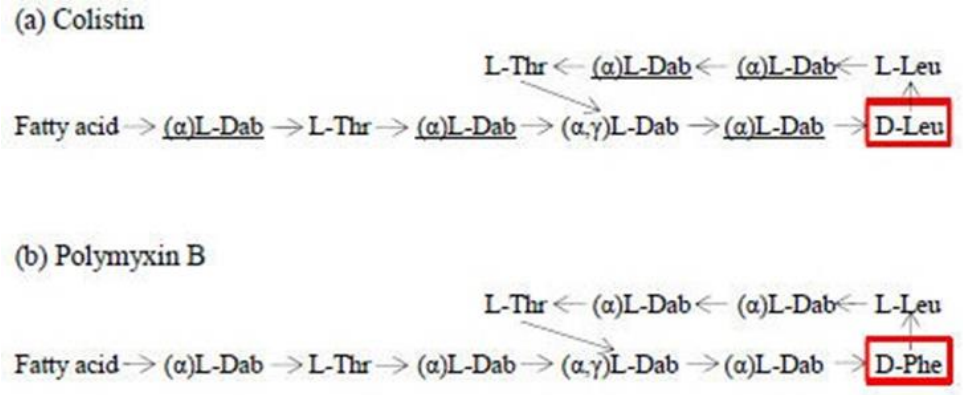

Prof. Dr. Zerrin Aktaş
İSTANBUL TIP FAKÜLTESİ
TIBBİ MİKROBİYOLOJİ AD

# SUNUM PLANI VE SORULAR

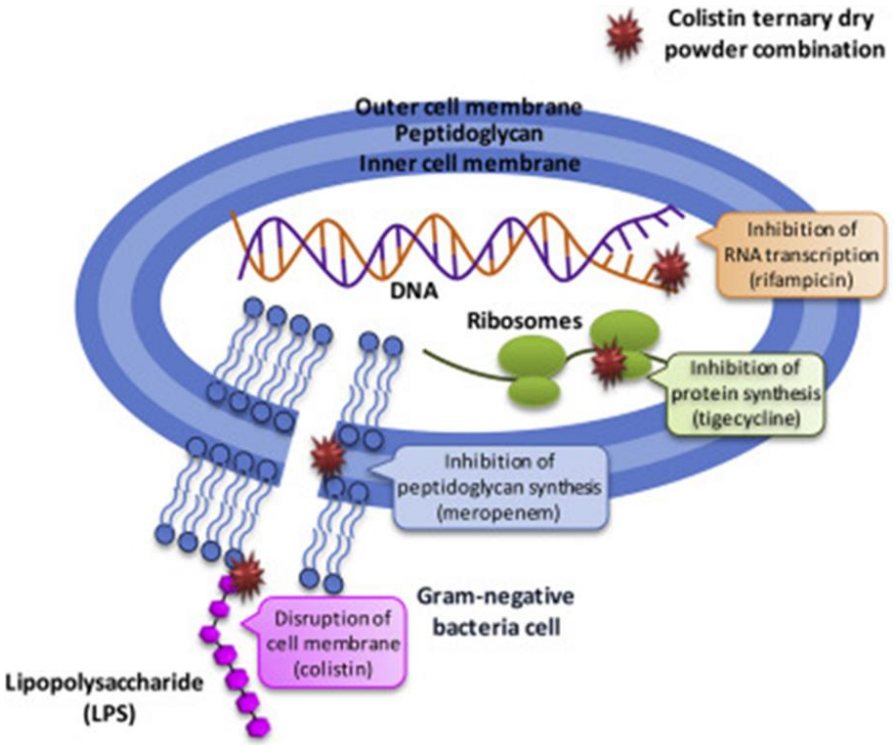
- KOLİSTİNİN ETKİ SPEKTRUMU
- DİRENÇ MEKANİZMALARINI BİLMEK ÖNEMLİ Mİ?
- PLAZMİT ANALİZLERİ ÖNEMLİ Mİ?
- PLAZMİDİK DİRENCİN KAYNAĞI?
- HANGİ DUYARLILIK YÖNTEMLERİNİ KULLANIYORUZ?
- KULLANDIĞIMIZ TESTLERE GÜVENMELİ MİYİZ?
- DÜNYADA DİRENÇ DURUMU?
- BİLİNENLER VE YAPILMASI GEREKENLER

# KOLİSTİN





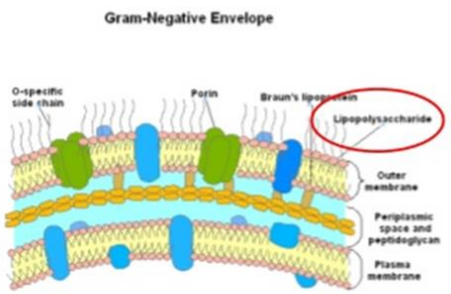

- 1950'de klinik kullanım
- Polimiksin E
- Günümüzde çoğul dirençli bakterilerle gelişen enfeksiyonlarda kullanım alanı

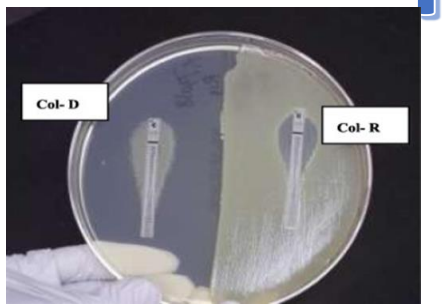


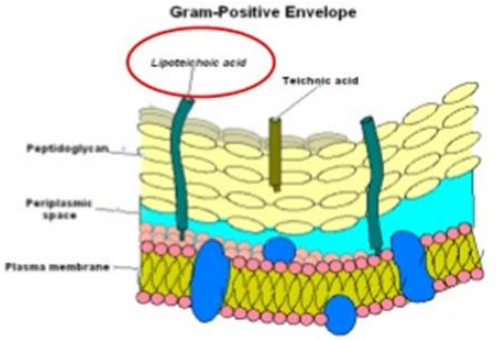

- ❖Karbapenemlere dirençli Enterobacteriaceae
- ❖Karbapenemlere dirençli *Acinetobacter baumannii*
- ❖Karbapenemlere dirençli *Pseudomonas aeruginosa*

Kolistin sülfat->Topikal tedavi
Kolistinmethan sulfonat->Parenteral

# Kolistinin Etki Spektrumu




## Etkili

- *P. aeruginosa, A. baumannii*
- *S. maltophilia, Aeromonas* spp
- ***Klebsiella* spp**, *Enterobacter* spp
- *E. coli*, *Citrobacter* spp
- *Salmonella* spp, *Shigella* spp
- *Legionella* spp.
- *H. influenzae*
- *Bordetella pertussis*
- *M. tuberculosis*
- *Mycobacterium* türlerinin bazıları

## Etkisiz

- Gram pozitif bakteriler
- *N. meningitidis*, *N. gonorrhoeae*
- *Proteus mirabilis, M. catarrhalis*
- *Serratia* spp, *M. morganii*
- *Burkholderia* spp.
- *Chromobacterium* spp.
- *Brucella* spp.
- Anaerop bakteriler

Expert Rev Anti Infect Ther. 2012;10(8):917-934.

# Gram Negatif Bakteriler

Kolistine Duyarlı
(0.12-2 mg/L)

Kolistine Dirençli
(4-≥128 mg/L)

**İntrinsik Direnç**

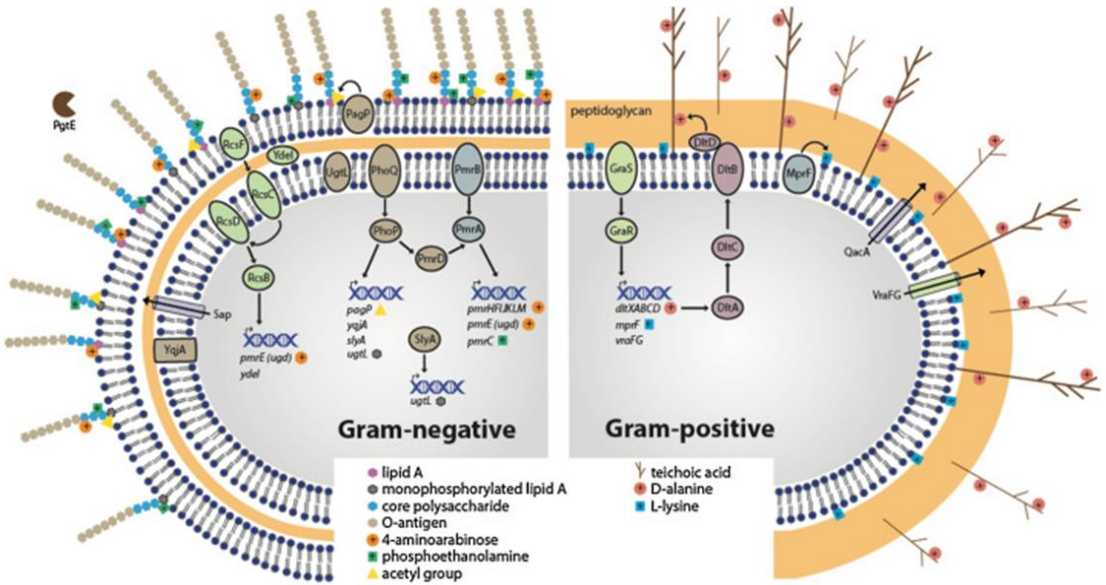
*Proteus, Morganella, Providencia, Serratia, Burkholderia*

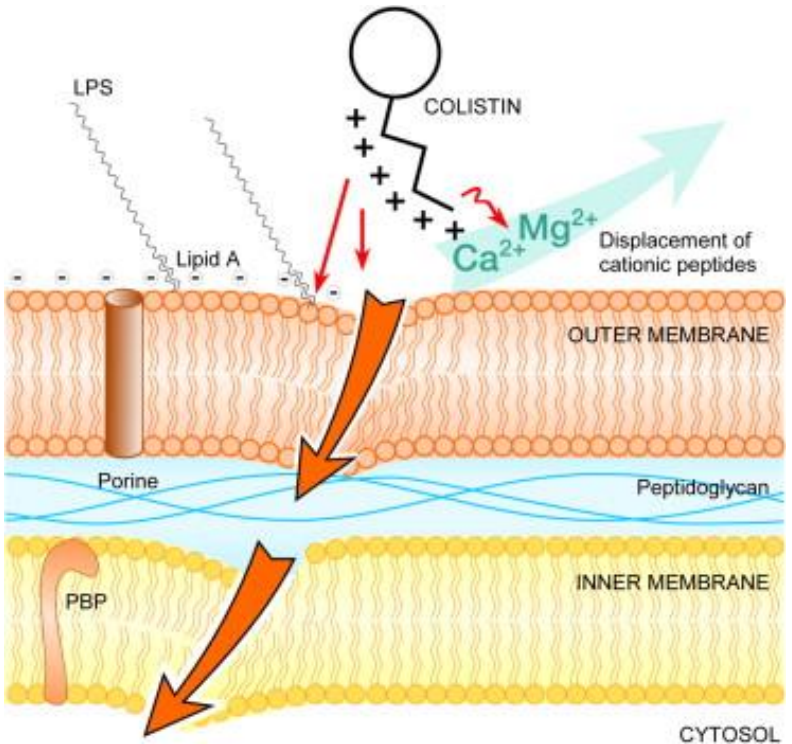
**Kazanılmış Direnç**
**A-Kromozomal, B-Plazmidik (mcr-1,2,3) C- Bilinmeyen**

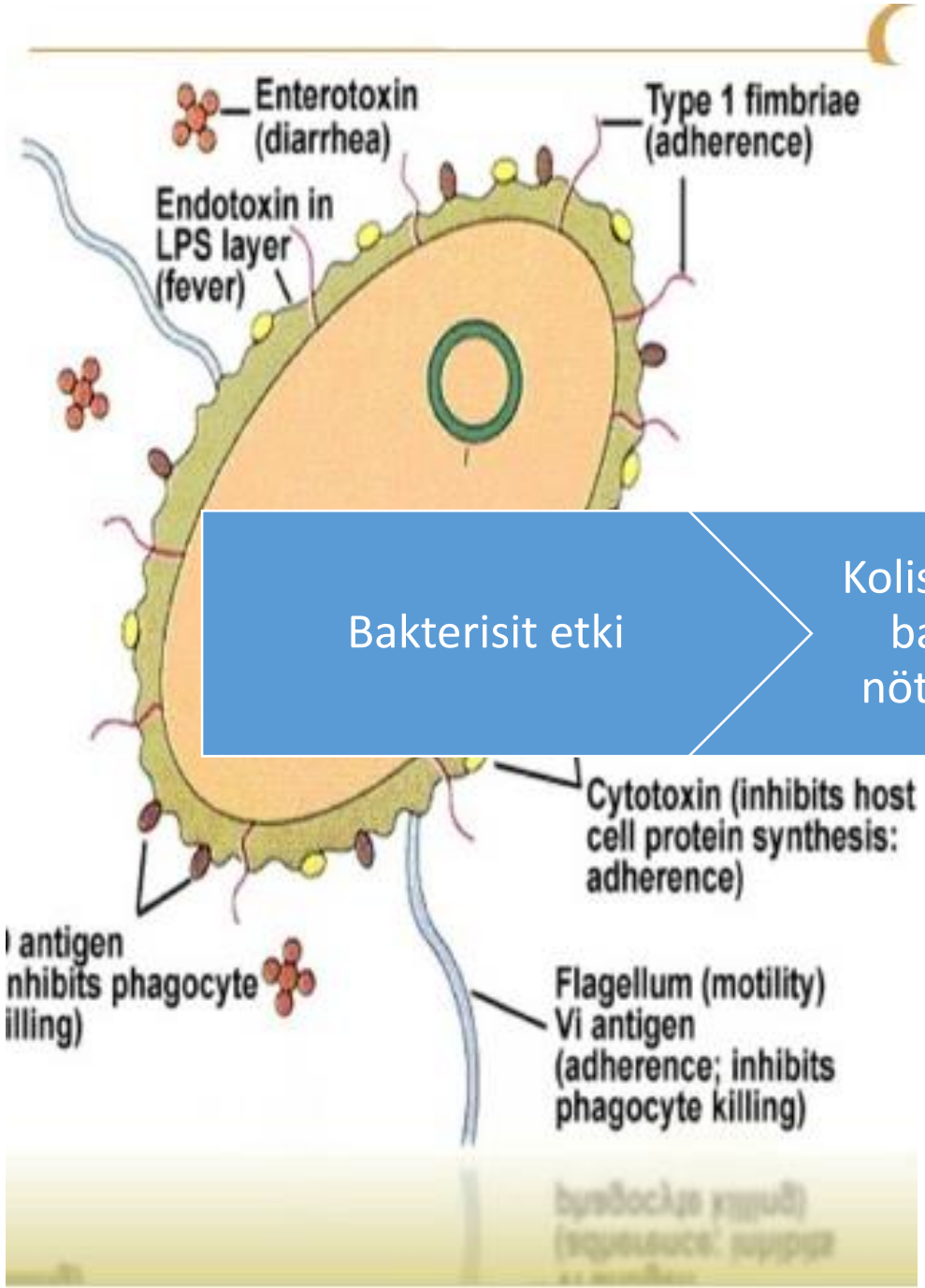
*E. coli, Klebsiella, Enterobacter, Hafnia, Acinetobacter, Pseudomonas, Stenotrophomonas*

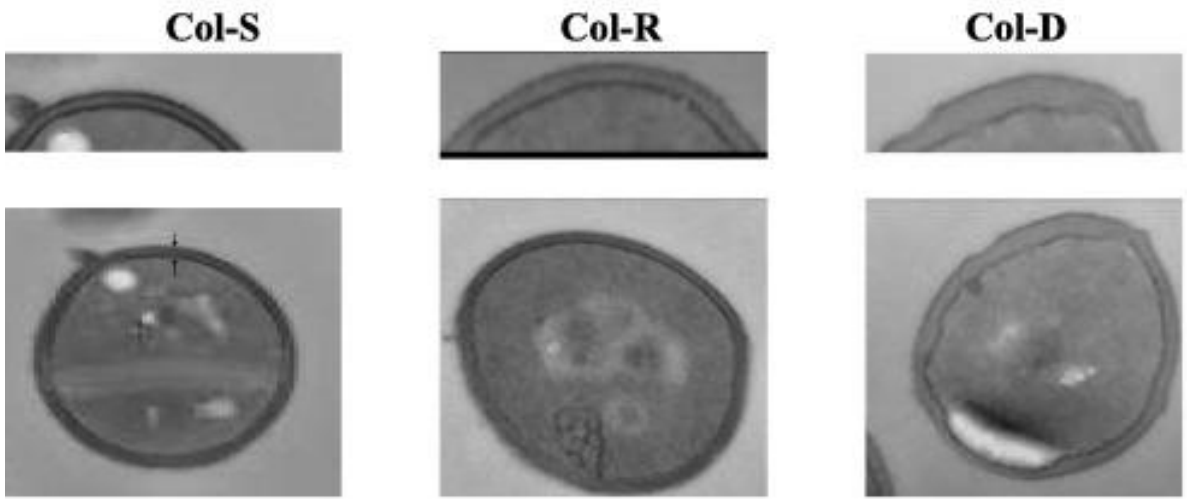
# Kolistinin etki mekanizması

- Kolistin LPS'deki anyonik LipitA'nın fosfat gruplarına bağlanarak
- Membran lipidlerinin fosfat gruplarından iki değerlikli katyonların (Ca, Mg) yer değişikliği sonucu dış ve iç hücre zarı parçalanır ve sitoplazma içeriği dışarı çıkarak bakterinin ölümü
- Kolistin dolaşımdaki serbest lipitA'ya bağlanarak nötralize eder ve dolayısıyla anti-endotoksin aktivitesi vardır.



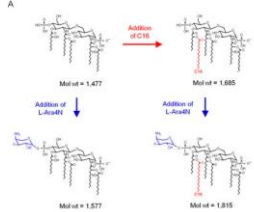






Bakterisit etki → Kolistin LPS'lere bağlanarak nötralize eder → Dolaşımdaki endotoksinin patofizyolojik etkilerini önler

# DİRENÇ MEKANİZMALARI



1-Lipid A biyosentezini yapan genlerin (lpxA/C/D) inaktivasyonu ile LPS kayıbı

2 Lipopolisakkaritlerin yapısında modifikasyonlar ile yapısal değişiklikler

PmrA/PmrB; PhoP/PhoQ ve mgrB genleri

Lipid A'ya 4-amino-4-deoksi-L-arabinoz (Lara4N) ve/veya fosfoetanolamin (pEtN) eklenmesi ile pozitif yükün arttırılmasıyla LPS'ye azalmış affinite

3--Dış membran proteinlerinde defektler

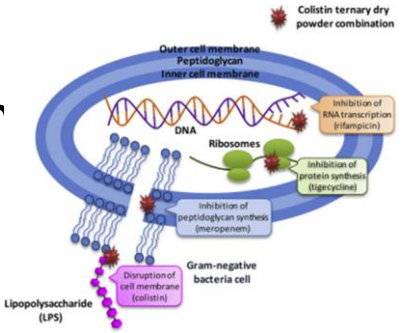
4-Aşırı kapsül sentezi ile lipopalisakkaritlere bağlanmasını önleme

5-mgr B geninde (IS5-like, IS1F-like, ISKpn14, ISEcp1-blaoxa-181) insersiyonlar veya delesyonlar ile inaktivasyon

6-Efluks pompalarıın aşırı çalışması

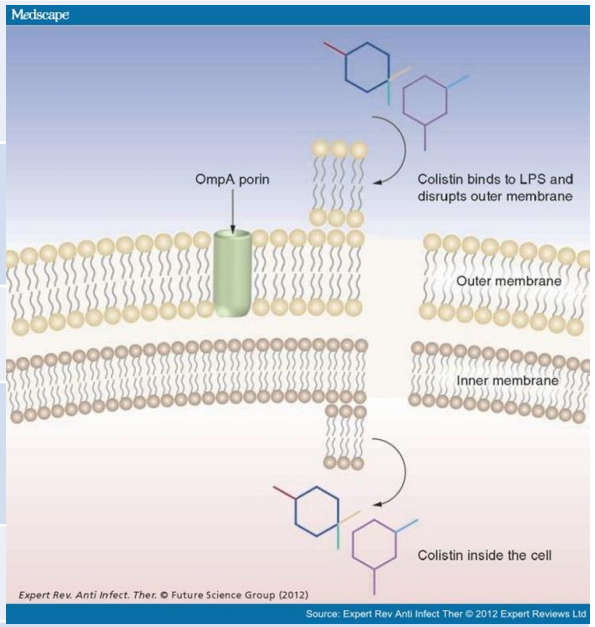
7-eptB, pagL, ve cdtA genleri kolistin direncinden sorumlu diğer ger

8-Bilinmeyen mekanizmalar

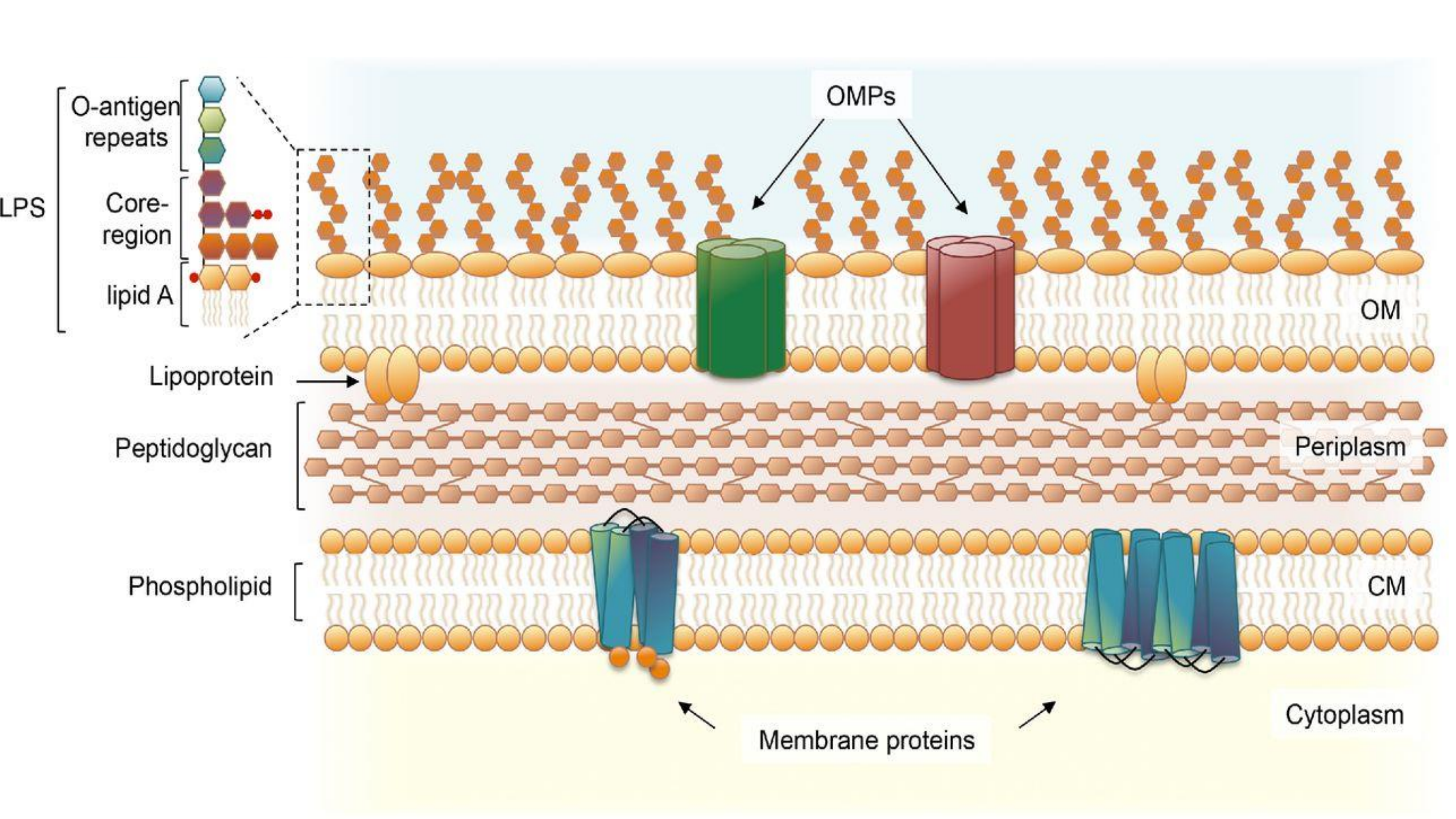


# POLİMİKSİN DİRENCİNİN MEKANİZMALARI

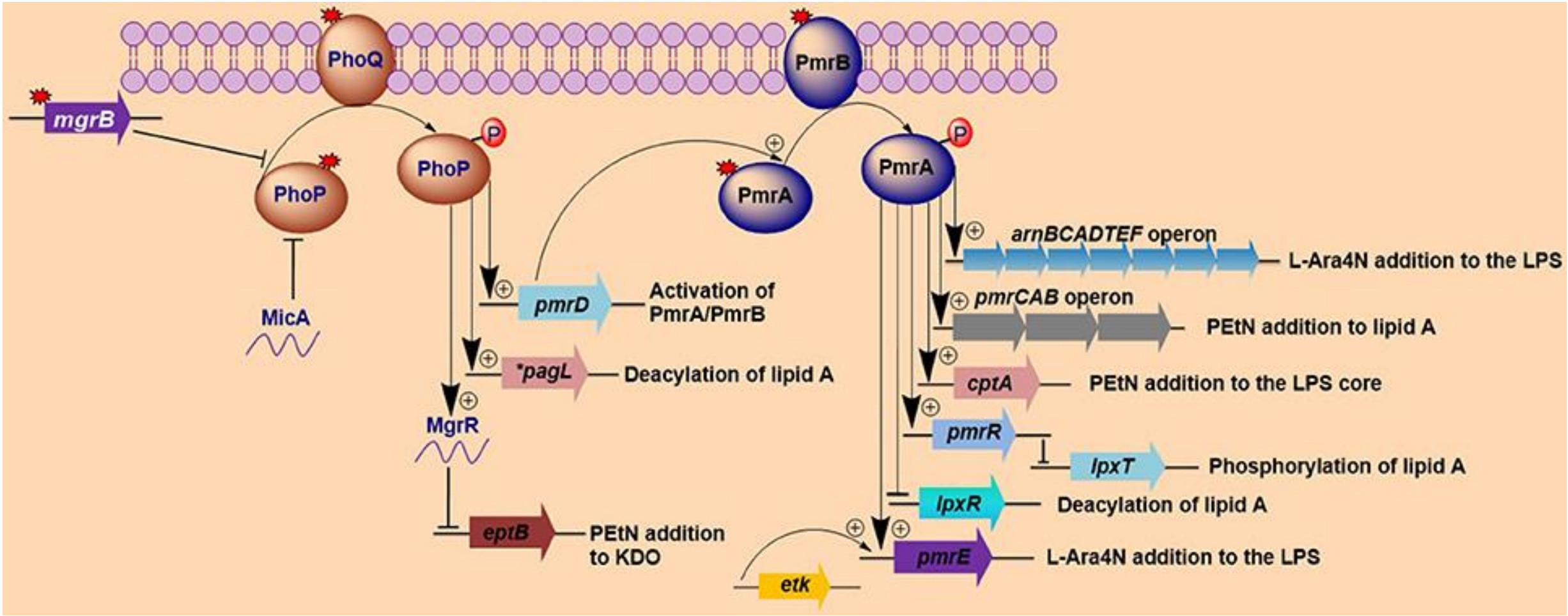
| Direnç Mekanizmaları | Bakteriler |
|---|---|
| LPS alteration | *E. coli, Salmonella, Klebsiella pneumoniae, P. aeruginosa A. baumannii* |
| pmrA ve pmrB genlerinde ve iki komponentli sinyal proteinlerinde mutasyonlar | *A. baumannii* |
| Mutations in lpxA, lpxC and lpxD induces loss of the lipid A component of lipopolysaccharide | *A. baumannii* |
| Role of OprH, an outer membrane protein altered | *P. aeruginosa* |
| Changes in negatively charged surface LPS induced by the regulatory loci pmrA and phoP | *Enterobacteriaceae* |
| Resistance by mutation in pmrA and PmrB genes | *Salmonella* |
| | |

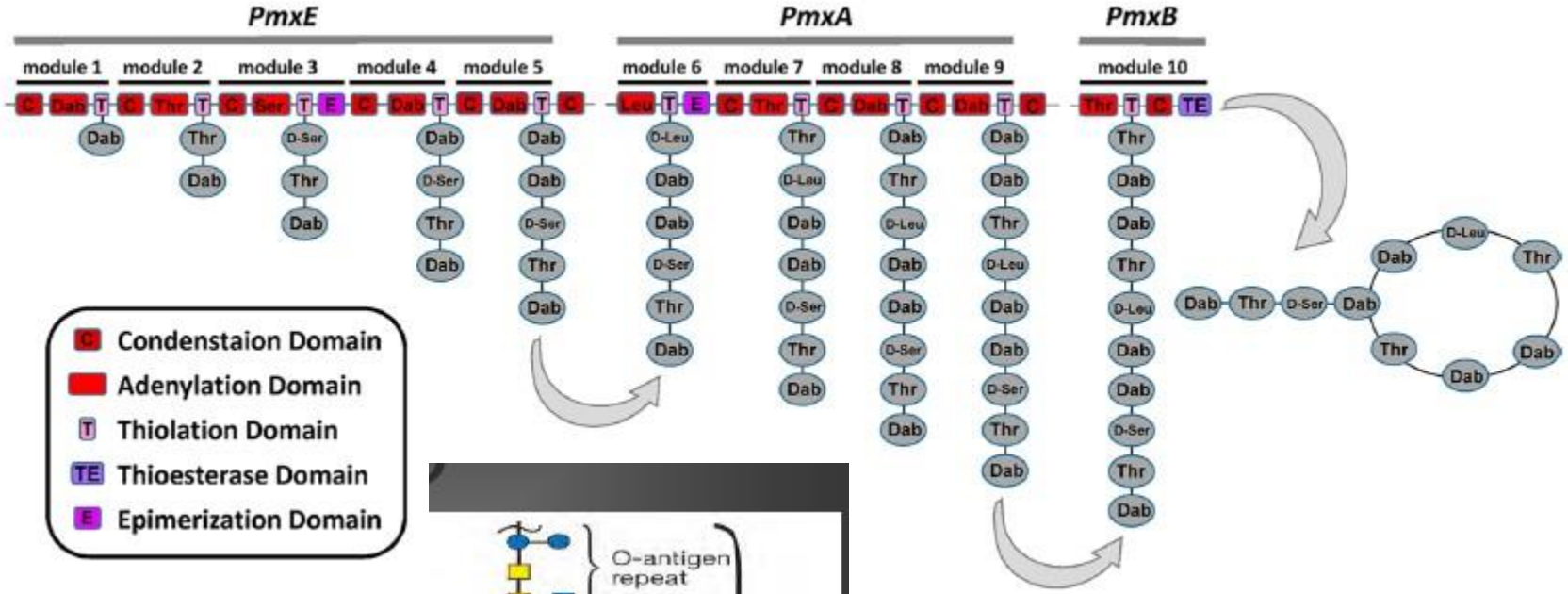


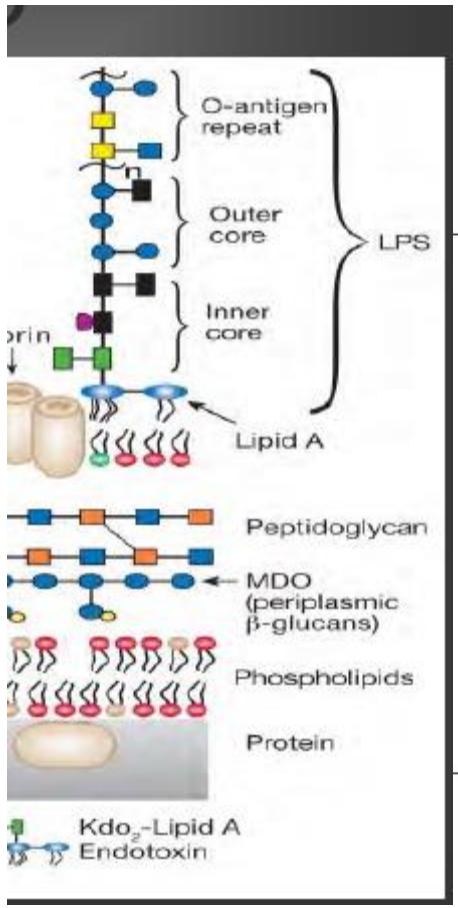

Expert Rev Anti Infect Ther. 2012;10(8):917-934.

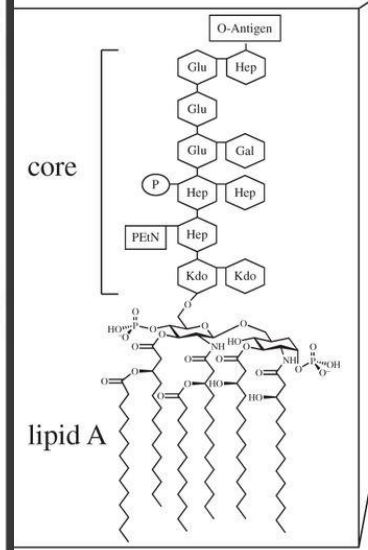

O-antigen repeats
Core-region
lipid A
LPS
OMPs
OM
Lipoprotein
Peptidoglycan
Periplasm
Phospholipid
CM
Membrane proteins
Cytoplasm

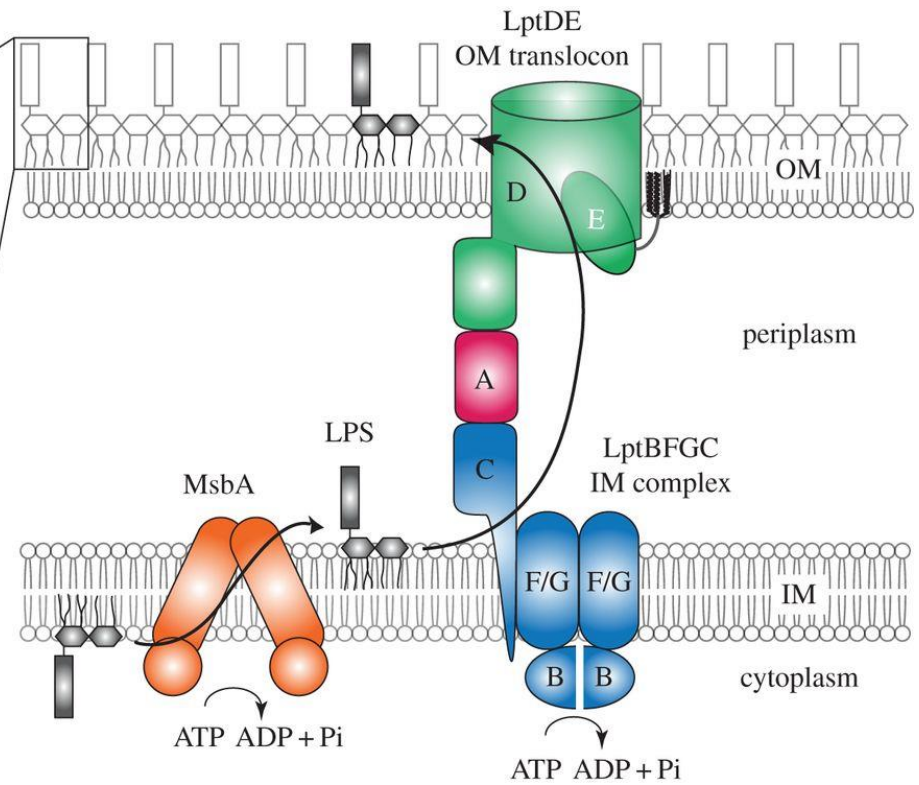
Kromozomal PmrA/PmrB ve PhoQ-PhoP'nin biribiriyle ilişkili iki düzenleyici sistem
Bu genlerde oluşan mutasyonlar lipopolisakkarit modifikasyonuna (fosfoetanolamin veya 4-amino-4deoksi -L-arabinoz)

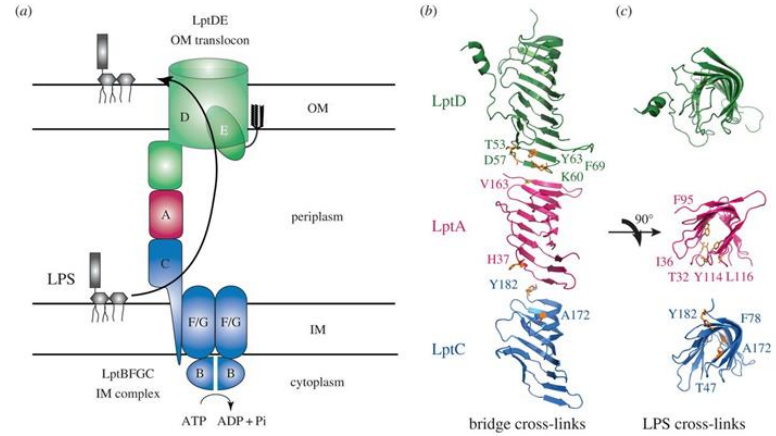



PmxE
PmxA
PmxB
module 1
module 2
module 3
module 4
module 5
module 6
module 7
module 8
module 9
module 10
Condenstaion Domain
Adenylation Domain
Thiolation Domain
Thioesterase Domain
Epimerization Domain


O-antigen repeat
Outer core
Inner core
LPS
Lipid A
Peptidoglycan
MDO (periplasmic β-glucans)
Phospholipids
Protein
Kdo2-Lipid A Endotoxin

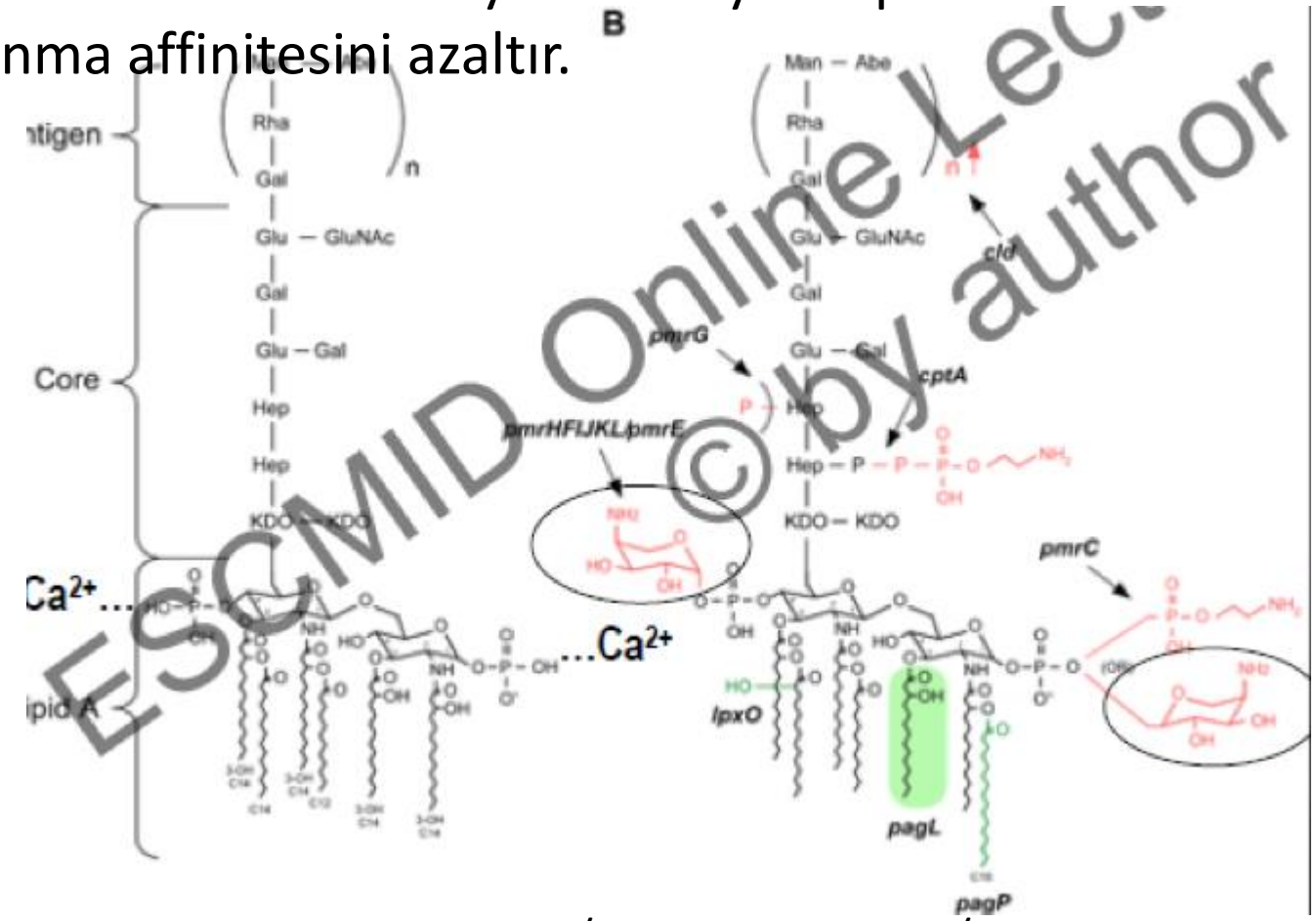

core
lipid A
O-Antigen
Glu
Hep
Gal
P
PEtN
Kdo


LptDE
OM translocon
OM
periplasm
IM
cytoplasm
LPS
MsbA
LptBFGC
IM complex
ATP ADP + Pi

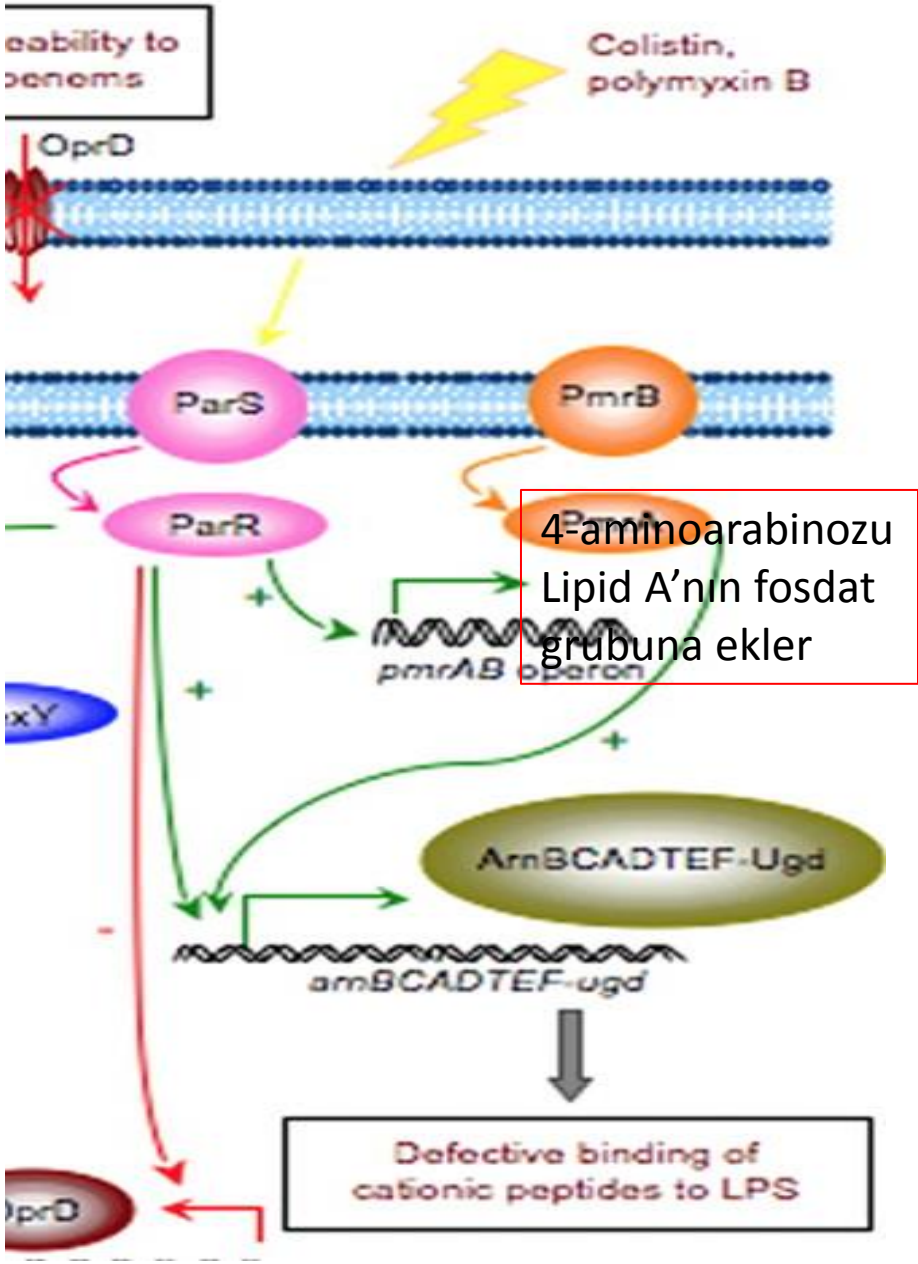

(a)
(b)
(c)
LptDE
OM translocon
LptD
LptA
LptC
bridge cross-links
LPS cross-links
90°

# *PSEUDOMONAS AERUGİNOSA'DA DİRENÇ*

-PmrE ve PmrHFIJKLM gen ekspresyonu ile
-LPS'in Lipid A'nınfosfat gruplarına etanolamin ve 4-amino-4-deoksi-L-arabinoz ekleyerek katyonik polimiksinlerin bağlanma affinitesini azaltır.

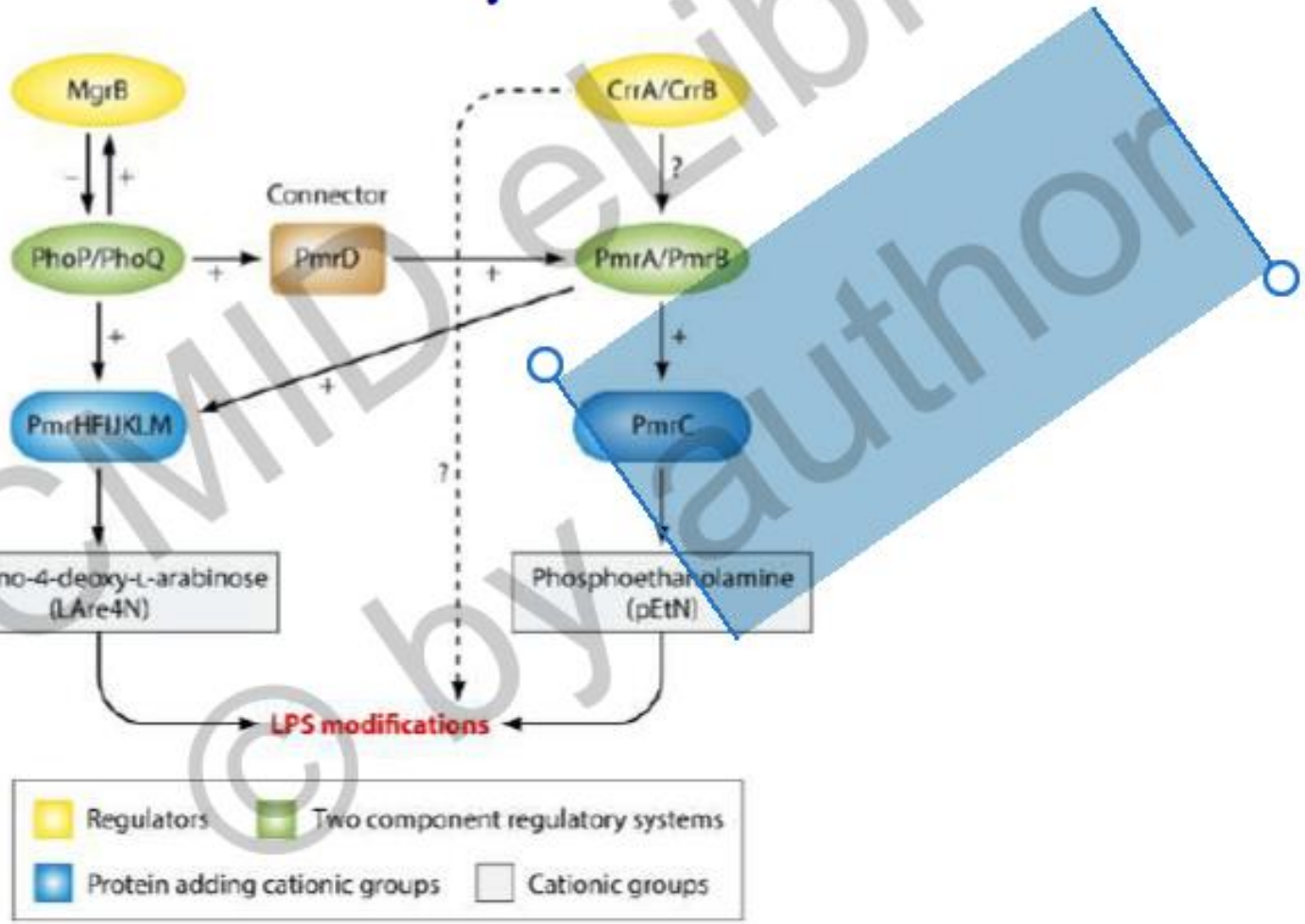



*Pmr*HFUKLM/pmrE=arnBCADTEF/pmrE




4-aminoarabinozu Lipid A'nın fosdat grubuna ekler

# Interplay of resistance mechanisms in *Klebsiella pneumoniae*




Poirel et al., Clin Microbiol Reviews 2017

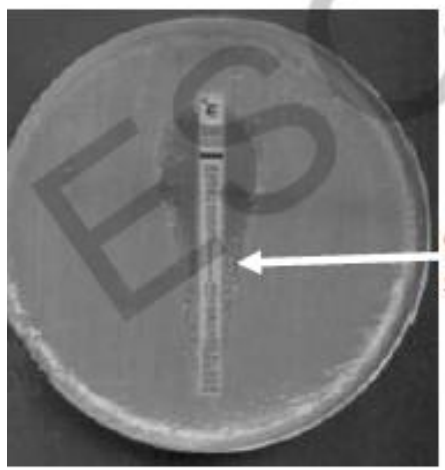
# Heteroresistance to colistin in *K. pneumoniae*

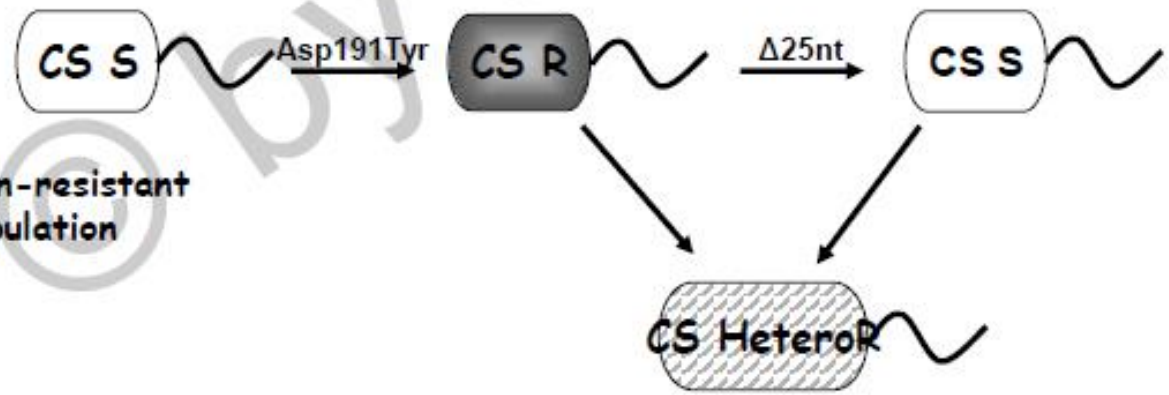
AAC
JournalsASM.org

Heteroresistance to Colistin in *Klebsiella pneumoniae* Associated with Alterations in the PhoPQ Regulatory System

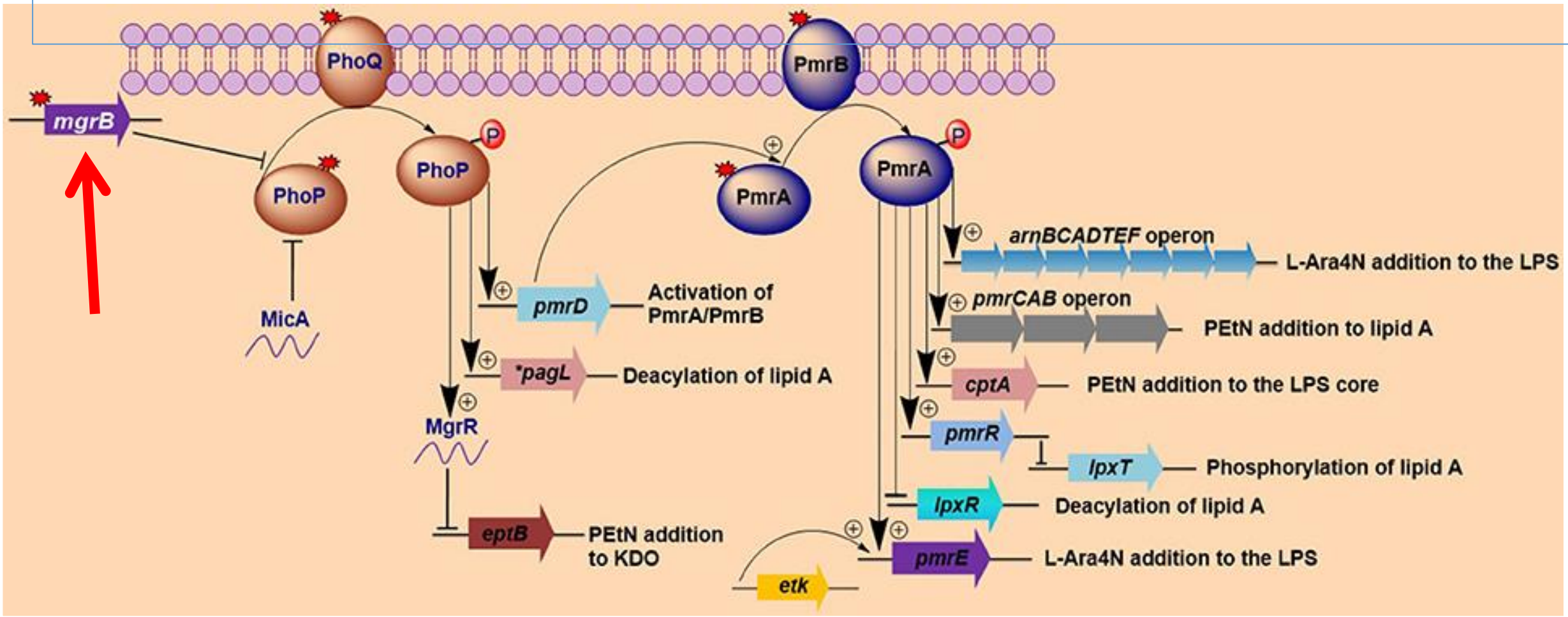
Aurélie Jayol,[a] Patrice Nordmann,[a,b] Adrian Brink,[c] Laurent Poirel[a]

Acinetobacter



- *mgrB* geninin insersiyonel (IS5-like, IS1F-like, ISKpn14, ISEcp1-blaoxa-181) inaktivasyonu kolistin direnciyle ilişkili bulunmuştur.
- OXA-181 geni karbapenemaz özelliğine de sahiptir ve ISEcp1 üzerinde taşınmaktadır.



| Bakteri (TÜRKİYE İZOLATLARI) | Direnç Mekanizması | MİK (mg/L) |
|---|---|---|
| *K. pneumoniae* | PhoQ R16C, PmrB L17Q | >128, 42 |
| *K. pneumoniae* | PmrB T157P, PmrB T157P, PmrB T157P | 32, 32, 16 |
| K. pneumoniae | MgrB W20R, | 32 |
| K. pneumoniae | MgrB W47R , MgrB truncated (46 aa) | 4, 32 |
| *K. pneumoniae* | mgrB IS2 between +44 and +45, mgrB IS1R between +44 and +45 | 64, 128 |
| *K. pneumoniae* | mgrB IS1R between +45 and +46 | 32 |
| *K. pneumoniae* | mgrB ISKpn26-likebetween +74 and +75 | 128 |
| *K. pneumoniae* | Bilinmiyor | 32 ve 4 |
| *K. pneumoniae* | mgrB ISKpn14 between +77 and +78 | 128 |
| K. pneumoniae | mgrB IS1R between +123 and +124 | 32 |
| *K. pneumoniae* | mgrB IS903b-like between +69 and +70 | 64 |
| *K. pneumoniae* | mgrB IS5-like between +74 and 75 | 128 |
| *K. pneumoniae* | mgrB ISKpn14 between +27 and +28 | 64 |
| *K. pneumoniae* | mgrB ISKpn14 between +28 and +29 | 64 |
| *K. pneumoniae* | Deletion nt 22 to 32 mgrB (>128 mg/L) | 128 |

Em. Inf. Dis.2016

# Plazmidik Direnç: mcr(1,2,3) genleri

## mcr-1

### Acquired resistance to colistin in *K. pneumoniae*

AAC

Resistance to Colistin Associated with a Single Amino Acid Change in Protein PmrB among *Klebsiella pneumoniae* Isolates of Worldwide Origin

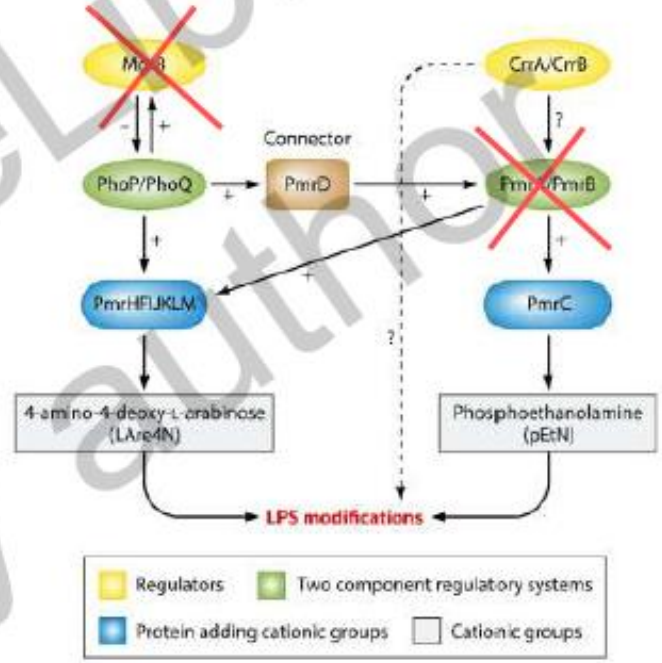
Aurélie Joyol,[a] Laurent Poirel,[a,b] Adrian Brink,[c] Maria-Virginia Villegas,[d] Mesut Yilmaz,[f] Patrice Nordmann[a,b,e]

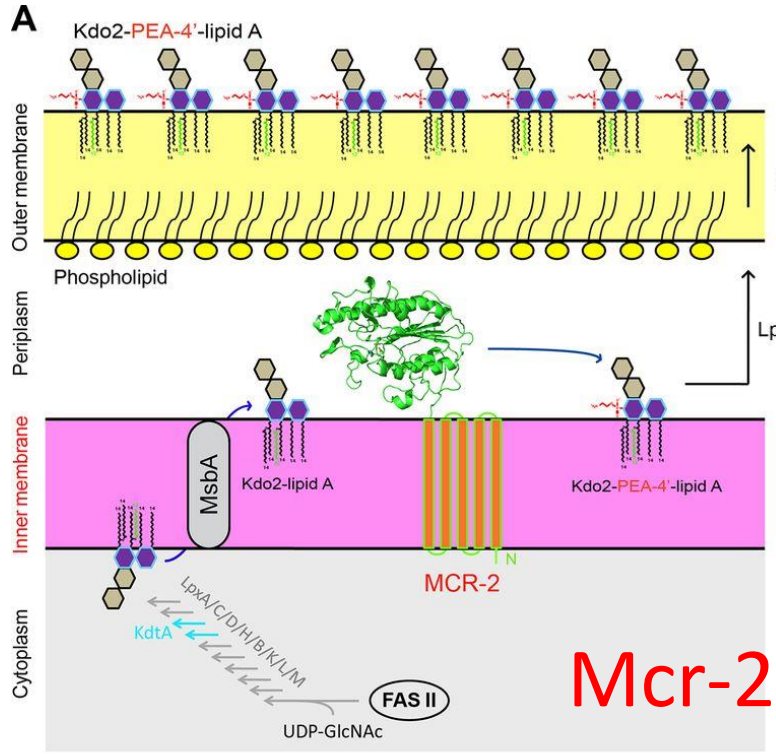
Journal of Antimicrobial Chemotherapy Advance Access published September 3, 2014

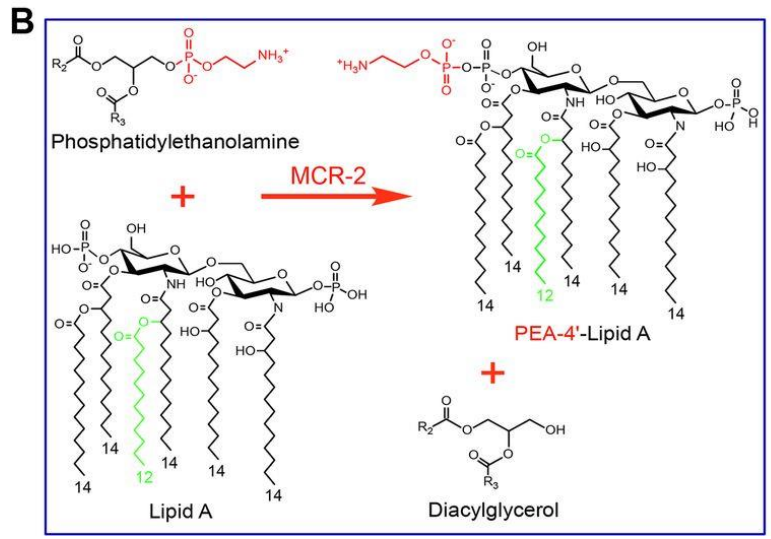
Journal of Antimicrobial Chemotherapy

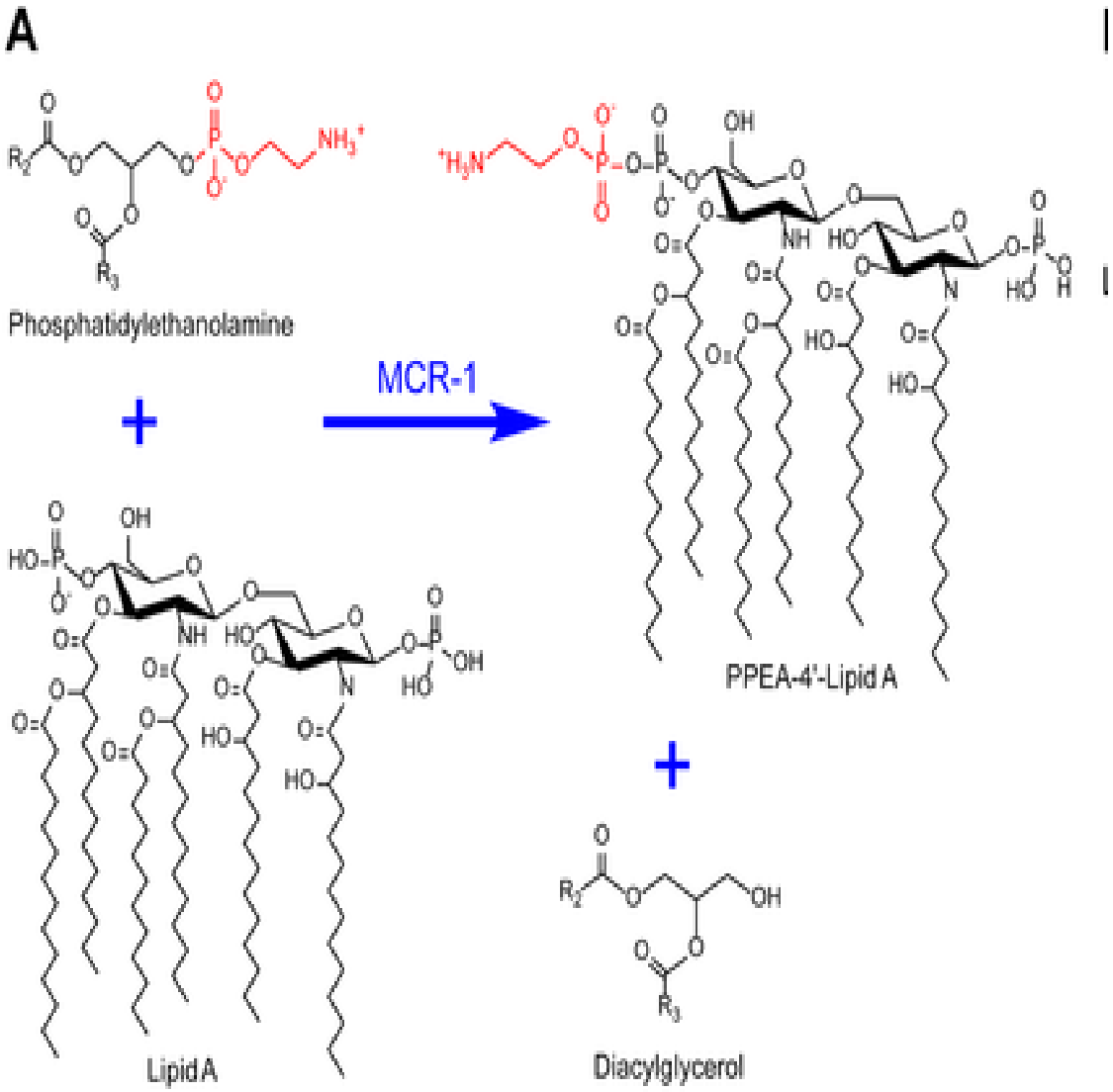
The *mgrB* gene as a key target for acquired resistance to colistin in *Klebsiella pneumoniae*

Laurent Poirel[1,2], Aurélie Joyol[1], Séverine Bontron[1], Maria-Virginia Villegas[3], Melda Ozdamar[4], Salih Türkoglu[4] and Patrice Nordmann[1,5]*

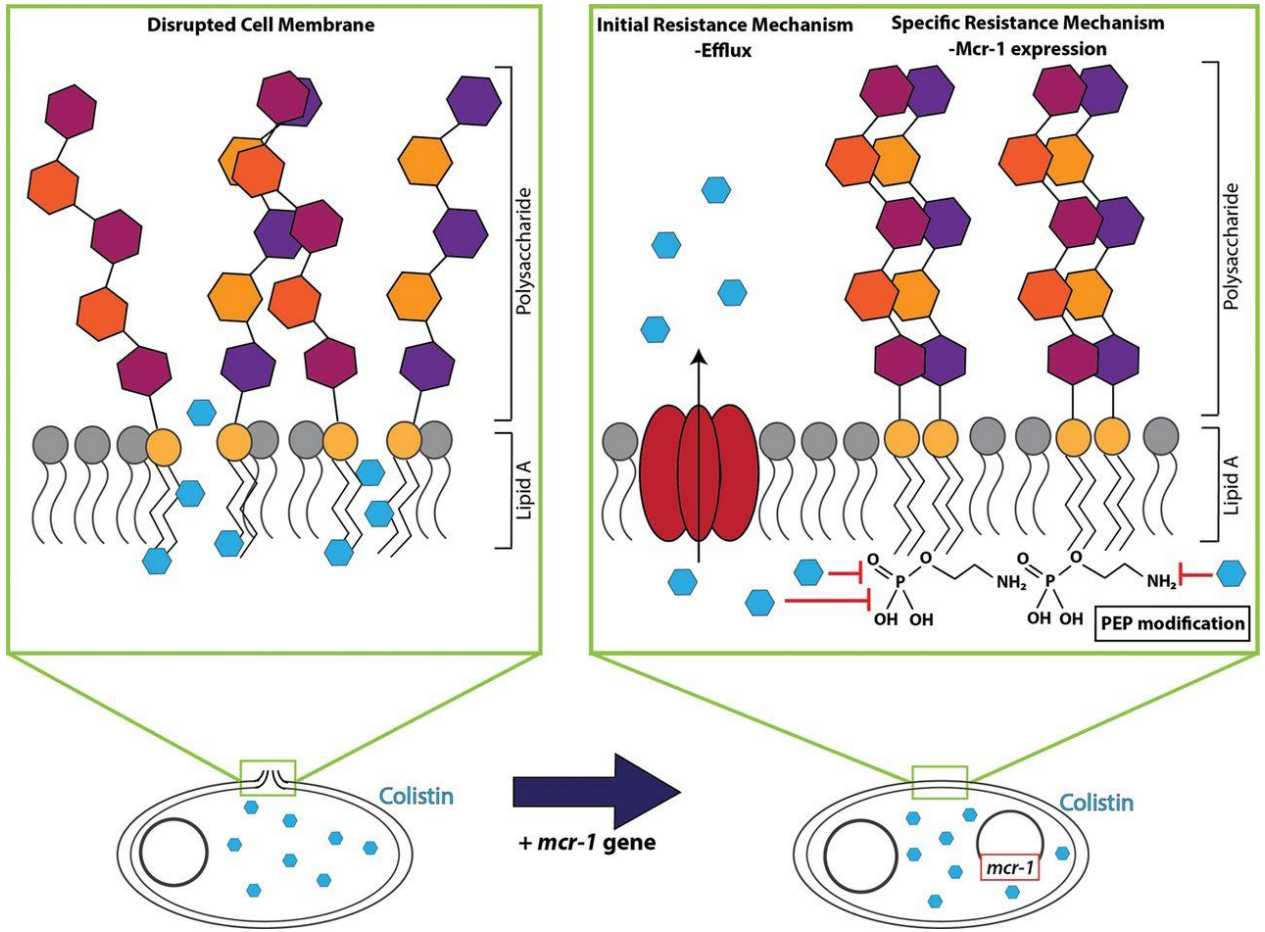




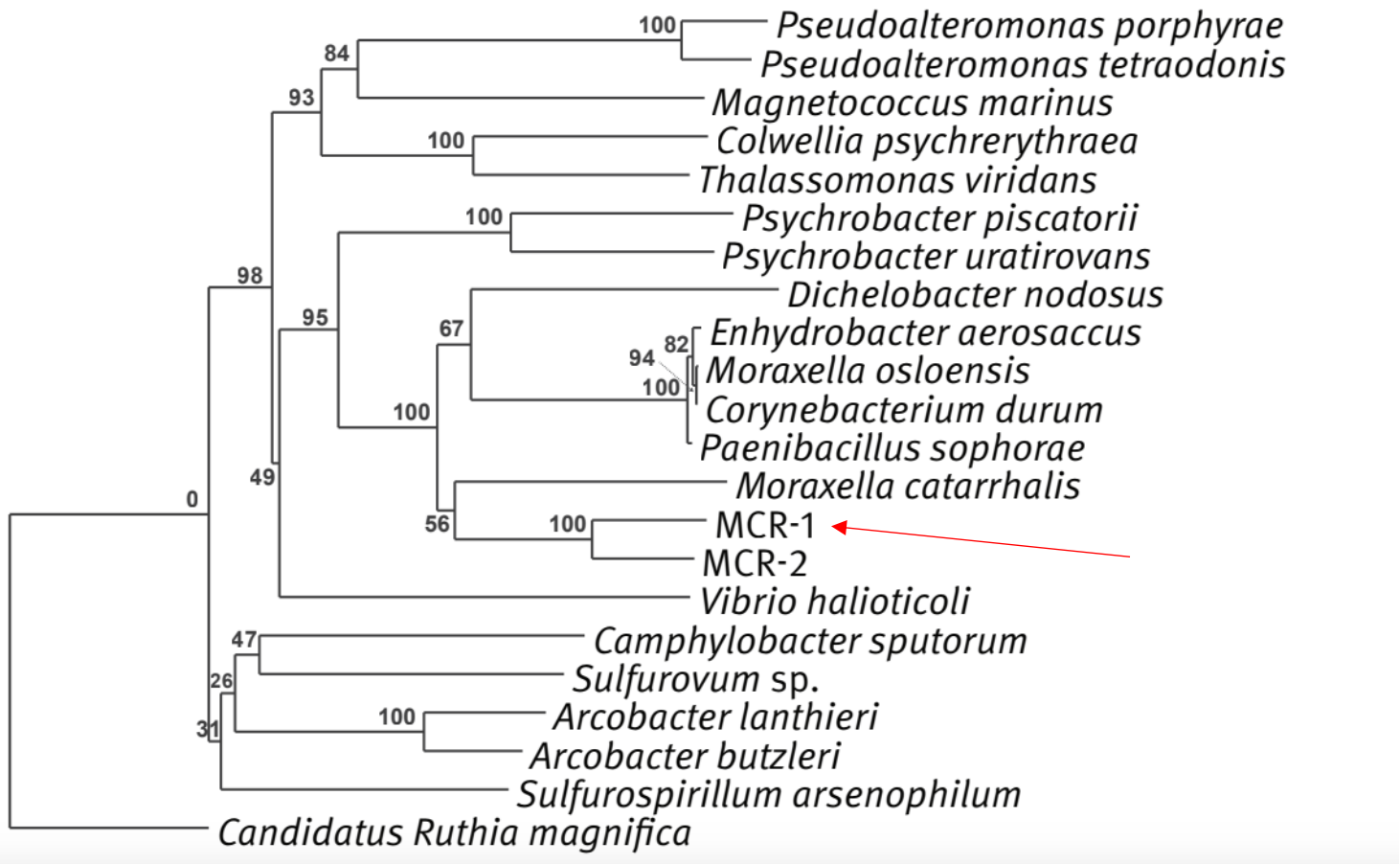

- 2016, mcr-1 geni kolistin direncinin horizontal yayılımından sorumlu
- Genelde *E. coli* de yaygın (insan ve hayvan izolatları)




Plazmit üzerindeki mcr-1 geni fosfoetanolamin transferazı kodlar ve bu enzim fosfoetanalaminin lipidA'ya ekler 16 kat MİK değerinde artış.

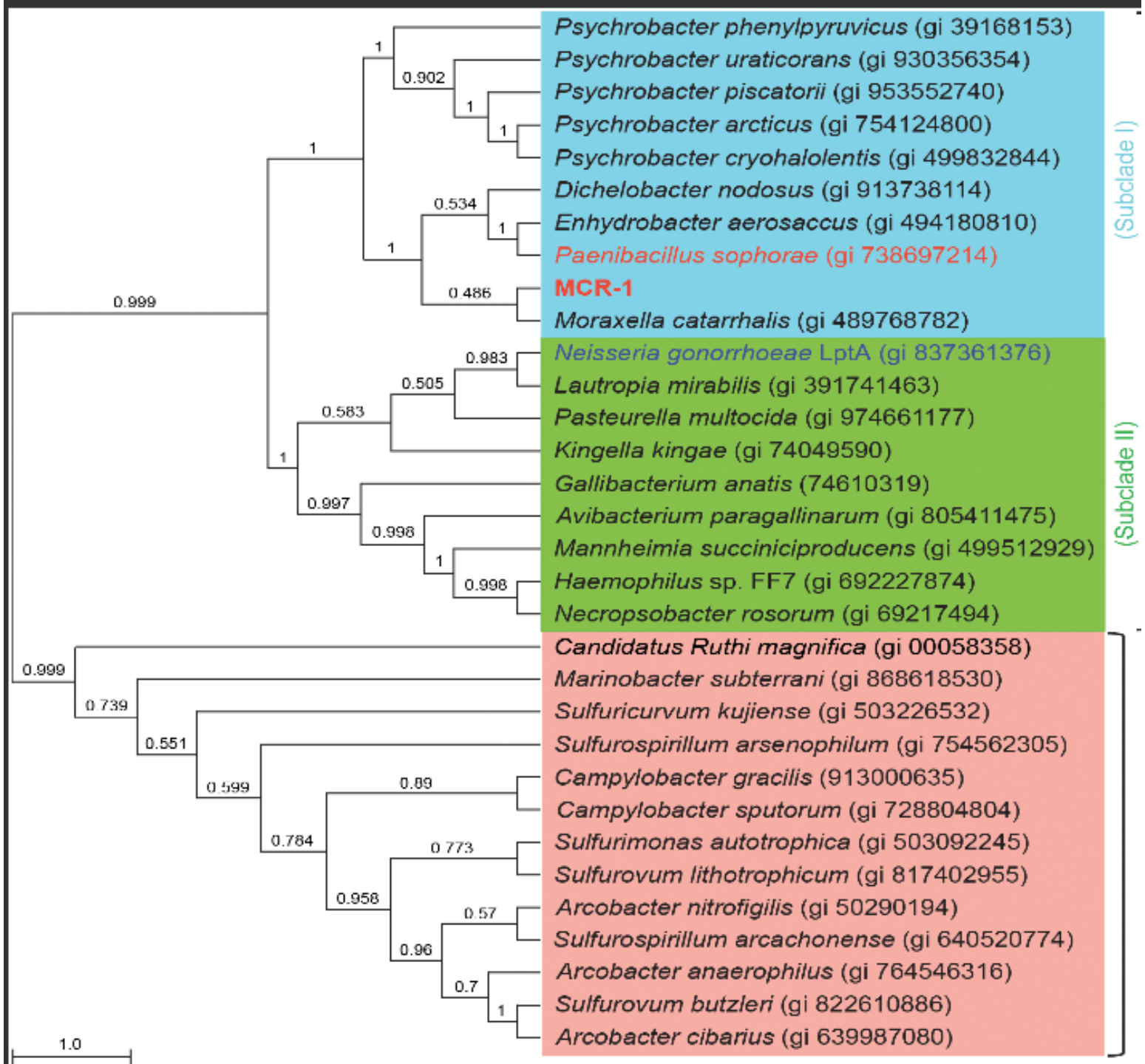
**FIGURE 3**

Phylogenetic analysis of the entire MCR-2 protein sequence




PEA lipid A transferase in Paenibacili, a known producer of polymyxins.
The fact that the plasmid-borne MCR-1 is placed in a subclade neighboring the chromosome-encoded colistin-resistant

Neisseria LptA (EptA) potentially implies parallel evolutionary paths for the two genes.




PLoS Pathog12(11)

Animal &
Plant Health
Agency

# *mcr-1* and *mcr-2* variant genes identified in *Moraxella* spp. isolated from pigs.

Dr. Muna Anjum

Molecular Lead: Antimicrobial Resistance and Enteric Pathogens,
Department of Bacteriology

Muna.Anjum@apha.gsi.gov.uk

# Kolistin ve PolimiksinB MİK Duyarlılık Sınırları
# CLSI ve EUCAST, 2017

| ➢ KRİTERLER<br>➢ BAKTERİLER | KOLİSTİN (MG/L) | | | POLİMİKSİN B (MG/L) | | |
|---|---|---|---|---|---|---|
| **CLSI (M100-S27)** | **Duyarlı** | **Orta Duyarlı** | **Dirençli** | **Duyarlı** | **Orta Duyarlı** | **Dirençli** |
| *Enterobacteriaceae* | - | - | - | - | - | - |
| *P. aeruginosa* | ≤2 | - | ≥4 | ≤2 | 4 | ≥8 |
| *A. baumannii* compleks | ≤2 | - | ≥4 | ≤2 | - | ≥4 |
| **EUCAST 2017 (V7.1)** | | | | | | |
| *Enterobacteriaceae* | ≤2 | - | >2 | - | - | - |
| *Pseudomonas* spp. | ≤2 | - | >2 | - | - | - |
| *A. baumannii* spp. | ≤2 | - | >2 | - | - | - |

# DUYARLILIK TESTLERİ

1-Mikrodilüsyon Broth Test → Altın Standart

2-Agarda dilüsyon

3-Disk diffusion

4-Gradiyent difüzyon (Biomerieux, Liofichem)

5-Sensititre [Thermo Fisher Scientific, MA, USA],

6-MicroScan [Beckman Coulter, CA, USA],

7-Vitek 2 system [bioMérieux, Marcy l'Etoile, France])

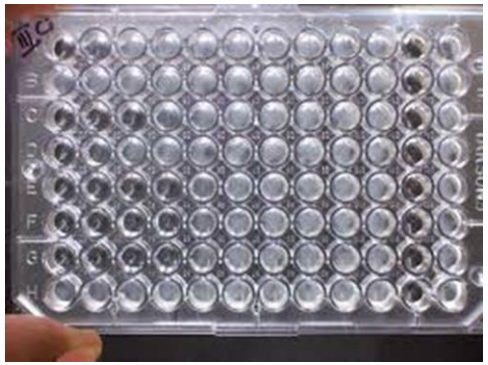
8-Kolistin ve Polymyxin B Etests (bioMérieux, Marcy l'Etoile, France)

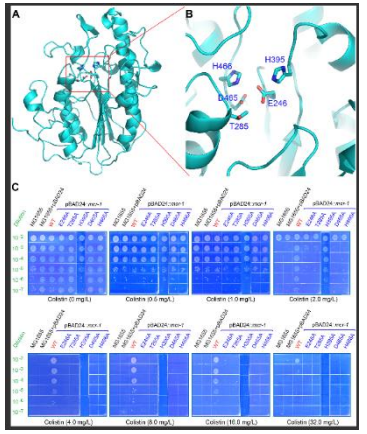
9-Colistin MicronautS ve Micronaut MIC-Strip (Merlin Diagnostika Mikrodilüsyon)

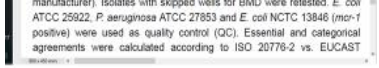
10-SEMPA1 (Thermo Scientific)

11-Rapid Polimiksin Pseudo/Stenotrophoöonas Test

12-Super Polimiksin NP Test (ELITech Microbiolgy)





Eur J Clin Microbiol Infect Dis DOI 10.1007/s10096-016-2846-y

# Mikrodilüsyon yöntemi

- Mikrodilüsyon Yöntemi-  ISOstandard(20776-1)
- Enterobacteriaceae
- *P. aeruginosa*
- *Acinetobacter* spp.
- Katyon katkılı  Mueller–Hinton buyyon
- Standart Polistiren Pleyt ( polysorbate-80 katkısız)
- Kolistin sülfat

# Conclusions

- BMD should be used for colistin MIC determination.
- The poor performance of disk diffusion was confirmed.
- EUCAST advices against using gradient tests at this point.
  - Even when QC results are within range!
- Quality control of colistin must be performed with both a susceptible QC strain and the colistin resistant *E. coli* NCTC 13846 (*mcr-1* positive).

# SİNERJİ TESTLERİ

1-Dama Tahtası Yöntemi (Checker Board Test)

2-Zamana Bağlı Öldürme Yöntemi (Time Kill Assay)

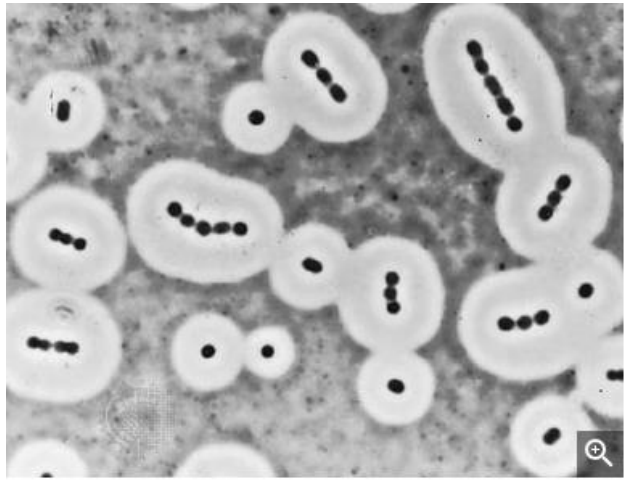
- Kolistine dirençli *K. pneumoniae* infeksiyonların tedavisinde

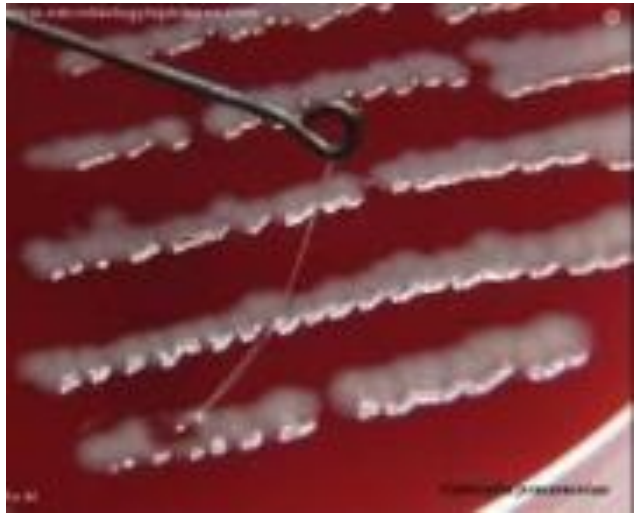
Tigesiklin, Fosfomisin, Karbapenemler, Kinolonlar
Çift karbapenem+kolistin (3'lü kombinasyon)

- Bu kombinasyonların doğru olarak seçilebilmesi için bakterilerin geliştirdiği **direnç mekanizmalarının bilinmesine** ve uygun kombinasyonların seçilmesine ihtiyaç vardır

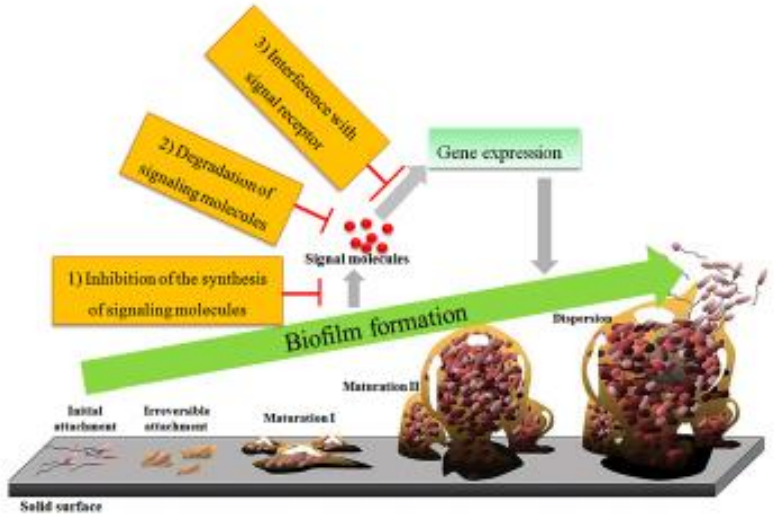
# • Biyofilm Oluşturan Suşlar??? Kapsüllü suşlar kolistine dirençli

- MİK değerleri serbest formlardan en az  100 kat daha yüksek

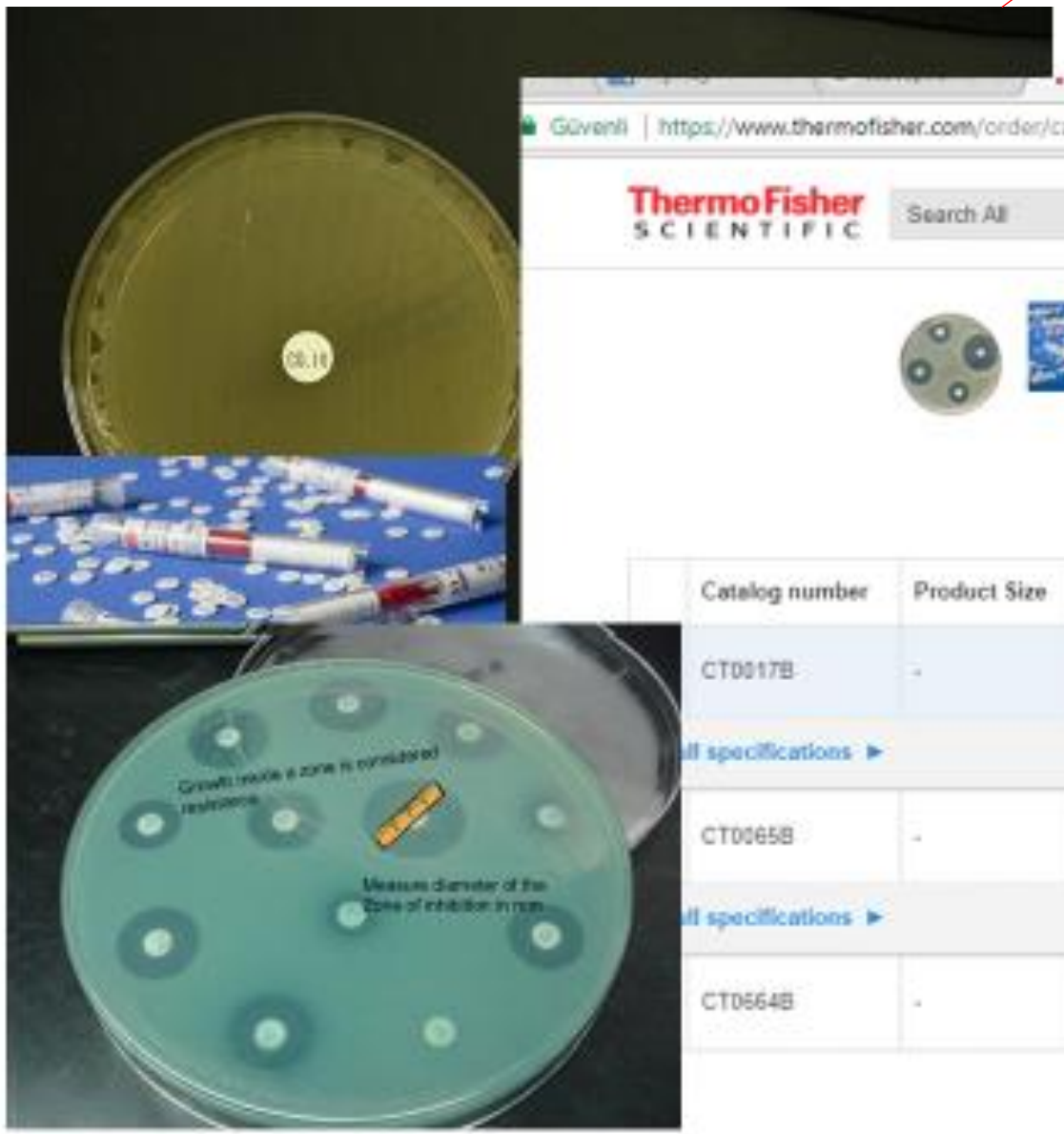




Colistin susceptibility was interpreted against EUCAST criteria (R > 2 mg/L).



# DİSK DİFÜZYON



Güvenli | https://www.thermofisher.com/order/catalog/product/CT0017B

ThermoFisher SCIENTIFIC

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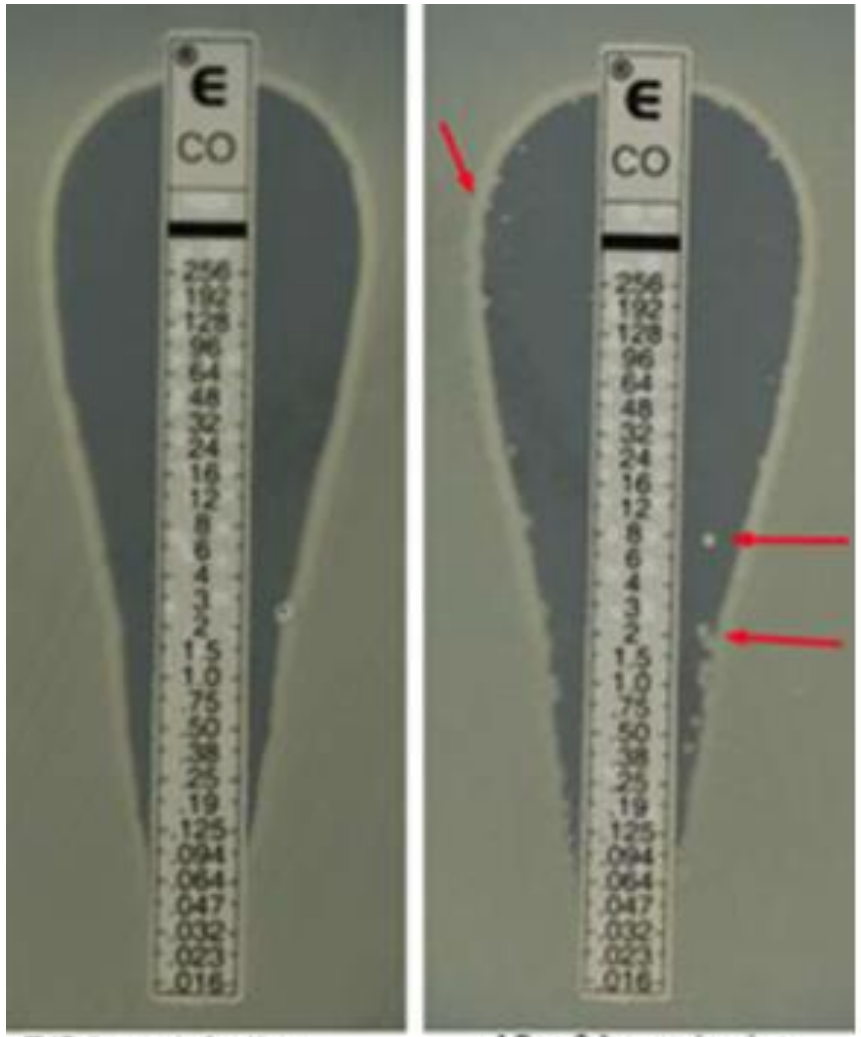
Contact Us Sign In

| Catalog number | Product Size | Description | Antibiotic Code | Concentration | Price |
|---|---|---|---|---|---|
| CT0017B | - | Oxoid Colistin Antimicrobial Susceptibility Disks | CT | 10µg | |
| CT0065B | - | Oxoid Colistin Antimicrobial Susceptibility Disks | CT | 25µg | |
| CT0664B | - | Oxoid Colistin Antimicrobial Susceptibility Disks | CT | 50µg | |

# Gradient Test




R/S-Ip preinfection




After 8 hours in vivo

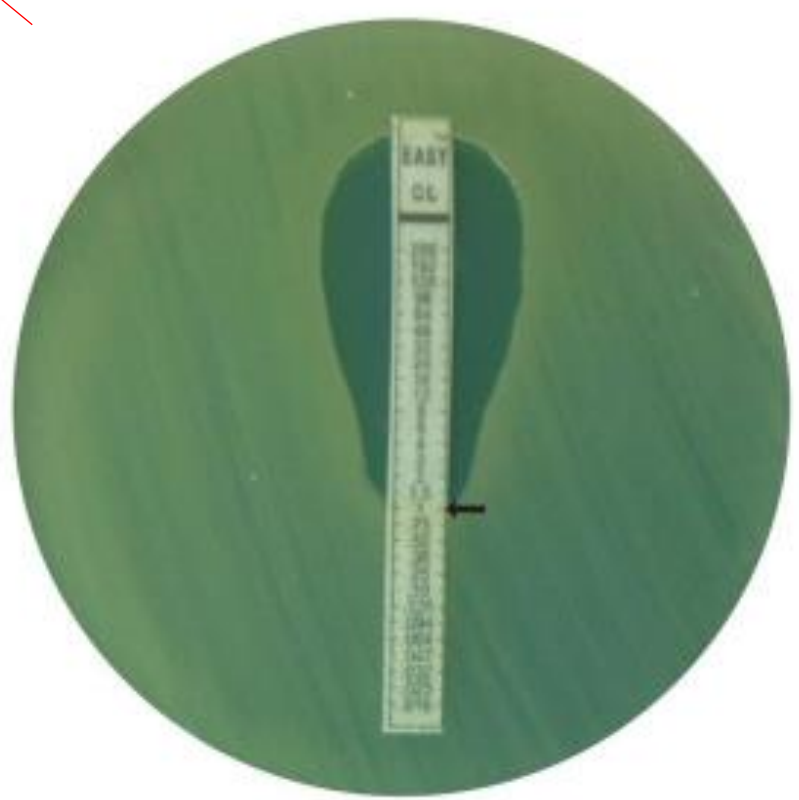


Fig. 2:
*Pseudomonas aeruginosa* ATCC 27853
MIC: 1.0 mcg/ml

## Lo-Ten-Foe JR et al. Comparative Evaluation of the VITEK 2, Disk Diffusion, Etest, Broth Microdilution, and Agar Dilution Susceptibility Testing Methods for Colistin in Clinical Isolates, Including Heteroresistant *Enterobacter cloacae* and *Acinetobacter baumannii* Strains

*Antimicrobial Agents and Chemotherapy 2007; 51:3726*



- Broth microdilution as reference method
- Disk diffusion – unreliable method
- Etest, agar dilution and VITEK showed a high level of agreement with the broth microdilution methods
- VITEK-2 could not detect heteroresistance

# Colistin and anti-Gram-positive bacterial agents against Acinetobacter baumannii

## Results

The minimum inhibitory concentrations (MICs) of vancomycin and norvancomycin for half of the isolates decreased below the susceptibility break point, and the MIC of linezolid for one isolate was decreased to the blood and epithelial lining fluid concentration using the current dosing regimen. When **vancomycin or norvancomycin** was combined with subinhibitory doses of colistin, the multidrug-resistant Acinetobacter baumannii test samples were eradicated. Transmission electron microscopy revealed that subinhibitory doses of colistin were able to disrupt the outer membrane, facilitating a disruption of the cell wall and leading to cell lysis.

## Conclusions

Subinhibitory doses of colistin significantly enhanced the antibacterial activity of vancomycin, norvancomycin, and linezolid against multidrug-resistant Acinetobacter baumannii.

Revista da Sociedade Brasileira de Medicina Tropical
Print version ISSN 0037-8682
Rev. Soc. Bras. Med. Trop. vol.47 no.4 Uberaba July/Aug. 2014
http://dx.doi.org/10.1590/0037-8682-0083-2014

MAJOR ARTICLES

**Colistin and anti-Gram-positive bacterial agents against *Acinetobacter baumannii***

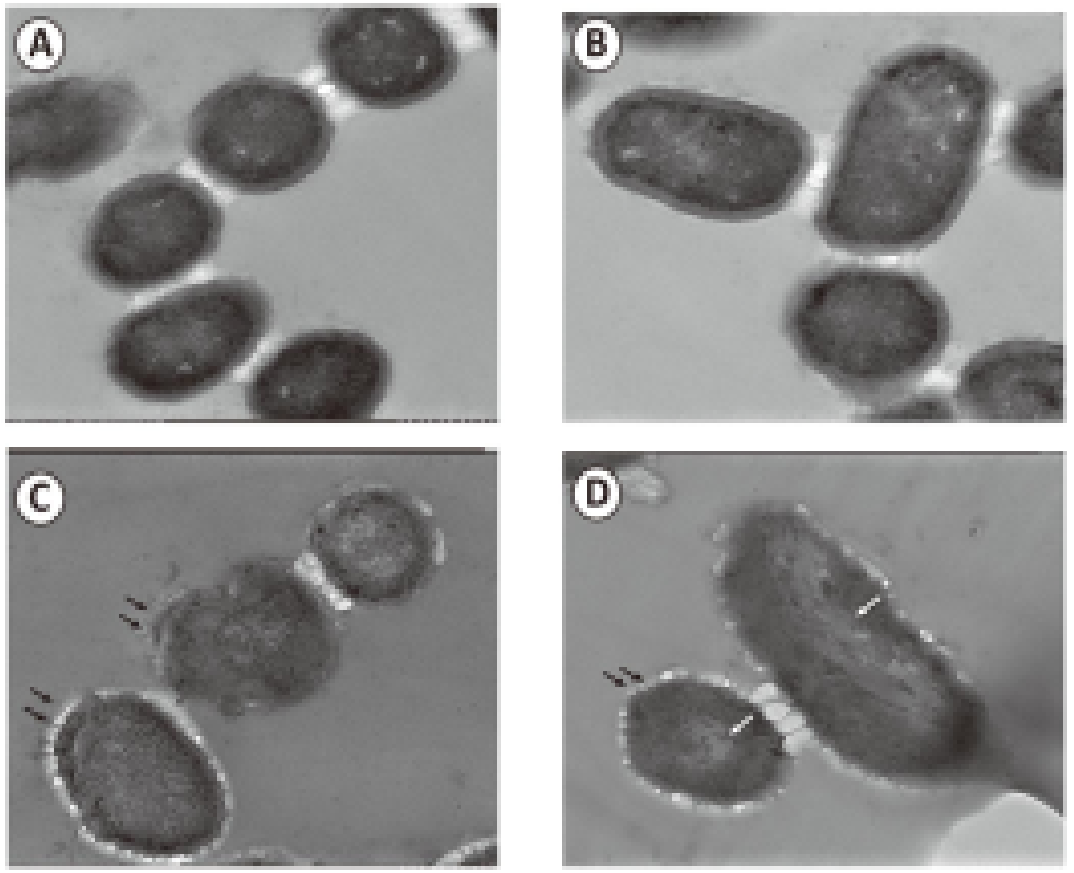
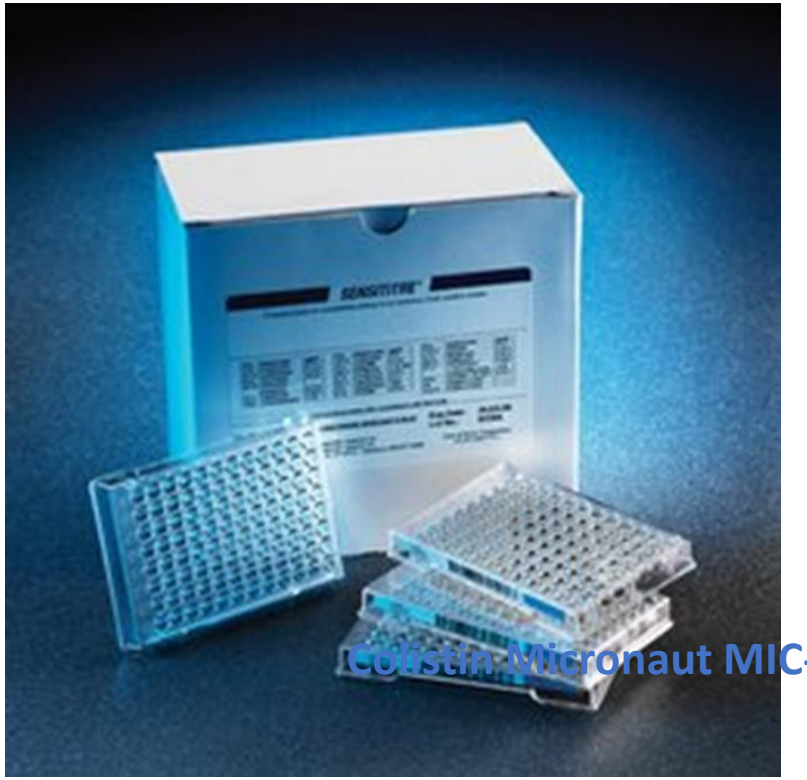



FIGURE 2- Transmission electron microscopy (TEM) of *Acinetobacter baumannii* after 4h of incubation in Mueller-Hinton II broth without drugs (A), with 16 µg/ml vancomycin (B), with 0.125µg/ml colistin (C), and with 0.125µg/ml colistin + 16µg/ml vancomycin (D). TEM Model: HC-ZOOM; TEM Mag: 12.0 K×; HV: 60.0 KV.

## Sensititre™ Gram Negative MIC Plate (Thermo Scientific)




## Colistin Micronaut MIC-Strip

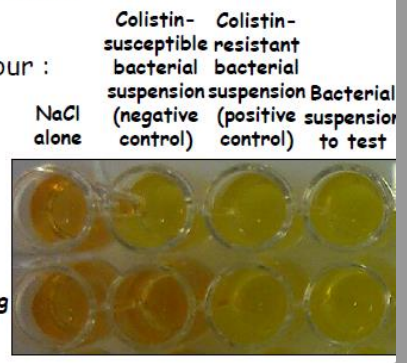
## 4. Tray incubation

The inoculated tray was incubated up to 4h at 35±2°C in ambient air, not sealed and without agitation.

## 5. Tray reading

Visual inspection of the tray was made every hour during 2 hours.

Result at 1 hour :



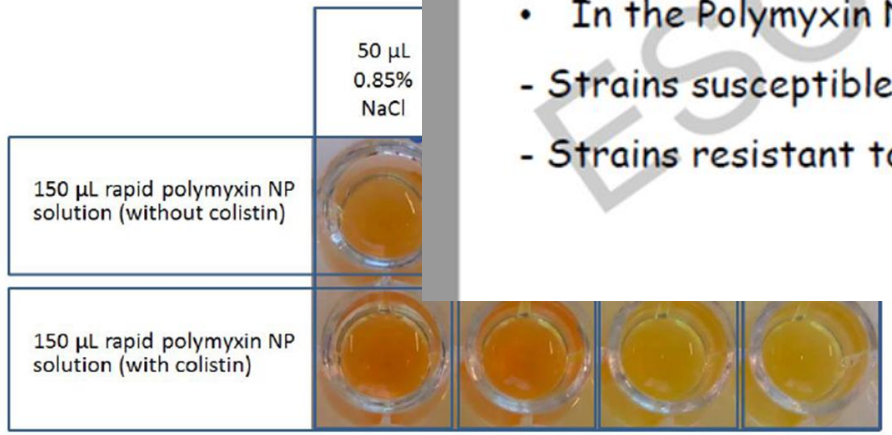




**Figure 1.** Distribution of solutions and bacterial suspension into wells for the rapid polymyxin NP test and positive result obtained with a colistin resistant test isolate.

## 3. Tray inoculation

- For each isolate, 2 wells are inoculated in parallel with the bacterial suspension, respectively with or without colistin.

# PREPARATION OF THE POLYMYXIN NP TEST

25 g of Mueller Hinton
25 g of phenol red and

in.
25 ml of D(+)-glucose

on.

repared using freshly-
uria-Bertani or Mueller-

l of sterile NaCl 0.85 %
$10^9$ CFU/ml).

## Test principle

- This test is based on the detection of the glucose metabolization related to bacterial growth in presence of a defined concentration of colistin.
- Formation of acid metabolites consecutive to the glucose metabolization was evidenced by a color change (orange to yellow) of a pH indicator (red phenol).
- In the Polymyxin NP solution with colistin :

- Strains susceptible to colistin are dead : test neg
- Strains resistant to colistin are alive : test pos

isolates (CS MICs
negative results,
ICs of 1 to 2 µg/ml
itive result).

between colistin
apid Polymyxin NP
and negativity of
specificity of the

Polymyxin NP test were excellent, being 99.3 and 92.3 %, respectively, as compared to the broth microdilution method taken as the gold standard.

- It was rapid (less than 2h) and reproducible.

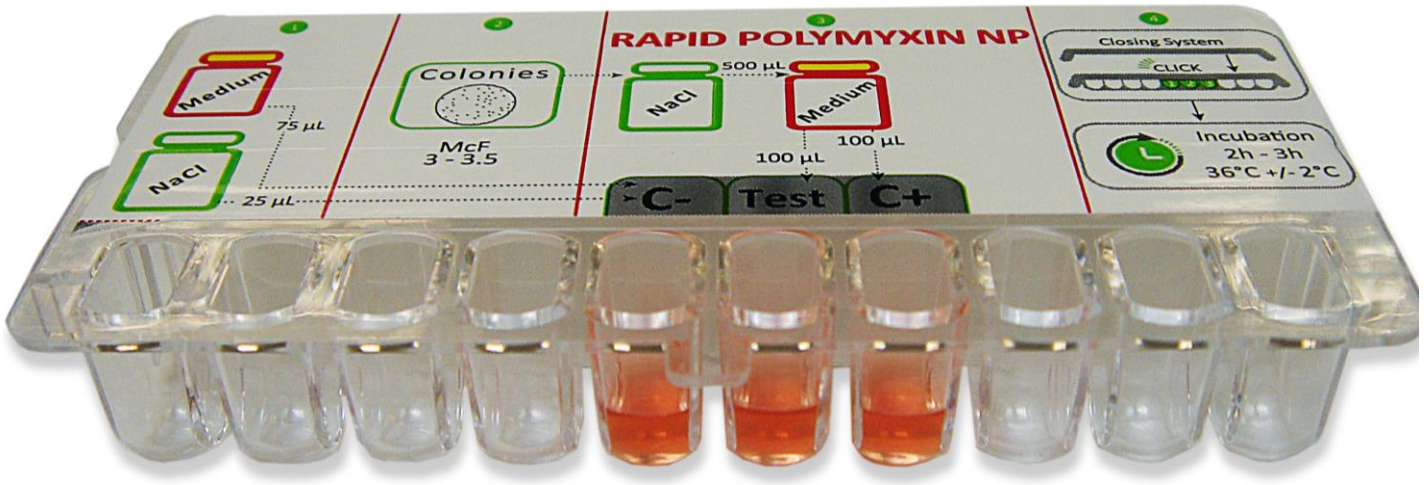
- Kolistine dirençli suşlar Süper polimiksin besiyerinde üreme: -24 saat

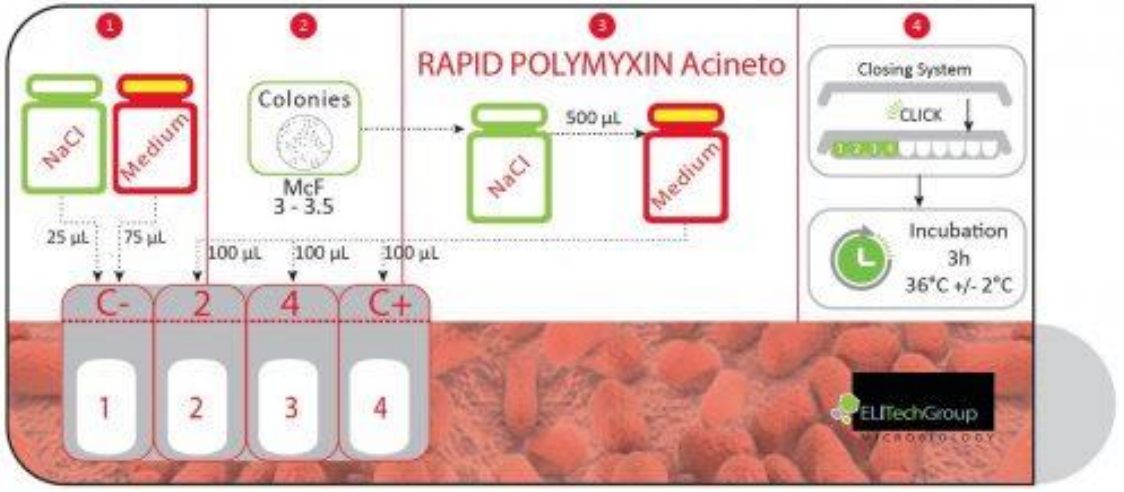
*P. aeruginosa, S. maltophilia, Burkholderia*->24-48 saat

Bakteri inokülümü:≈$10^9$ CFU/mL): 3, 3.5 Mc Farland

- Duyarlılığı ve özgüllüğü %90-100

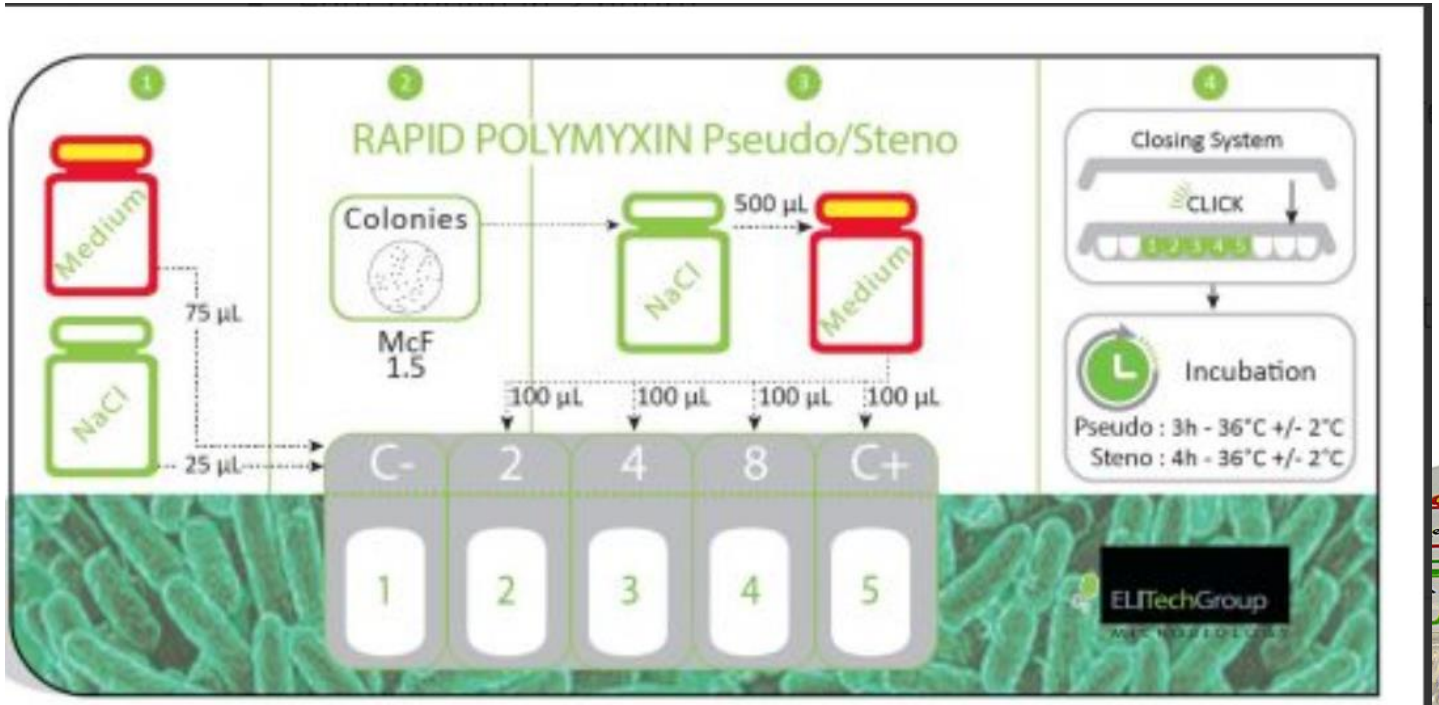
(Mekanizmaların tümü için;(intrinsik, kromozomal, plazmidik), bilinmeyenler hariç



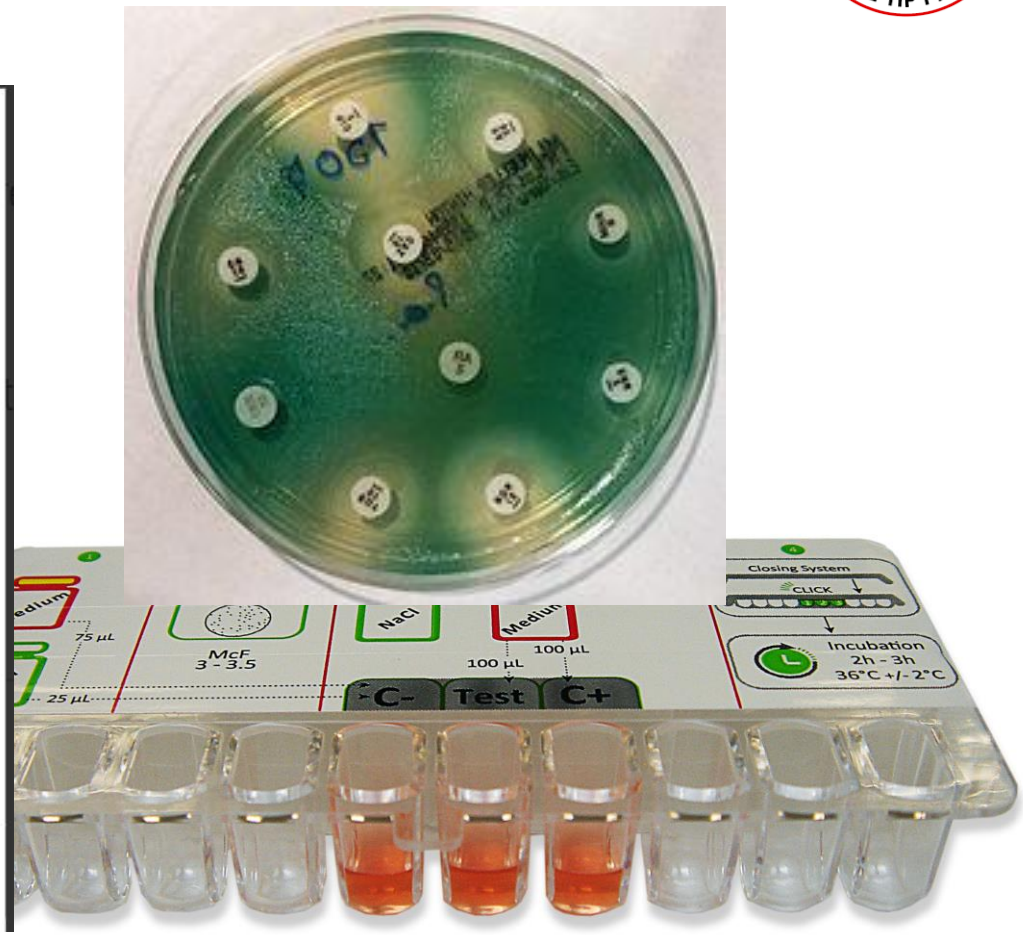

PhoQ R16C

# Rapid Polymyxin™ Pseudo/Steno




s://www.elitechgroup.com/wp-content/uploads/2017/05/Galerie-RP-ıdo-Steno-e1495552319318.jpg

CLOSE ✕



# 2049 Enterobacteriaceae izolatı

*E. coli* (*n* = 1704)
*K. pneumoniae* (*n* = 151)
*P. mirabilis* (*n*= 73),
*Citrobacter sp* (*n* = 32)
*K. oxytoca* (*n* = 22)
*E. cloacae*(*n* = 18)
*M. morganii* (*n* = 13)
*E. aerogenes* (*n* = 15)
*P. vulgaris* (*n* = 7)
*Serratia sp* (*n* = 7
*P. rettgeri* (*n* = 3)
Salmonella group D (*n* = 2)
*Hafnia alvei* (*n* = 1)
*Kluyvera ascorbata* (*n* = 1)

**NP TEST**

ELSEVIER
Volume 51, October 2016, Pages 4-5

Short Communication

## Very low prevalence of MCR-1/MCR-2 plasmid-mediated colistin resistance in urinary tract *Enterobacteriaceae* in Switzerland

Nadia Liassine [a], Laetititia Assouvie [b, c], Marie-Christine Descombes [d], Valérie Dénervaud Tendon [b, c], Nicolas Kieffer [b, c], Laurent Poirel [b, c], Patrice Nordmann [b, c, e]
Show more

https://doi.org/10.1016/j.ijid.2016.08.008


### Highlights

- This is the first prospective analysis of the prevalence of MCR-1 and MCR-2 in clinical samples.
- The new rapid polymyxin NP test was used to detect colistin resistance in *Enterobacteriaceae*.
- Point prevalence rate of spread of MCR-producers in clinically significant isolates.

# Süper Polimiksin NP testinin en az 4 kısıtı var:

1. Polimiksin için düşük MİK'li heterojen dirençli izolatları algılamakta sorunlu
2. Dirençli bakteriler için kolayca görülebilen bir turuncu-sarı renk değişikliği içerir; ancak düşük seviyeli olanlat için yorumlanma güçlükler, tecrübe gerekli, güvenilirliğini tam olarak değerlendirmek için farklı laboratuvarlarda daha büyük ölçekli çalışmalara ihtiyaç var.

3. Kolistin direncinin bilinmeyen fatklı mekanizması var olabilir ve çeşitli şekillerde ifade edilebilir.

4. Enterobacteriaceae ailesinden farklı metabolik yolları olan bakteri türlerinde hızlı polimiksin NP testi değerlendirilememiş.

5. Farklı metabolik yollara sahip olan polimiksin dirençli Pseudomonas aeruginosa ve Acinetobacter baumannii'nin saptanmasına adapte etmek için daha fazla çalışmaya ihtiyaç duyulmaktadır

Format: Abstract

Send to

J Clin Microbiol. 2017 Oct;55(10):3016-3020. doi: 10.1128/JCM.00934-17. Epub 2017 Jul 26.

# Evaluation of the Rapid Polymyxin NP Test for Polymyxin B Resistance D… Enterobacter cloacae and Enterobacter aerogenes Isolates.

Simar S[1], Sibley D[1], Ashcraft D[1], Pankey G[2].

Author information

## Abstract

Polymyxin resistance is an increasing problem worldwid… …oblematic and lengthy. Polymyxins diffuse poorly into agar, potentiall… …pid screening test (2 h) for the detection of polymyxin resistance in *Enterob*… …lymyxin NP test) in 2016, detects glucose metabolization in the pres… …sitivity and specificity were 99.3 and 95.4%, respecti… …st larger numbers of *Enterobacter* A total of 143 nonc… …cluding 136 collected from Oc… Health Syste… …isolates from JMI… were determi… …to y… change is dee… …. The sens… µg/ml), includin… determined to b… were 25 and 100… …Although the rapid poly… polymyxin resistan… …icrodilution (16 to 20 h), our stud… testing *Enterobacte*…



**KEYWORDS:** Enterobac… …g resistance; microbial drug resistance; polymyxin b…

PMID: 28747375 PMCID: PMC5625387 [Available on 2018-04-01] DOI: 10.1128/JCM.00934-17

www.ncbi.nlm.nih.gov/pubmed/28747375#

NP test: 2 – 4 saat
MBD:16 - 20 saat

S. marcescens,
1- P. vulgaris
1- H. alvei
102 Enterobacter cloacae compleks
41 E. aerogenes

NPtestinin BMD testine göre
Duyarlılığı 25
Özgüllüğü %100,
Enterobacter için dikkat

Although the… …ompared to (2 to 4 h) for … broth microdil… …udy indicates that it may be subject to li… …ten testing Enterobacter.

Format: Abstract

Send to

J Clin Microbiol. 2016 Sep;54(9):2273-7. doi: 10.1128/JCM.00918-16. Epub 2016 J

# Rapid Detection of Polymyxin-Resistant Ente

Jayol A[1], Dubois V[2], Poirel L[3], Nordmann P[4].

Author information

## Abstract

Enterobacteriai
resistant isolates s
reliable. Recently, the
based on the detecti
B). The formation
polymyxin NP test was evaluated for detection of colistin-resistant Enterobacteri
with 73 blood culture sets (either spiked or clinical blood cultures) with various enterc
discrimination between polymyxin-resistant and polymyxin-susceptible en bacter
within 4 h. It is easy to perform and requires neither subculture nor a centri rapid,
early identification of polymyxin-resistant Enterobacteriaceae directly from bl



PMID: 27307457 PMCID: PMC5005486 [Available on 2017-03-01] DOI: 10.1128/JCM.00918-16

[Indexed for MEDLINE] Free PMC Article

**2015-2016- 73 Kan kültürü**

- Direkt üreme pozitif kan kültürü
- Subkültür ve santrifüj etmek gerekmiyo
- 4 saatten az zamanda sonuç
- Hızlı, kolay, spesifik
- Erken olarak
- Ente

2- S. marcescens,
1- P. vülgaris
1- H. alvei

E. coli,
Klebsiella,
Enterobacter
Citrobacter

Acinetobacter ,
Pseudomonas
spp.sonuçları
yorumlanamamış

test
l,
E. coli negatif
miyor)
nsantrasyon 3.75 mg/L

| | Organism | *E. coli* and *K. pneumoniae* (n=32) | *P. aeruginosa* (n=21) | *Acinetobacter* spp. (n=22) | All isolates (n=75) |
|---|---|---|---|---|---|
| | Colistin MIC range (mg/L) | 0.25-32 | 0.25-128 | 0.5-32 | 0.25-128 |
| Essential agreement (EA) | Sensititre custom plate* | 27 | 19 | 20 | 66 (96%) |
| | MICRONAUT-S | 31 | 21 | 20 | 72 (96%) |
| | MICRONAUT MIC-Strip | 31 | 21 | 22 | 74 (99%) |
| | Etest, Oxoid MH | 27 | 13 | 13 | 53 (71%) |
| | Etest, BBL MH | 20 | 11 | 1 | 32 (43%) |
| | Etest, MHE | 24 | 9 | 2 | 35 (47%) |
| | MTS, Oxoid MH | 19 | 12 | 9 | 40 (53%) |
| | MTS, BBL MH | 24 | 12 | 13 | 49 (65%) |
| Major Errors (ME) | Sensititre custom plate | 1 | 1 | 2 | 4 |
| | MICRONAUT-S | 2 | 1 | 3 | 6 |
| | MICRONAUT MIC-Strip | 2 | 0 | 3 | 5 |
| | Etest, Oxoid MH | 2 | 0 | 0 | 2 |
| | Etest, BBL MH | 1 | 0 | 0 | 1 |
| | Etest, MHE | 2 | 0 | 0 | 2 |
| | MTS, Oxoid MH | 0 | 0 | 0 | 0 |
| | MTS, BBL MH | 0 | 0 | 0 | 0 |
| Very Major Errors (VME) | Sensititre custom plate | 0 | 0 | 0 | 0 |
| | MICRONAUT-S | 0 | 2 | 0 | 2 |
| | MICRONAUT MIC-Strip | 0 | 2 | 0 | 2 |
| | Etest, Oxoid MH | 0 | 6 | 6 | 12 |
| | Etest, BBL MH | 1 | 7 | 7 | 15 |
| | Etest, MHE | 0 | 5 | 4 | 9 |
| | MTS, Oxoid MH | 6 | 6 | 4 | 16 |
| | MTS, BBL MH | 5 | 6 | 7 | 18 |

Comparison of Vitek 2®, E-test, and MICRONAUT Prepreparad Plates With

Etest Misse

## Error Rates for Vitek 2 Are Una

Vitek 2

All species

| BMD | | S | R | EA | ME | VME |
|---|---|---|---|---|---|---|
| S | 856 | 852 | 4 | 95 | 0.5 | 29. |
| R | 106 | 31 | 75 | | | |
| | | 883 | 79 | | | |

*K. pne*

| | | | | | | |
|---|---|---|---|---|---|---|
| S | 438 | 435 | | | | |
| R | 81 | 13 | 6 | | | |
| | | 448 | 71 | | | |

*E. c*

| | | | | | | |
|---|---|---|---|---|---|---|
| S | 186 | 186 | 0 | | | |
| R | 5 | 3 | 2 | | | |
| | | 189 | 2 | | | |

considered (2.5%, 16%, and 4% for E-test, Vitek 2 and MICRONAUT).

## MICRONAUT (Merlin) Shows Lower Error Rates

MICRONAU

| BMD | | | | | | |
|---|---|---|---|---|---|---|
| S | | | | | | |
| R | | | | | | |
| | | | | | | |

0.8 3.7

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 78 | | | | |
| | 128 | 79 | | | | |

*E. coli* only

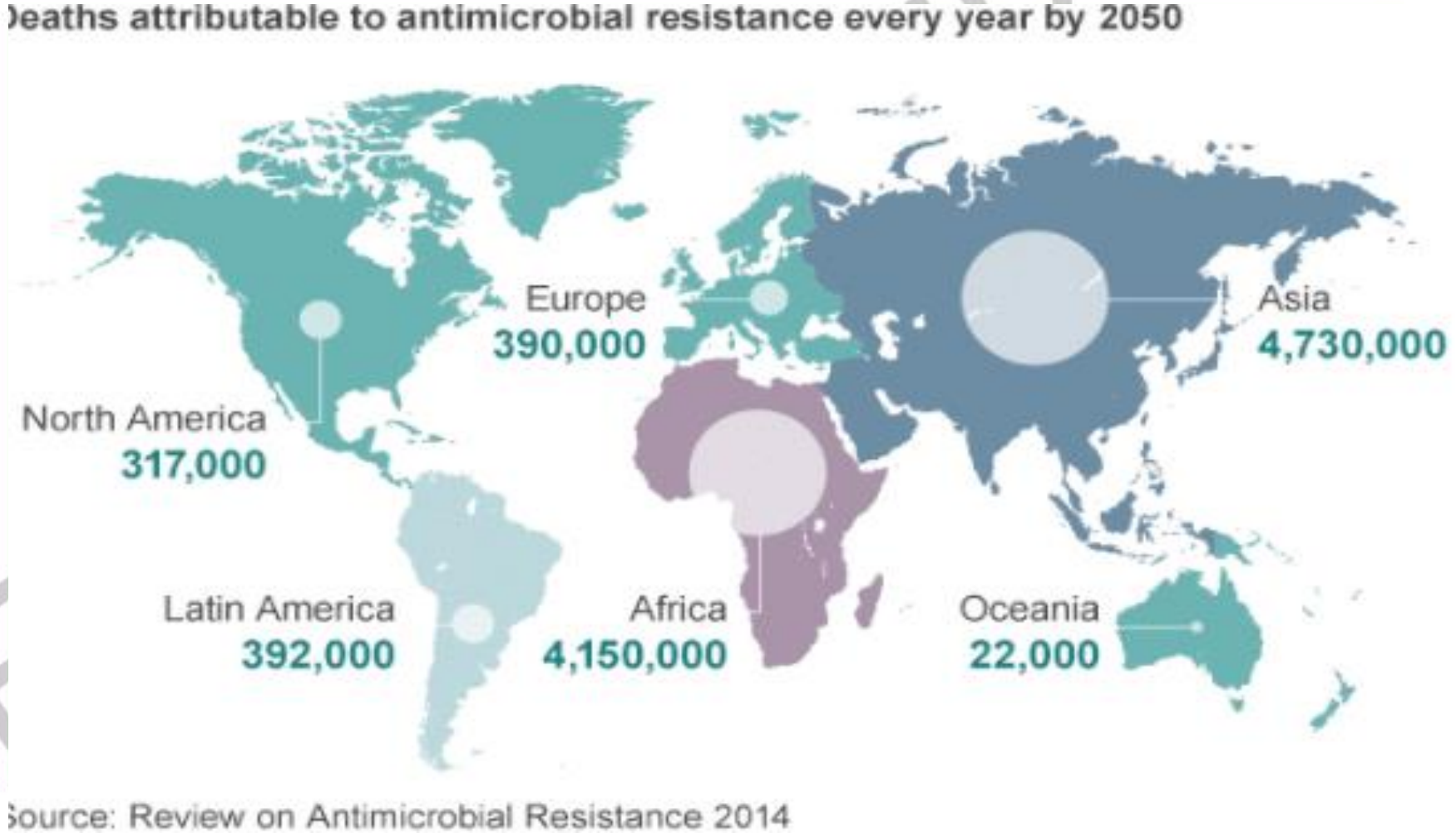
| | | | | | | |
|---|---|---|---|---|---|---|
| S | 48 | 48 | 0 | 79.2 | | 20 |
| R | 5 | 1 | 4 | | | |
| | | 49 | 4 | | | |

### Conclusions

E-test showed the lowest VME rates if only *K. pneumoniae* were considered. For all enterobacterial species the commercial BMD plates showed the lowest VME rate. For Vitek 2 the errors were always unacceptably high. When colistin susceptibility of Enterobacteriacese is tested, an BMD system should be preferred, for *K. pneumoniae* E-test on MH-E agar may be an acceptable alternative.

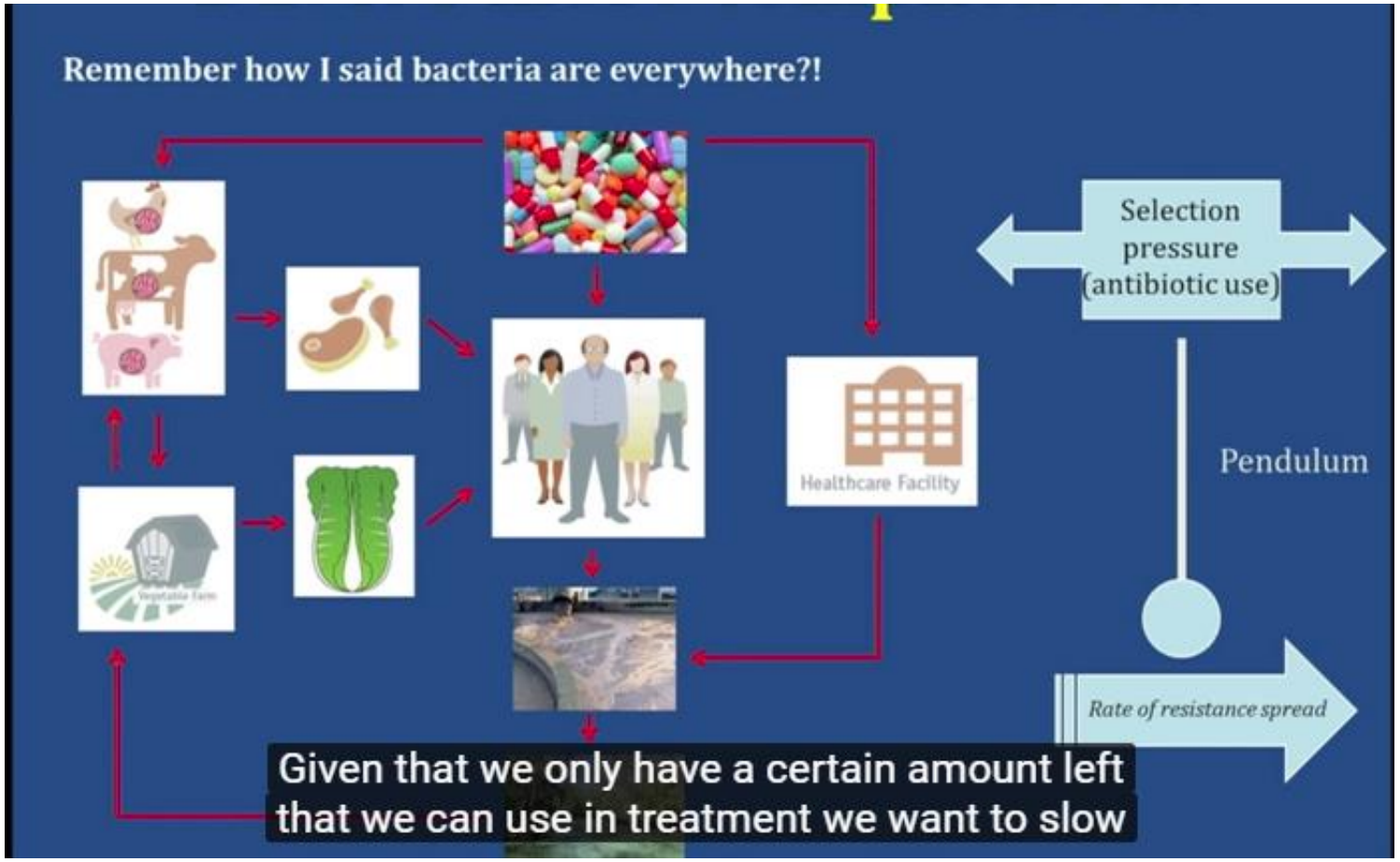
# *Deaths Attributable to Antimicrobial Resistance*

## Every Year by 2050 (>10 Million)




Review on Antimicrobial Resistance 2014

- Mcr genlerinin hayvanlardan ➡ İnsanlara
- Hayvancılıkta kolistin kullanımının durdurulması



# Background: Colistin use in food-producing animals

## Veterinary medicine:

**Extensive use for decades:**

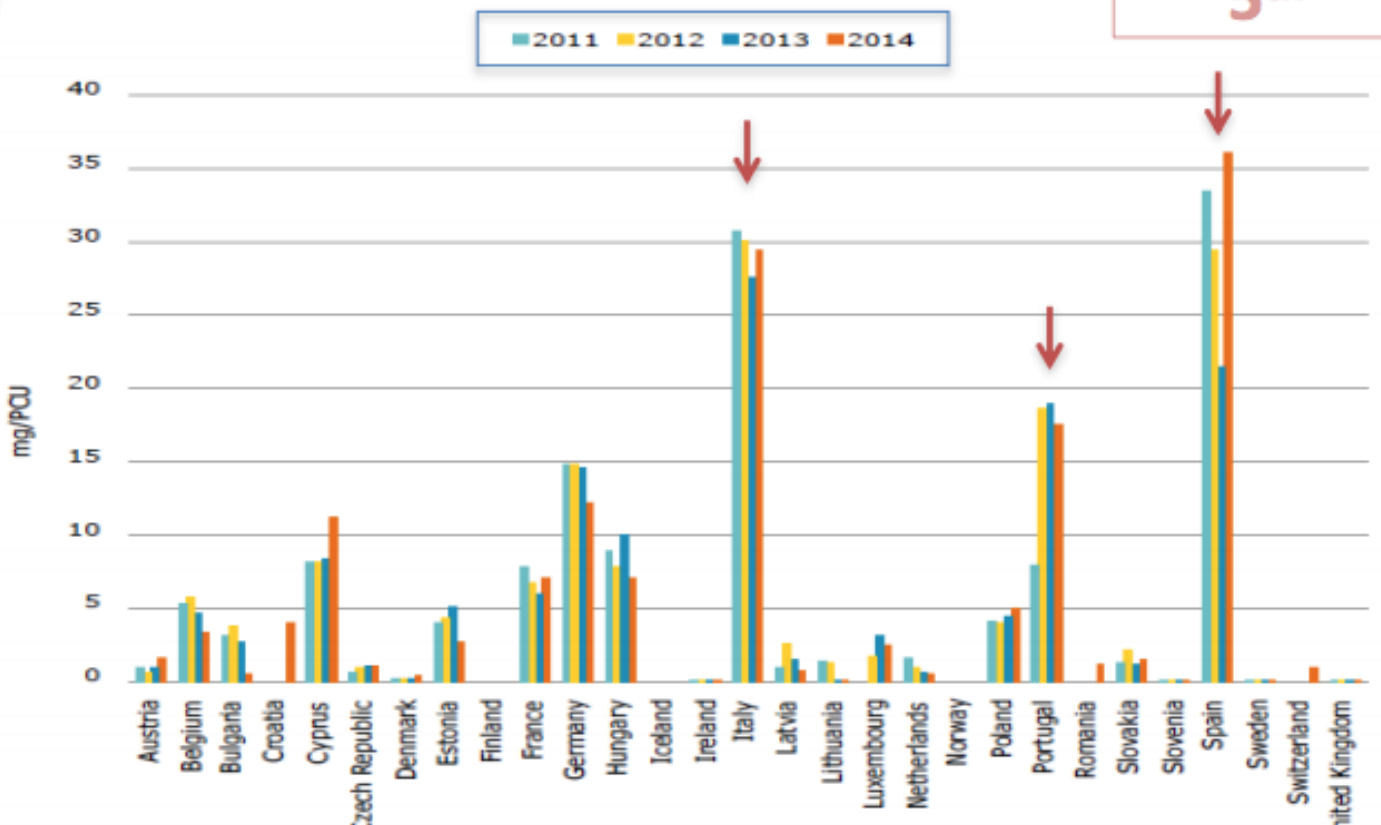
**Specially in swine**

**(treatment and prevention of infectious diseases due to *E.coli* and *Salmonella* spp.)**

**Colistin,**
**AB critically important:**
**Category 2**
EMA/CVMP/CHMP/231573/2016

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

**EU: Colistin 5th**




Sales of polymyxins, (in 2014 the only polymyxin sold was colistin) for use in animals, in mg/PCU, in 29 European countries, from 2011 to 2014. EMA/61769/2016

# Background: Metal use in food-producing animals

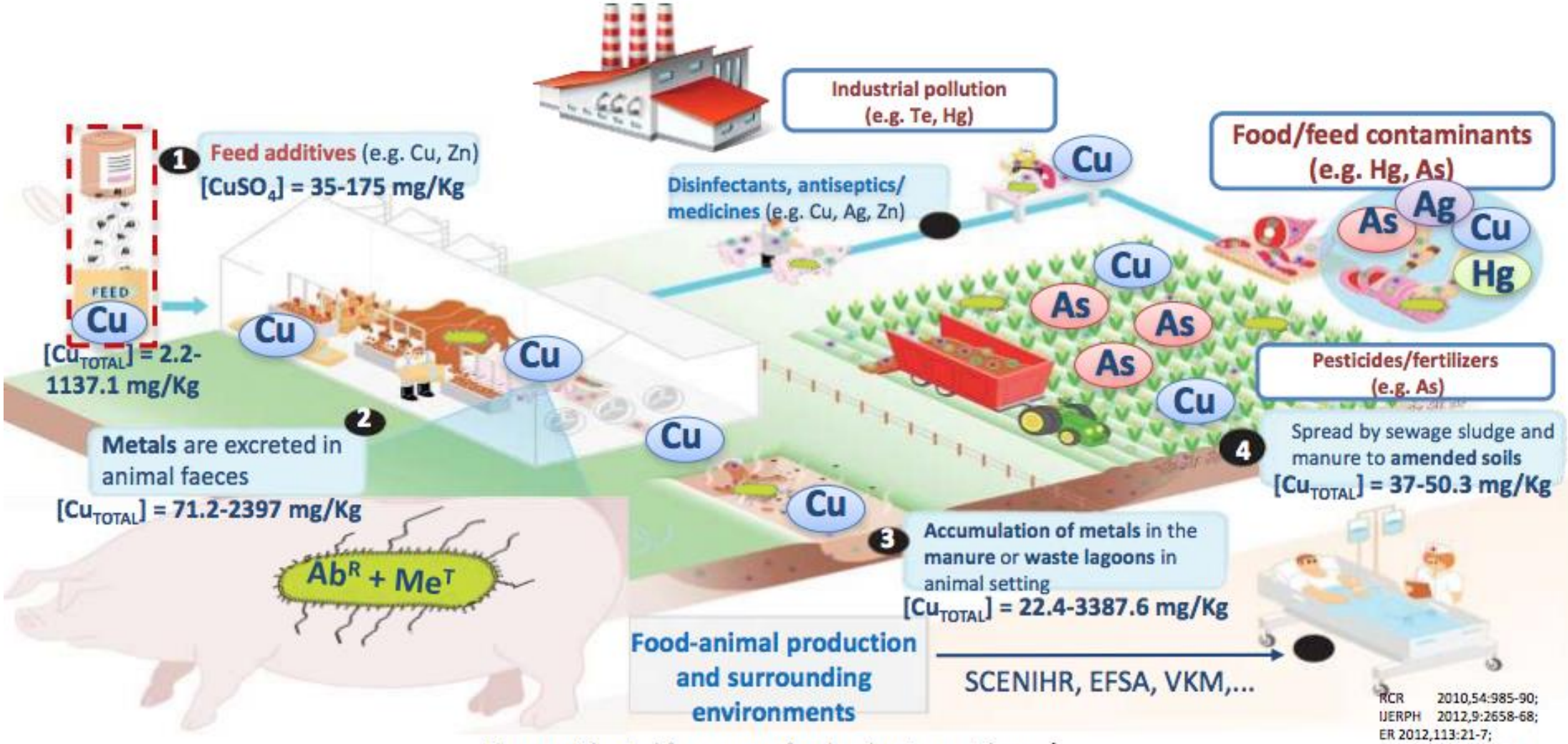



**Figure:** Adapted from www.foodandwaterwatch.org/

RCR 2010,54:985-90;
IJERPH 2012,9:2658-68;
ER 2012,113:21-7;
PNAS 2013,110:3435-40;
JHM 2012,178-185;
MEHD 2014;25:1-7

# Background: Worldwide dissemination of *mcr-1*

UK

The Netherlands

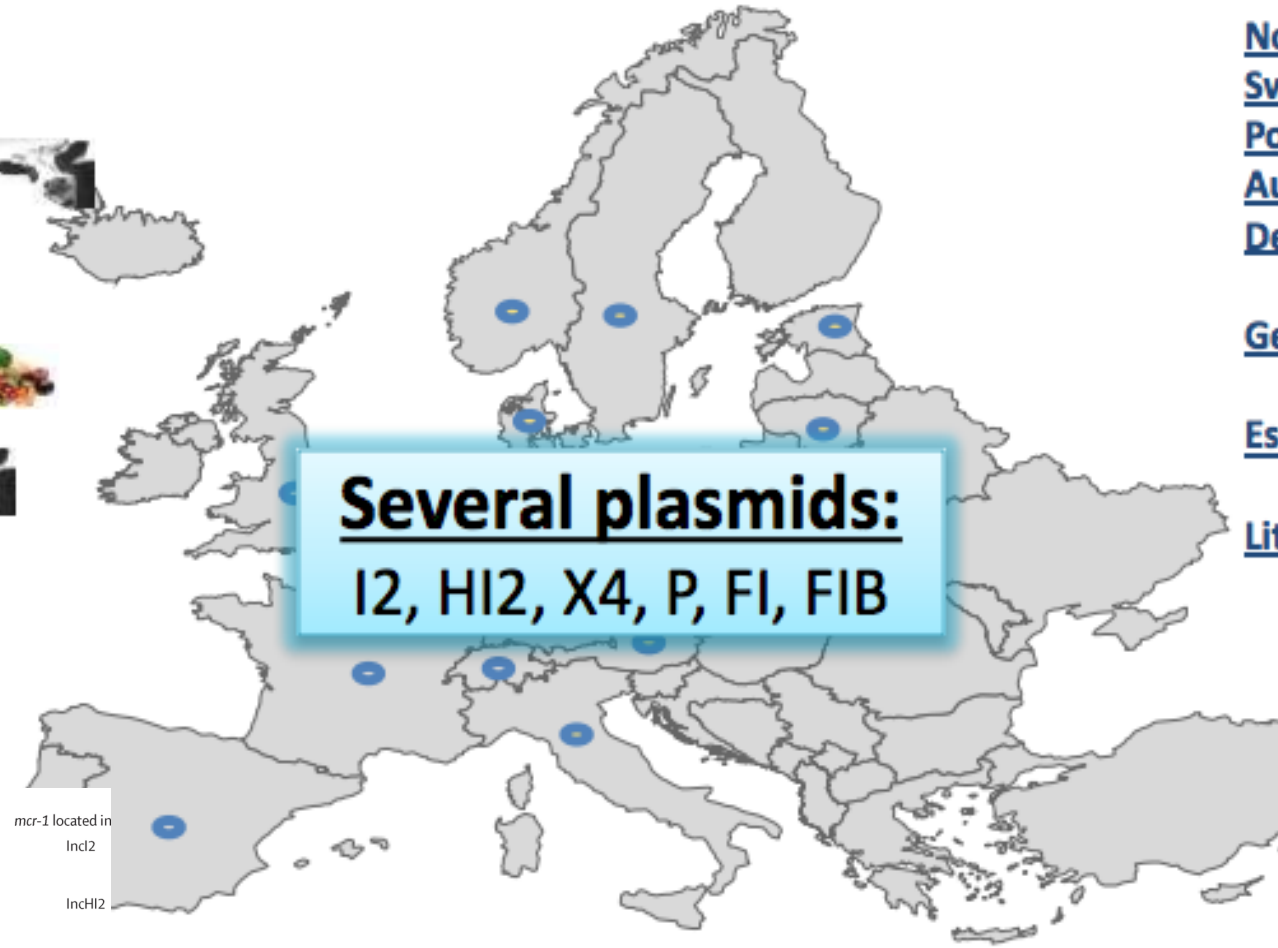
Belgium

Switzerland

Italy

France

Spain

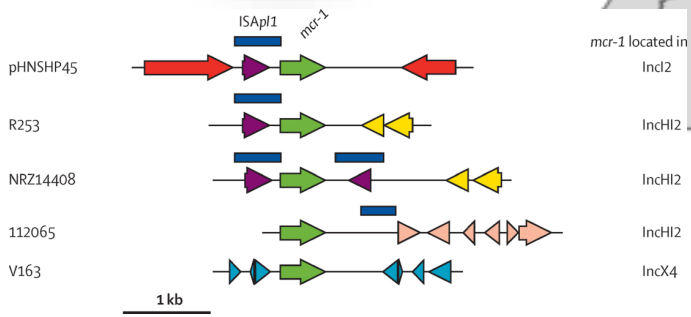


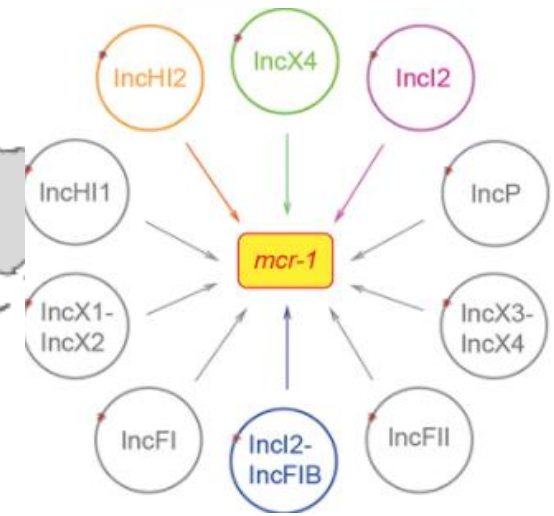

Norway
Sweden
Poland
Austria
Denmark

Germany

Estonia

Lithuania



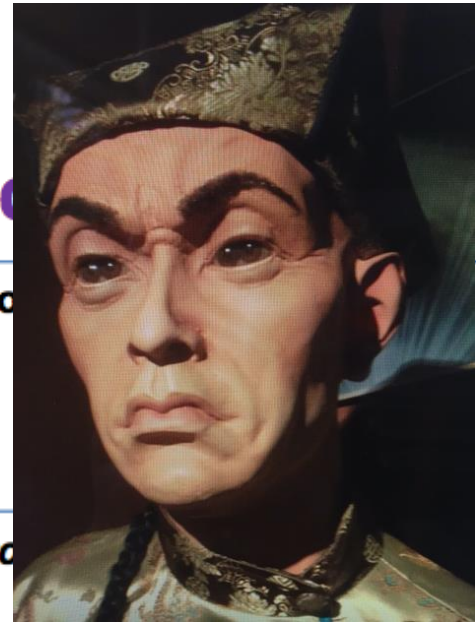




https://www.ncbi.nlm.nih.gov/pubmed/

# IncI2 plasmid

## Chromosomal locati

Colistin-resistant *mcr-1* positive *E. coli* iso



İSTANBUL ÜNİVERSİTESİ · KÜLTESİ ·

pMcp0221

repA hok

## IncI2 plasmid

Characterising of *mcr-1* isolates by WGS using APHA SeqFinder pipeline (Duggett et al, JAC 2017).

| Isolate and origin | Inc-types present | Estimated size of *mcr-1* containing plasmid (kb)** | Similarity to pHNSHP45 (%) | Maximum similarity to publicly available *mcr-1* plasmids (%) | Colistin resistance genes | Other AMR genes |
|---|---|---|---|---|---|---|
| E4 # O139:K82 2015 Veterinary submission [4] | IncX4, IncIFIB(K), repB, **pO111**, IncI1, IncX1, IncFIA(HI1), IncA/C2 | 78.5 | 16 | *89* | *pmrA*, pmrB*, phoP*, phoQ*, etk** and *mcr-1* | *aadA2, aac3-Iva, aph4-Ia, aph3-Ib, aph6-Id, blaTEM-1, blaLAT-1, cml, dfrA12, sul1, sul2, tet(A), gyrA** |
| Clinical isolate ^ O149:H10 2015 Veterinary submission | IncI, **IncX4**, IncFII(pCoo), IncFIB(AP001918), IncFIC(FII), IncY | 32.7 | 28 | *99* | *phoP*, phoQ*, pmrA*, etk** and *mcr-1* | *aadA1b, ant3-Ia, dfrA1, folP*, sul2* |
| PO155 ^ -:H56 2015 surveillance study | IncI1, Col8282, **pO111**, IncX1 | 91.2 | 19 | *90* | *mcr-1* | *aac3-IVa, aadA2, ant3-Ia, aph3-Ib, aph4-Ia, aph6-Id, blaTEM-1, cml, dfrA12, inuF, sul2, tetA* |
| PO169 ^-:H2 2015 surveillance study | IncX1, **IncI2**, IncFII(pCoo), IncB/O/K/Z | 59.2 | 90 | *97* | *acrR*, phoP** and *mcr-1* | *blaTEM-1, gyrA*, qnrS1, tetA* |

ethods

wiss human faeces (n=3) (Pires J et al. IJAA, April 2017)
(Bernasconi OJ et al. AAC, July 2016)
wiss chicken caecum (n=2)
wiss calf caecum (n=1)
wiss chicken retail meat (n=1)
nported (GER) retail chicken meat (n=2)

mid sequencing (WPS) with MinION (Oxford
ed by correction with mapped Illumina reads

l chicken meat (GER)

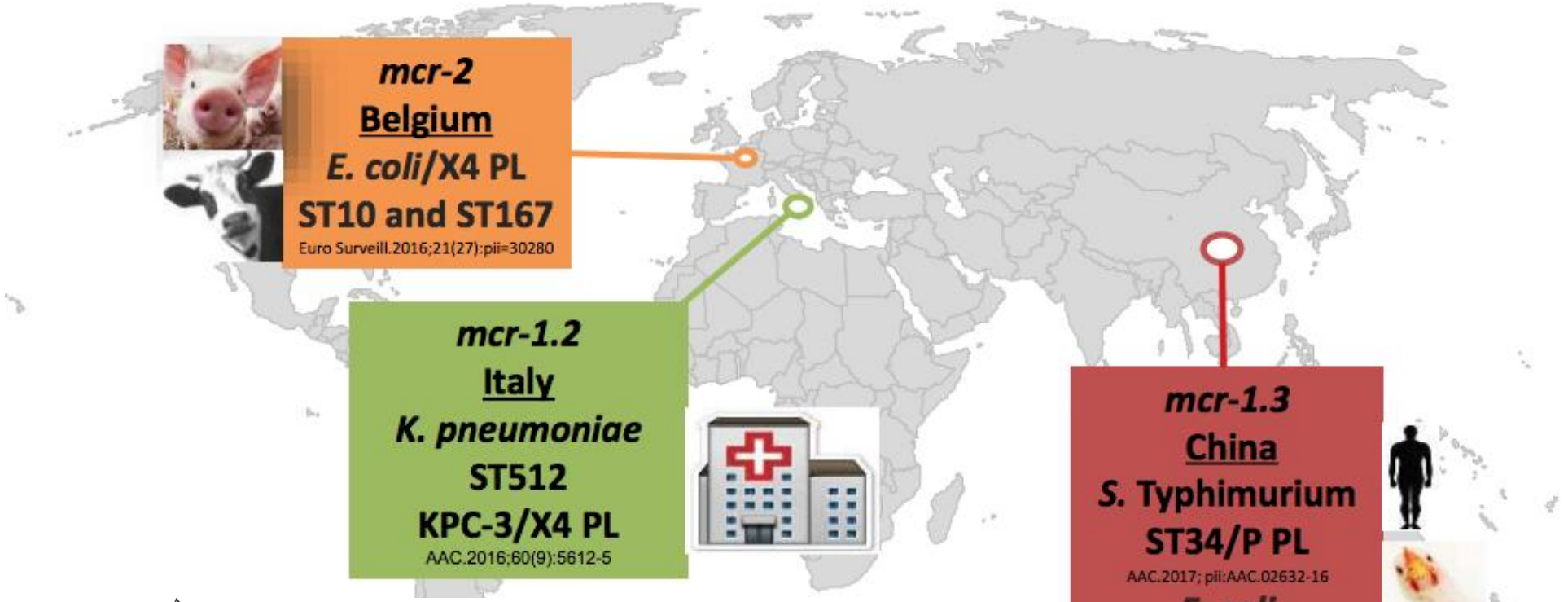
equence-typing with the online
l platforms, respectively

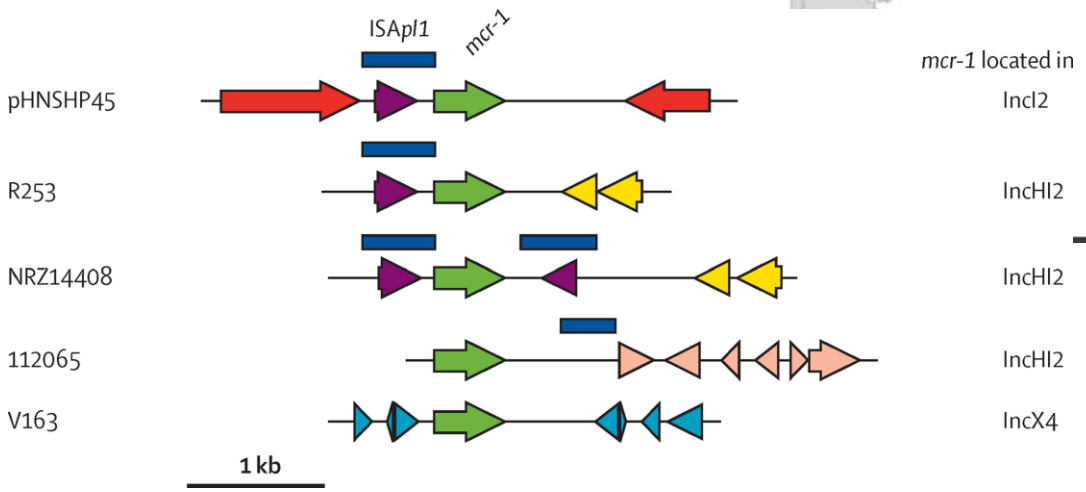
asmid (64 kb)

Skov RL et al. Euro Surveill., March 2016

# Heterogeneous location of the *mcr-1* gene in Colistin-Resistant *Escherichia coli* strains of Human and Chicken Meat Origin

# Background: *mcr* genes variants

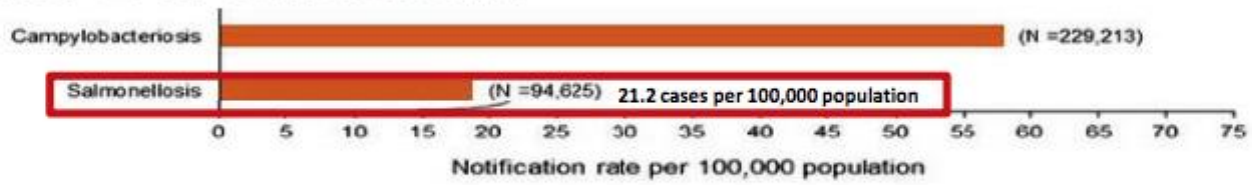


# Background: *mcr-1* in *Salmonella*

**Non-typhoidal *Salmonella***

**Zoonotic Foodborne Pathogen**




EFSA Journal 2016;14(12):4634

**UK**
*S.* Typhimurium
*S.* 1,4,[5],12:i:-
*S.* Virchow
*S.* Paratyphi B
JAC 2016;71(8):2300-5
JAC 2016;71(8):2306-13

**The Netherlands**
*S.* Anatum
*S.* Schwartzengrund
*S.* Java
JAC 2016;71(8):2340-2

**Denmark**
*S.* 4,5,12:i:-
*S.* 4,12:i:-
IJAA 2017;49(2):261-2

**China**
*S.* Typhimurium
*S.* London
*S.*Heidelberg
*S.* Enteritidis
AAC 2017 ;pii:AAC.02471-16
AAC 2017; pii:AAC.02632-16
EID 2017;23(2):291-5

**Germany**
*Salmonella* spp
CMI 2017;3(1):51

**France**
*S.* 1,4,[5],12:i:-
*S.* Derby
*S.* Paratyphi B
LID 2016;16(2):144-5

**Portugal**
*S.* Typhimurium
JAC 2016;71(8):2338-40

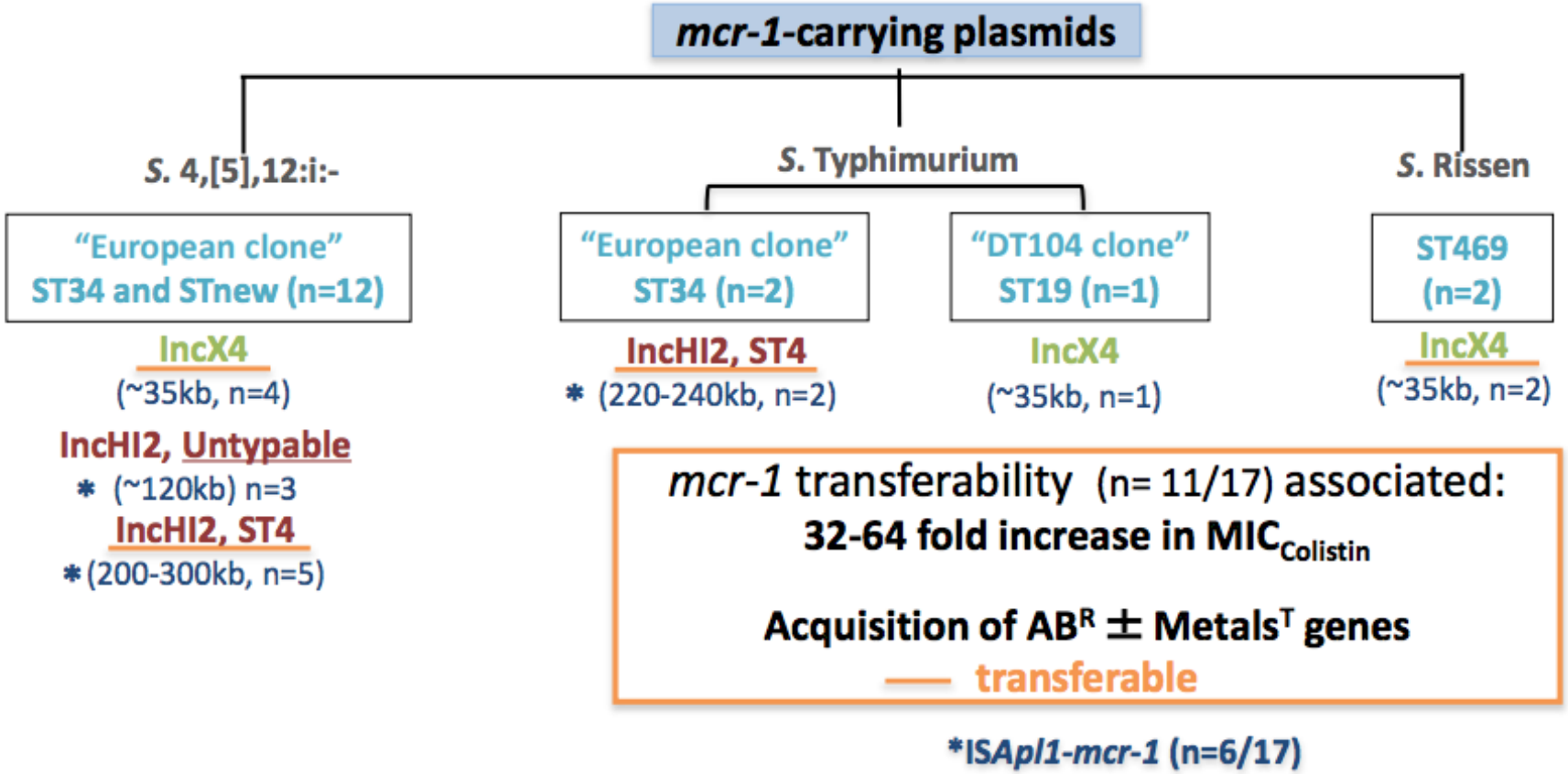
**Spain**
*S.* Typhimurium
*S.* Rissen

**Italy**
*S.* Schwartzengrund
*S.* 1,4,[5],12:i:-
*S.* Bovismorbificans
S. Newport
*Salmonella* spp
CMI 2017;3(1):51
AAC 2016;60(12):7532-34

**Japan**
S. Typhimurium
LID 2016;16(3):284-

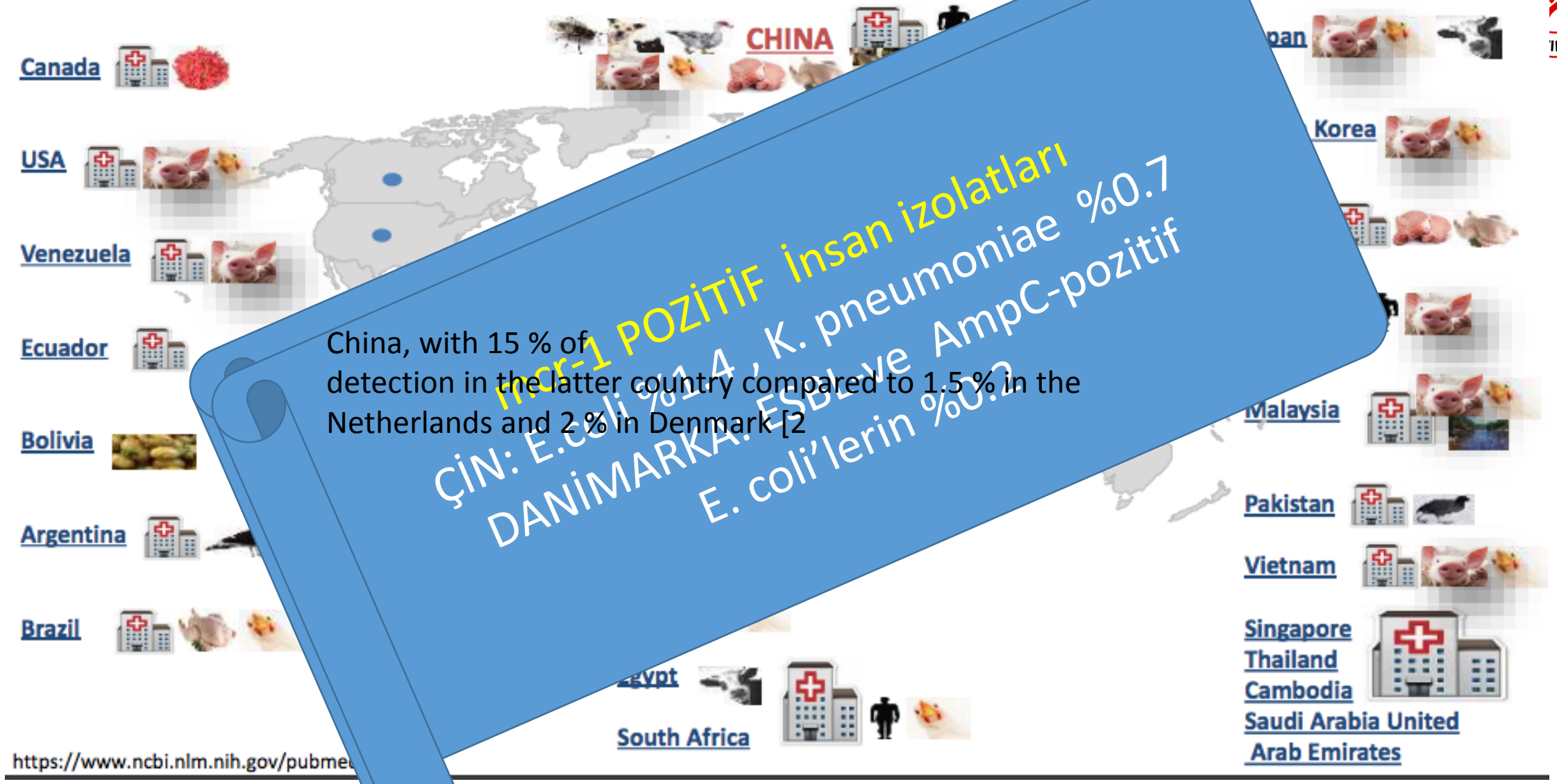
https://www.ncbi.nlm.nih.gov/pubmed/

# Results and Discussion: Plasmid characterization




- Genomic location (*I-CeuI*/S1-PFGE-hybridization)
- Plasmid characterization (*ISApl1* presence/PCR-PBRT/pMLST)
- Conjugation assays (*E. coli* HB101 – recipient strain ($MIC_{Colistin}$: 0.125 mg/L)

# Background: Worldwide dissemination of mcr-1 24 ülke




China, with 15 % of
detection in the latter country compared to 1.5 % in the
Netherlands and 2 % in Denmark [2

mcr-1 POZİTİF İnsan izolatları
ÇİN: E.coli %1.4 , K. pneumoniae %0.7
DANİMARKA: ESBL ve AmpC-pozitif
E. coli'lerin %0.2

Eur J Clin Microbiol Infect Dis
DOI 10.1007/s10096-016-2846-y

Eurosurveillance
Europe's journal on infectious disease epidemiology, prevention and control
Impact factor 5·9

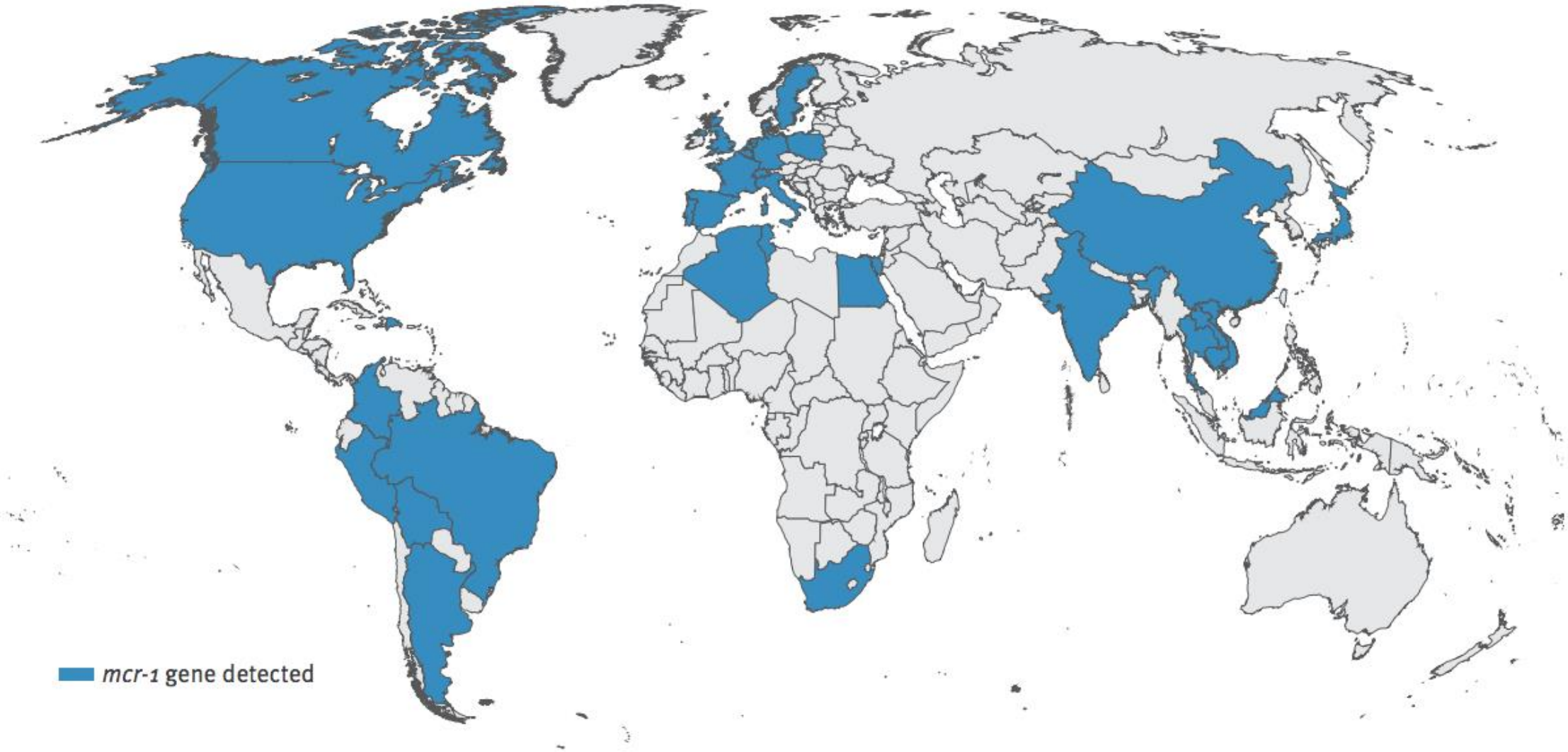

Special edition:

# Plasmid-mediated colistin resistance (*mcr-1* gene) in food animals, food and humans

July 2016

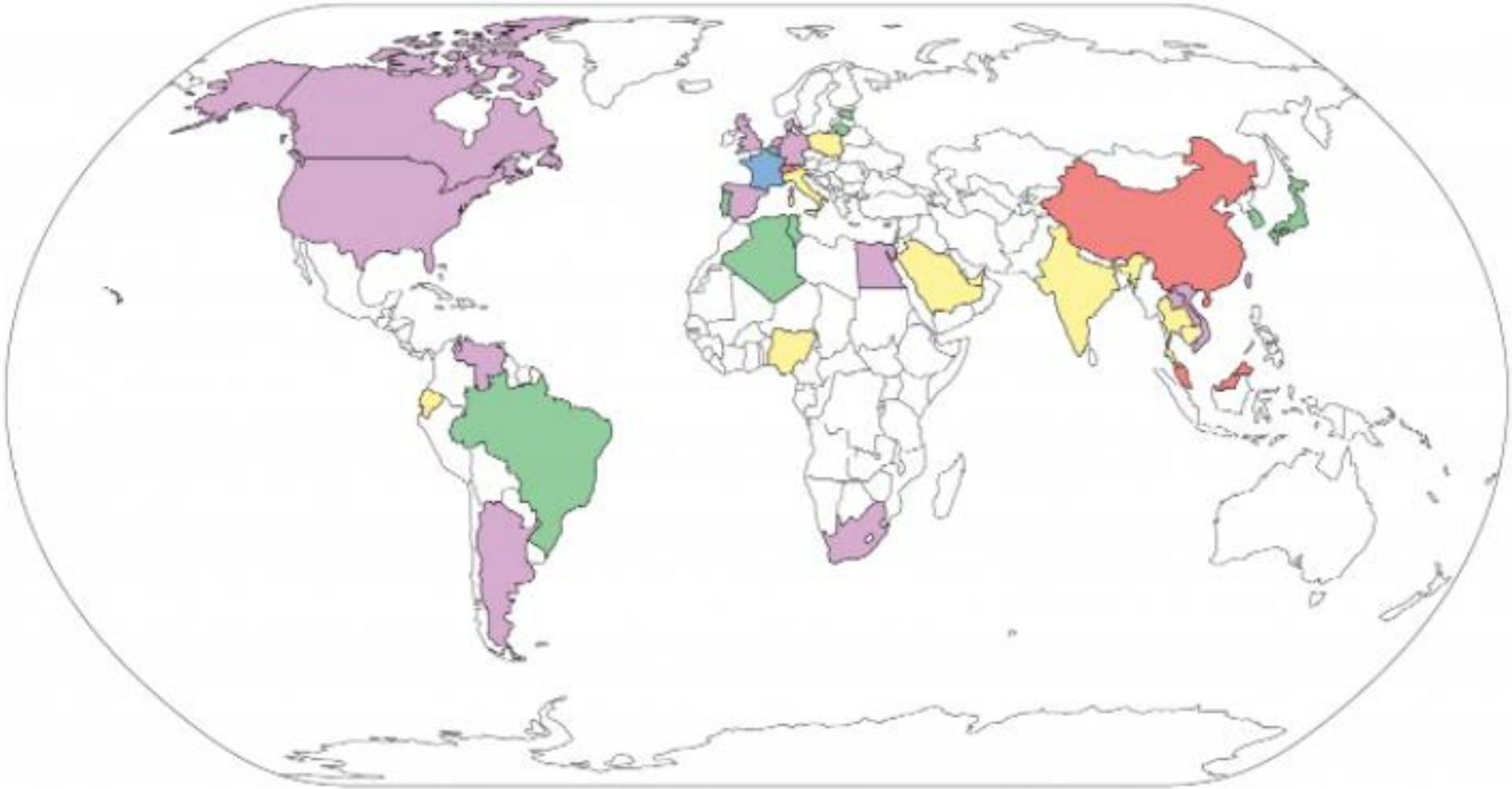
**FIGURE 4**

Countries (n = 30) reporting presence of *mcr-1* in samples of animal, environmental or human origin (data collected till 27 June 2016)




Adapted from [15]; updated using data from [14,16,17,25-27].

## Countries reporting plasmid-mediated colistin resistance encoded by *mcr-1*



Isolate source(s):

- Animals
- Humans
- Animals and humans
- Animals and environment
- Animals, humans and environment

Data source: Al-Tawfiq, J. A., Laxminarayan, R. & Mendelson, M. How should we respond to the emergence of plasmid-mediated colistin resistance in humans and animals? Int. J. Infect. Dis. [2016]. doi:10.1016/j.ijid.2016.11.415

CDDEP THE CENTER FOR Disease Dynamics, Economics & Policy

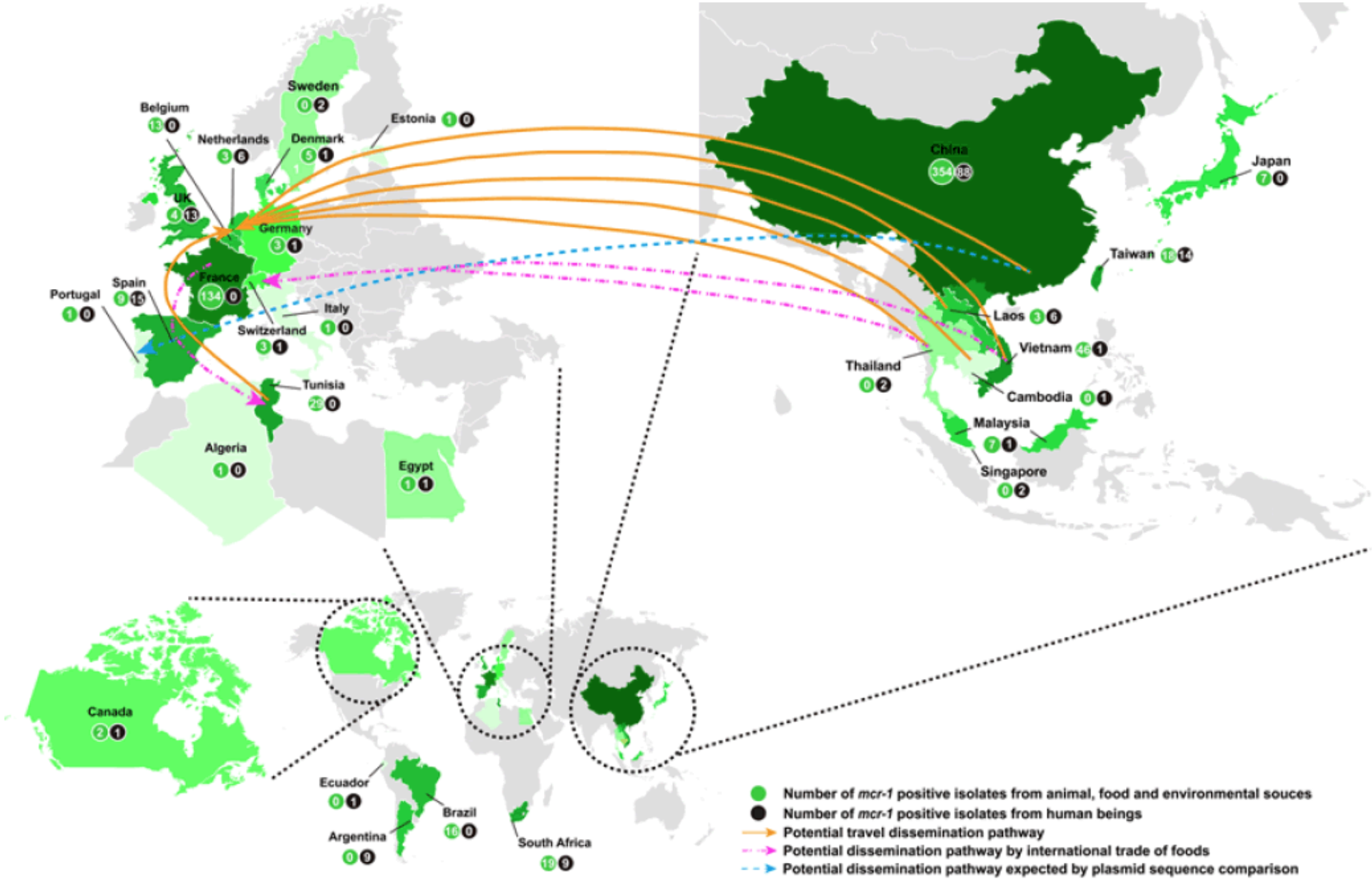



***Figure 1***. *Epidemiological features of mcr-1-harbouring Enterobacteriaceae (as of 4th May 2016). The size of circle is not proportional to the amount of mcr-1 positive isolates. The deeper color means the more amounts of mcr-1 positive isolates.*

# Silent dissemination of colistin-resistant *Escherichia coli* in South America could contribute to the global spread of the *mcr-1* gene

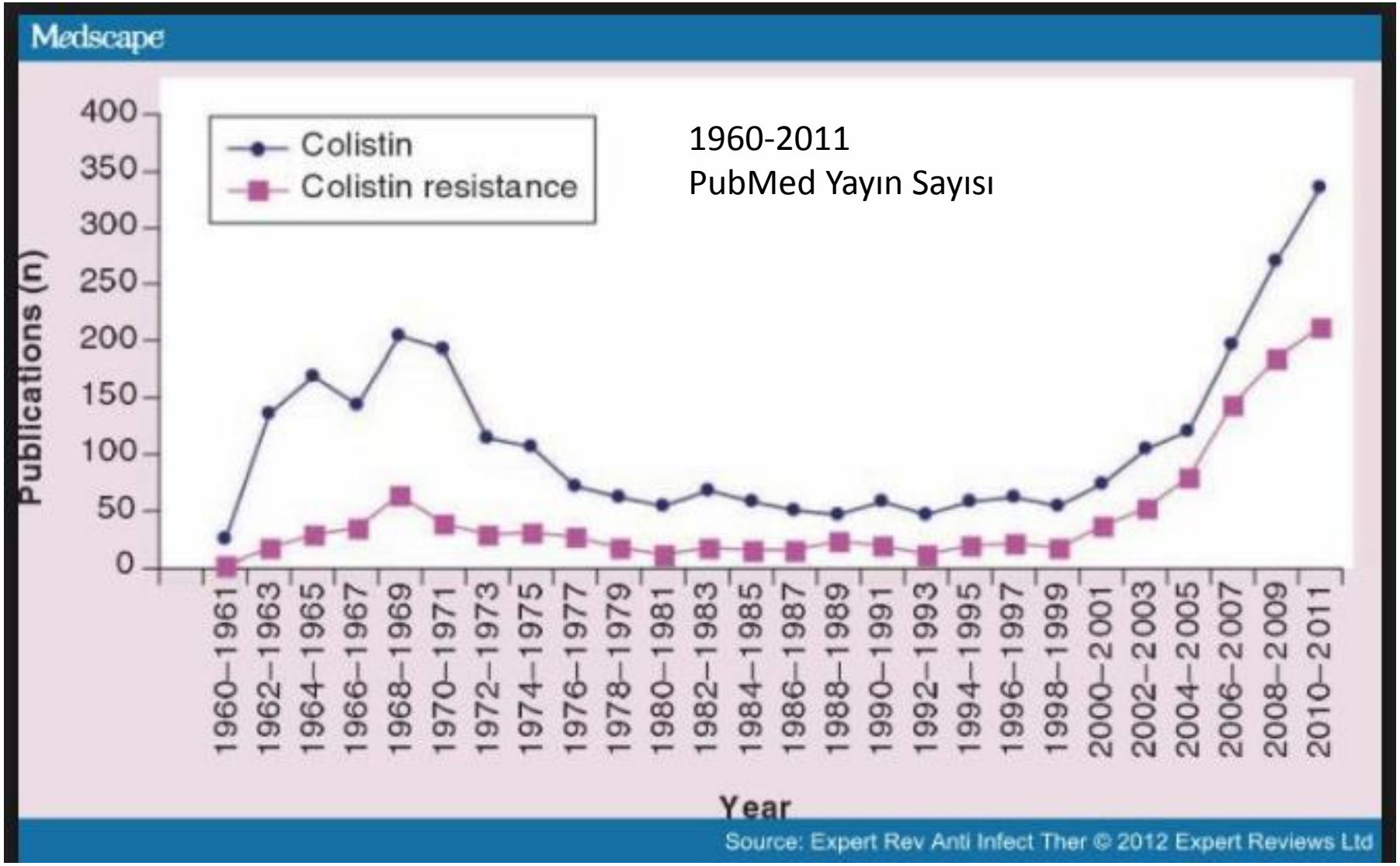
MR Fernand[illegible], MH Matté [5]

[illegible]a [3], MP Cunha [3], F Esposito [1], R Lopes [2], LK Otutumi [4], DD Gonçalves [4], M Dropa [5], [illegible]ncisco [7], MF Bueno [7], D de Oliveira Garcia [7], T Knöbl [3], AM Moreno [3], N Lincopan [1]

FIGURE 2
Geographical distribution of *mcr*-1-positive *Escherichia coli* isolates reported from South America, 2012–2016

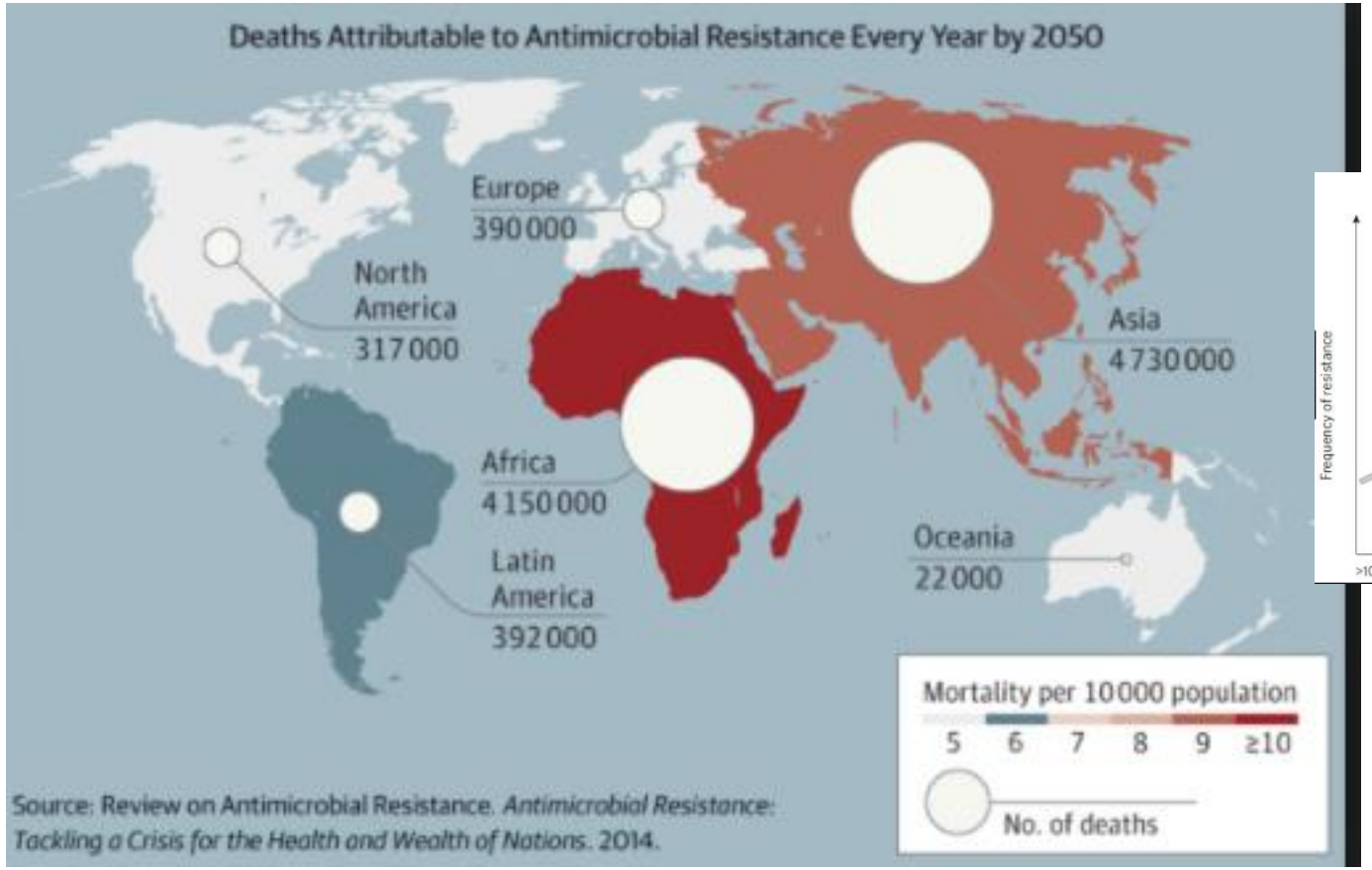

A light grey colour is used for Brazil, where this study was conducted. The dark grey colour indicates countries (Bolivia, Colombia and Peru) visited between November 2012 and November 2013, by unrelated Dutch travellers, for whom acquisition of faecal colonisation and carriage with MCR-1 and extended-spectrum beta-lactamase (ESBL)-producing E. coli was shown one to two weeks after their return to the Netherlands [12]. A dark grey colour is used for Ecuador, where subsequent to the identification of a human mcr-1-positive isolate, a sequence was deposited in GenBank in March 2016 (GenBank accession number: KU886144.1).

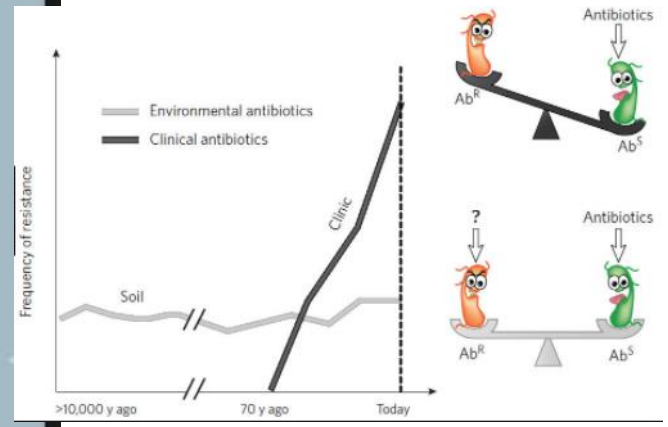
The 4,620 isolates were screened using MacConkey agar plates supplemented with colistin (2 mg/L). A total of 515 isolates, which had grown on the screening plates were obtained. These originated from



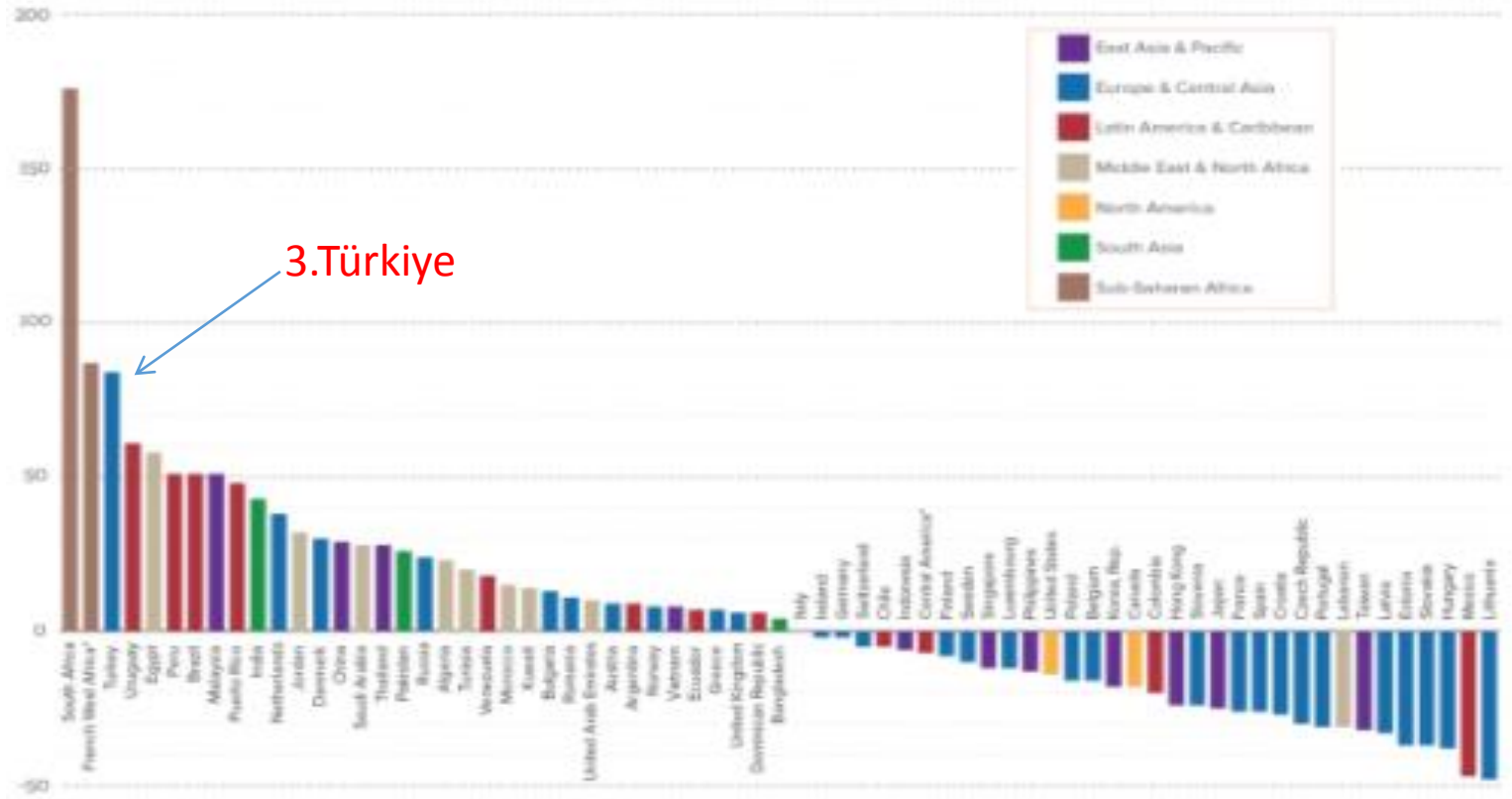

1960-2011
PubMed Yayın Sayısı

## 2050 yılına kadar dirençli bakterilerle enfeksiyonlardan ölüm >10 milyon



# Most high-income countries maintained or decreased their antibiotic consumption from 2000–2010*; BRICS countries had the highest upsurge.

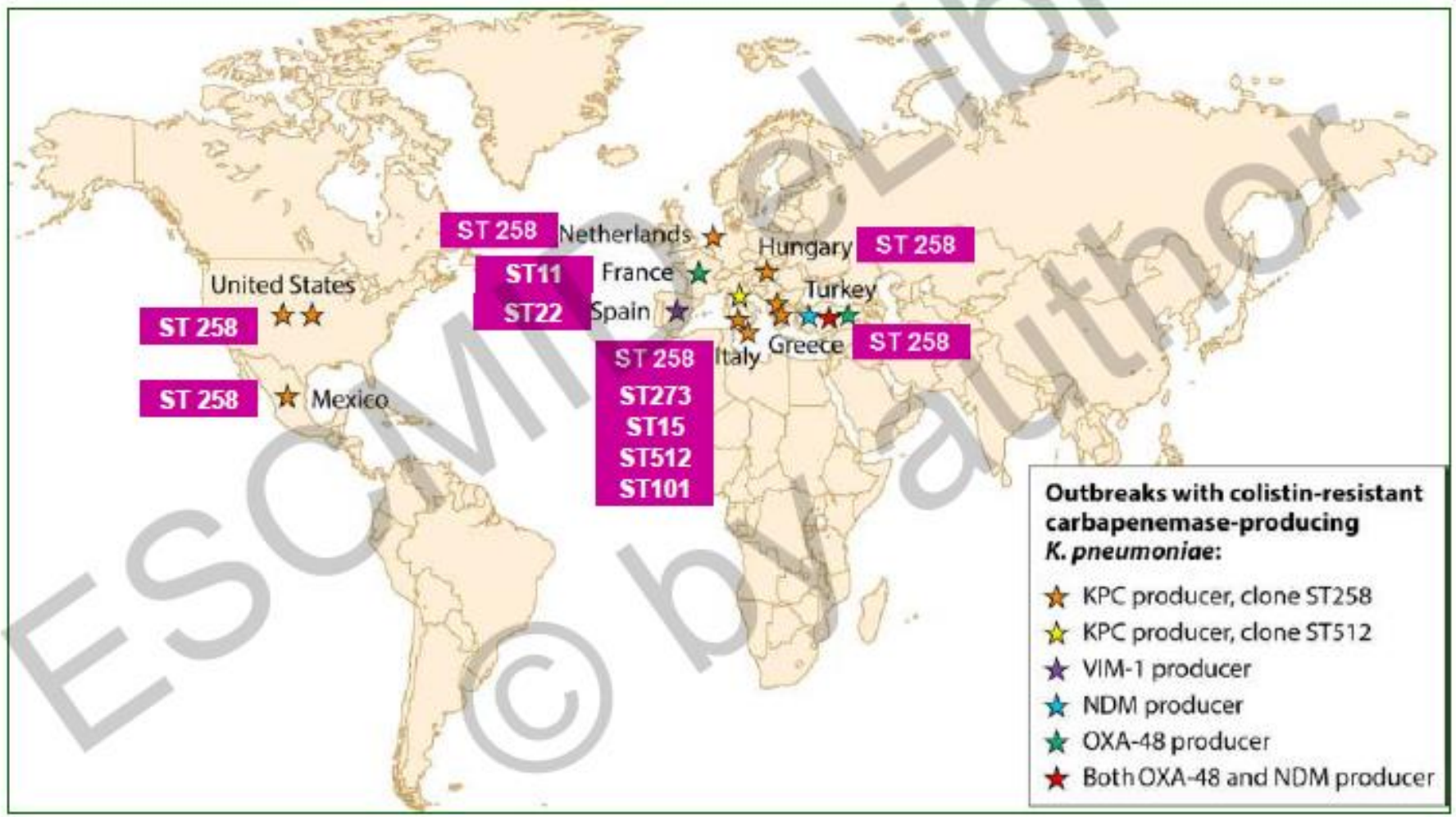



Data for Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua and Panama were available only as a group classified as Central America. Similarly, data for Benin, Burkina Faso, Cameroon, Côte d'Ivoire, Gabon, Guinea, Mali, Republic of the Congo, Senegal, and Togo were grouped and classified as French West Africa. The data for these countries represent the estimates for the corresponding regional groupings they belong to. For countries that did not have data available for 2000, the values for the earliest years for which data were available after 2000 were used to calculate the percentage changes. These countries and initial years are Algeria (2002), Bangladesh (2007), Croatia (2005), Netherlands (2005), and Vietnam (2005).

Data source: Van Boeckel, T. P., Gandra, S., Ashok, A., Caudron, Q.,Grenfell, B. T., Levin, S. A., and Laxminarayan, R. 2014. Global Antibiotic Consumption 2000 to 2010: An Analysis of National Pharmaceutical Sales Data. *The Lancet Infectious Diseases*, 309(14). The study was based on IMS MIDAS International Prescription Data, January 2000–December 2010. IMS Health Incorporated. All Rights Reserved. The statements, findings, conclusions, views, and opinions contained and expressed herein are not necessarily those of IMS Health Incorporated or any of its affiliated or subsidiary entities.

CDDEP Center for Disease Dynamics, Economics & Policy

# *Outbreaks Caused by Colistin-resistant, Carbapenemase-producing K. pneumoniae*




Each star indicates a single report

Poirel L et al. *Clin Microbiol Rev* 2017;30:557-596.

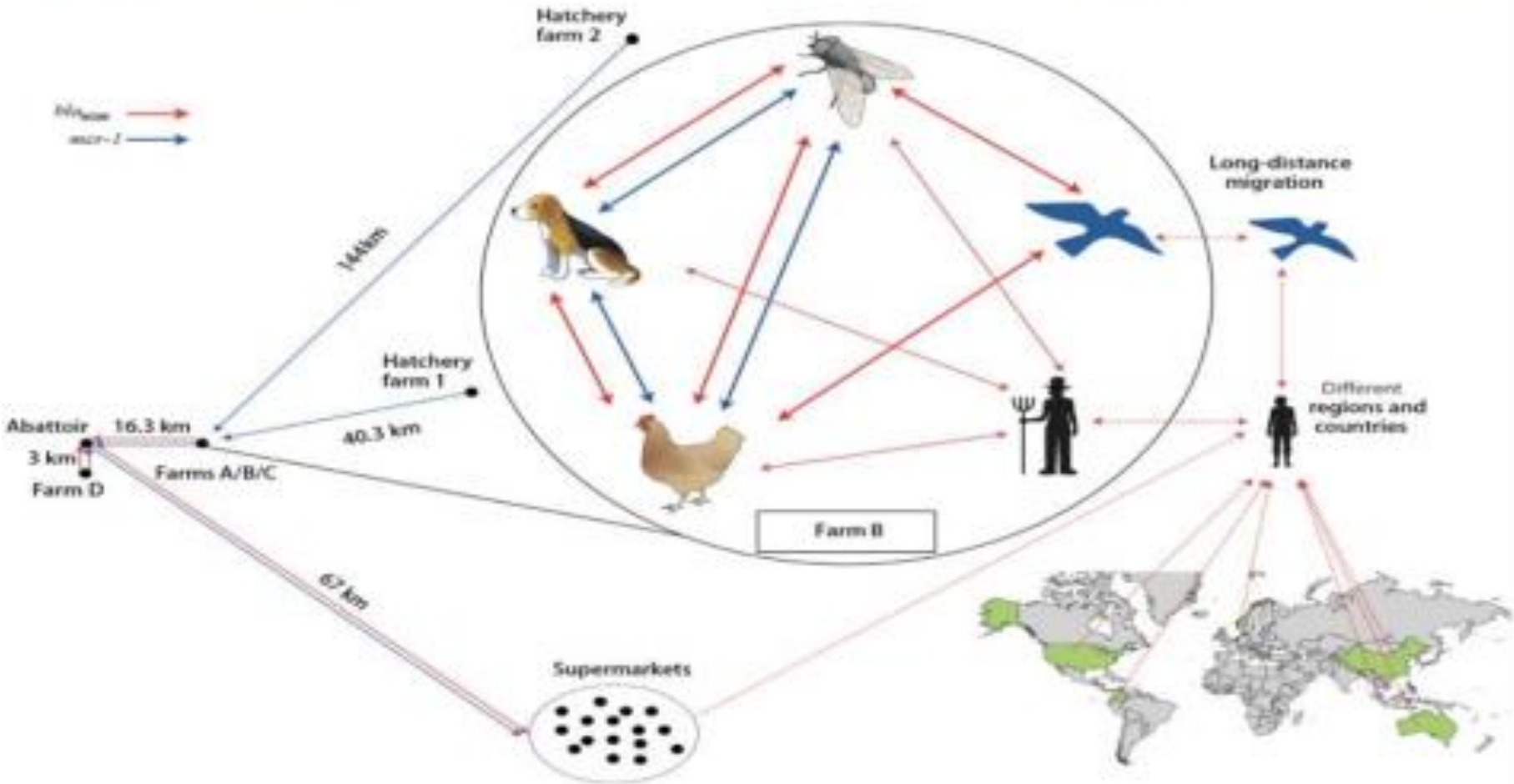
# Plazmidik mcr-1 genlerinin yiyecek, çevre, hayvan ve insanlarda küresel dağılımı
## Kasım 2015-Nisan 2016

- **Yiyecek:474**
- **Hayvan:577**
- **İnsan:184**
- **Çevre 75**


**TOPLAM:1010 İzolat**

IJAA:2016;48:583

**The possible transmission routes of NDM and *mcr-1* in poultry production chain**



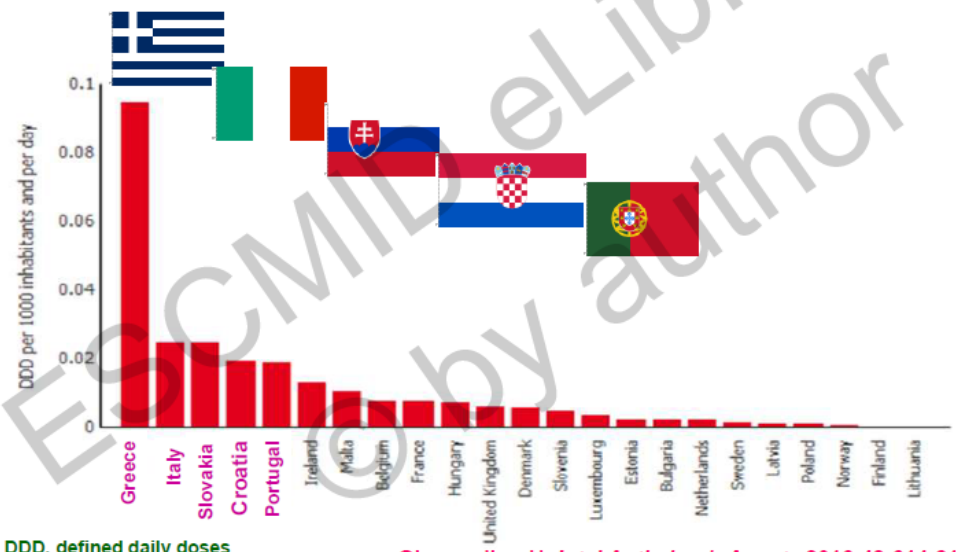

- *mcr-1* can be transferred from hatcheries to supermarket, NDM entered into the commercial farms through environmental media (bird, fly and human), then further contaminated the abattoir and supermarkets.

## Trends in consumption of antibacterials for systemic use in the hospital sector (expressed in DDD per 1000 inhabitants and per day), EU/EEA, 2009-2013

| | 2009 | 2010 | 2011 | 2012 | 2013 | Trends in antimicrobial consumption, 2009–2013 | Average annual change 2009–2013 | Statistical significance |
|---|---|---|---|---|---|---|---|---|
| **Total** (J01) | 2.1 | 2.0 | 2.0 | 2.0 | 2.0 | | -0.02 | n. s. |
| **Carbapenems** (J01DH) | 0.043 | 0.050 | 0.049 | 0.054 | 0.056 | | 0.003 | significant |
| **Polymyxins** (J01XB) | 0.008 | 0.008 | 0.011 | 0.014 | 0.014 | | 0.002 | significant |

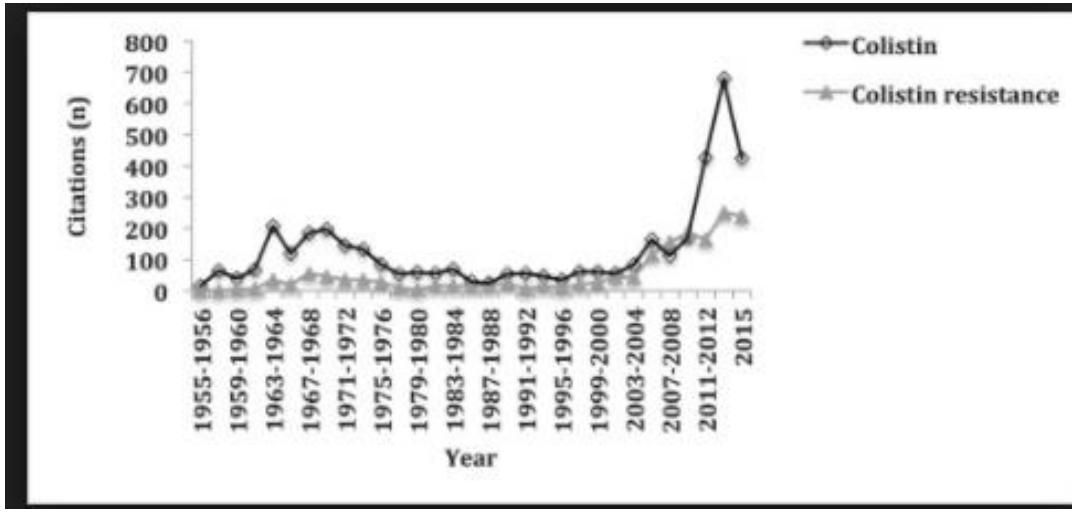
Source: ESAC-Net, 2014. http://ecdc.europa.eu/en/eaad/Pages/antibiotics-data-reports.aspx

## *Trends in Consumption of Polymyxins* European Countries, ECDC, 2014




DDD, defined daily doses

Giamarellou H. *Int J Antimicrob Agents* 2016;48:614-21.



# *P. aeruginosa* ve *A. baumannii*'de kolistin direnci

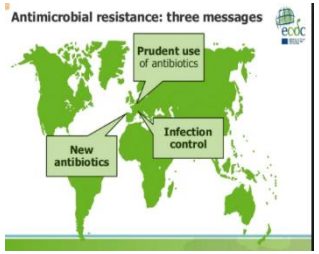
- *P. aeruginosa*
  - SENTRY 2009-2011 (N=2383) < %7
  - 2011-2012 13 AVRUPA ÜLKESİ 32 MERKEZ (n=2191), MDR- %0.2
  - Yunanistan:2011-2012 (n=881) %6.3
  - Kanada: 2008-2015 (n=2906), %5.1
  - Hindistan:2011-2013☹n=352), %2.2-3.8

## *A. baumannii*

- Güney Avrupa ve Güney Doğu Asya (%15-40)
- Heterojen Direnç %18.7-100
- Çin: 2009-2014:%3
- Yunanistan: 2014, %7.9
- SENTRY: 2006-2009-%0.9-3.5 (Kore%30.6)

Infect Cheother 2016;48:190
Chin Med. J. 2017:130☹6):659
IJAA 2017, April
Diag. Microbiol Infect Dis. 2017:87;60IJAA 2016:48;614

# STANDART ENFEKSİYON KONROL ÖNLEMLERİNE



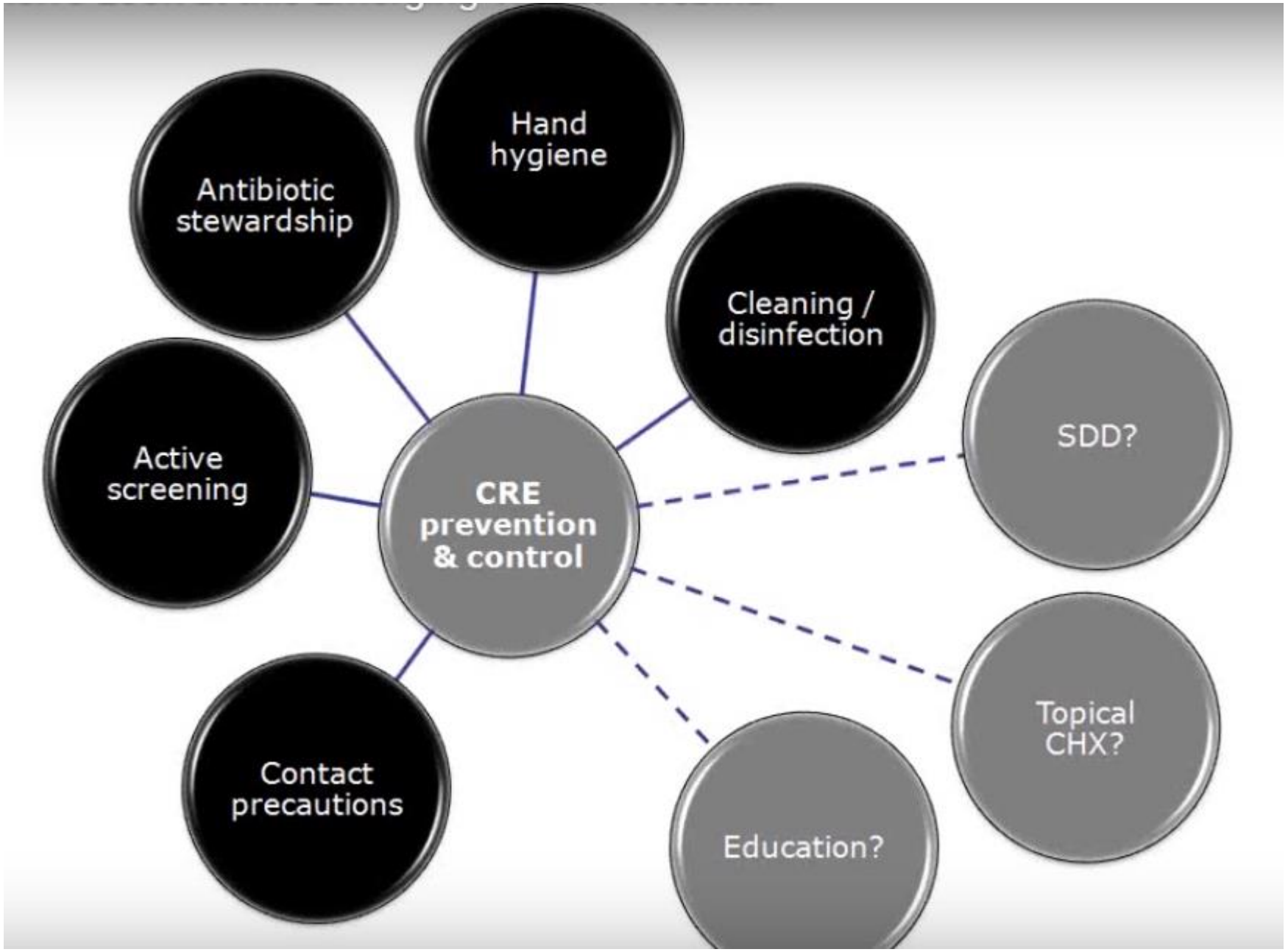

## BİLİNENLER

- Direnç hızla artıyor
- Direnç mekanizmalarının bilinmesi önemli (OXA-181)
- Ülkemizde çok merkezli çalışmalara ihtiyaç var
- mcr genlerinin Enterobacteriaceae ailesinde yayılımı devam etmekte
- Horizontal gen aktarımı
- *P. aeruginosa ve A. baumannii*


## YAPILMASI GEREKENLER

- Hastanelerde rasyonel antibiyotik kullanımı
- Hayvanlarda promotor veya proflaksi amacıyla kullanımın yasaklanması
- Duyarlılık testlerinin doğru yöntemle yapılması
- Tarama çalışmalarının yapılması
- Taşıyıcı ve enfekte hastaların izolasyonu (mcr geni pozitif ise)
- Sürveyans çalışmalarının ve özellikle moleküler epidemiyolojik çalışmaların yapılması
- EĞİTİM

**TEŞEKKÜRLER**


Hand hygiene
Antibiotic stewardship
Cleaning / disinfection
Active screening
CRE prevention & control
SDD?
Topical CHX?
Contact precautions
Education?

Rapid promotion of strict adherence to your Carbapenemase-producing Enterobacteriaceae Management Plan (Card B.1) should take place, including the need for compliance with its recommendations

*ENSURE THAT*:

1. All staff fully understand isolation procedures and adhere to standard precautions *as a norm* including:
    - hand hygiene
    - personal protective equipment
    - aseptic technique
    - laundry management
    - safe use of sharps
    - waste disposal (especially faeces)
2. Scrupulous IP&C practices are emphasised as being particularly important when using and caring for devices / equipment such as:
    - intravenous / peripheral line
    - central venous catheter line
    - urinary catheter
    - ventilators
    - renal dialysis equipment
    - enteral feeding equipment
    - colostomy or ileostomy
    - any re-usable diagnostic equipment (Card A.7)

NOTE: Loose stools or diarrhoea (for any reason) increase the risk of spread of the bacteria from the gut, therefore:

- observe strict IP&C measures

# CMS Dosing: Alternative US (Weight-based) Algorithm

CrCl>50: 5mg/kg/d

CrCl 30-49: 3.5 mg/kg/d

CrCl 10-29: 2.5 mg/kg/d

CrCl<10 or HD: 1.5 mg/kg/d

This algorithm leads to doses similar to those obtained using Nation, EMA dosing algorithms