Colistin heteroresistance in carbapenem-resistant
*Klebsiella pneumoniae*

Jean-Marc Rolain

Aix Marseille University, IRD, APHM, MEPHI, IHU-Méditerranée Infection – Marseille – France

Email: jean-marc.rolain@univ-amu.fr
No conflict of interest
Outline of the lecture

– History of emergence of resistance to colistin
– Mechanism of action and resistance
  • General mechanisms
  • Definition of heteroresistance
– What is known in *K. pneumoniae* and related bacteria: a review of the literature
– Heteroresistance: does it matter?
– Conclusion
Consequences of 3GC resistance: increase use of carbapenems and increase of carbapenem resistance in Gram-negative bacteria

[ESBL or carbapenemase] and K. pneumoniae keywords


Increasing use of colistin

Increase Use of colistin

And ......
Colistin therapy for 3 – 6 months for MDR P. aeruginosa orthopedic infections
Polymyxins:
- Polymyxin B and colistin (polymyxin E)
- discovered in 1947 in *Paenibacillus polymyxa* (formerly *Bacillus polymyxa* var. *colistinus*)
- available since 1959 for treatment of Gram negative bacterial infections
- cyclic polypeptide antibiotics
- secondary metabolite from nonribosomal peptide synthase (NRPS)
- mode of action: on bacterial cell membrane, binds to LPS and phospholipids
- in the outer cell membrane and displaces divalent cations (Ca and Mg)
- **Cationic** antimicrobial polypeptides
- **Bactericidal** activity against most *Gram-negative bacteria*

Spectrum of activity: Two major categories of Gram-negative bacteria

i. **Naturally susceptible bacteria** (*Escherichia coli, Klebsiella pneumoniae, Salmonella, Acinetobacter baumannii, Pseudomonas aeruginosa*)

ii. **Intrinsically resistant bacteria** (*Proteus, Providencia, Morganella, Serratia, Burkholderia*)
General Mechanism of Colistin Resistance through Lipid A Modifications In Gram-negative Bacteria


\[ \text{4-amino-4-deoxy-L-arabinose} \]

\( \text{arnT-pmrE operon} \)

\( \text{pmrA-pmrB mutation} \)

\( \text{pmrD mutation} \)

\( \text{phoP-phoQ mutation} \)

\( \text{mgrB} \) (negative regulator of \text{phoPQ})

\( \text{pmrC} \) mutation

\( \text{Phosphoethanolamine} \)
Background

Overall colistin resistance mechanism

1st description of a transferable gene conferring colistin resistance - \textit{mcr-1}

\textit{Molecular mechanisms of polymyxin resistance: knowns and unknowns.}
Baron S, Hadjadj L, Rolain JM, Olaitan AO.
• **Heteroresistance**: phenomenon wherein a subpopulation of bacteria in an otherwise homogenous population display antibiotic resistance.
Constant Colistin Heteroresistance

- Disk diffusion method or E-test: first inhibition zone followed by regrowth in high colistin concentrations
- Broth microdilution method: resistant or ‘skipped’ wells
- Described in Gram negative bacteria for:
  - *Serratia marcescens* -> Intrinsic resistance
  - *Enterobacter spp.* -> Acquired resistance

Role of efflux?

Baron SA, J. of Antimicrob Chemother., 2018

Telke A, J. of Antimicrob Chemother., 2017

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PAPs: Population Analysis Profiles

Colistin mg/L

Control 0,125 0,25 0,5 1 2

MIC

0,5 McF suspension

True Colistin susceptibility?

Persistance of colonies = Heteroresistance

Colistin susceptible

Colistin Heteroresistant

Colistin mg/L

Control 0,125 0,25 0,5 1 2 4 8 16 32

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Regrowth observed after 4h in all the heteroresistant (HR) isolates
• Concentrations needed to kill HR isolates unachievable in vivo
Development of Diagnostic tools: the problematic of colistin resistance

- Colistin Resistance defined as MIC > 2µg/ml –isolates with borderline MICs
- Disk diffusion method is not reproducible for detection ➔ broth microdilution

![Graph showing K. pneumoniae strains from Greece MIC Colistin (n=288)]

- Carba R (MIC > 2mg/L)
- n = 256
  89 %
Colistin heteroresistance in carbapenem-resistant *K. pneumoniae* – Review of the literature

20 carbapenem resistant *K. pneumoniae*

16 colistin-susceptible isolates by BMD

12 isolates colistin-heteroresistant (PAPs)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Colistin treatment</th>
<th>Carbapenemase type</th>
<th>Broth MIC (mg/L)</th>
<th>Highest concentration of growth in PAPs (mg/L)</th>
<th>Proportion of resistant subpopulations</th>
<th>Resistant colonies MIC after 2 week daily passages onto colistin-free medium (mg/L)</th>
<th>Resistant colonies MIC after 2 week daily passages onto colistin-free medium (mg/L)</th>
<th>Susceptibility</th>
<th>PFGE group</th>
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Heteroresistance in *K. pneumoniae*

Emergence of a colistin-resistant subclone due to mutation Asp191Tyr in protein PhoP
Genomic Characterization of Colistin Heteroresistance in *Klebsiella pneumoniae* during a Nosocomial Outbreak

Toysir Halaby,⁎⁎ Emre Kucukkose,⁎⁎ Axel B. Janssen,⁎ Malbert R. C. Rogers,⁎ Dennis A. Doorduijn,⁎ Adri G. M. van der Zanden,⁎ Nashwan al Naimi,⁎⁎ Christina M. J. E. Vandenbroucke-Grauls,⁎ Willem van Schaik⁎

<table>
<thead>
<tr>
<th>Isolate identifier</th>
<th>Yr of isolation</th>
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Mutations found in *mgrB*, *phoQ*, *yciM*, *lpxM*

*yciM* contributes to cell wall integrity by regulating LPS biosynthesis

LpxM is responsible for the addition of one of the secondary acyl chains to lipid A in *Enterobacteriaceae*

Emergence of a colistin-resistant subclone due to mutations in \textit{mgrB}
Typing subpopulation

1) Same AST
2) MLST: ST86
3) Same PFGE (XbaI)
4) Same MALDI-TOF Gel View

Bardet et al., Antimicrob Agents Chemother., 2017
soxRS induces colistin hetero-resistance in Enterobacter asburiae and Enterobacter cloacae by regulating the acrAB-tolC efflux pump

Amar A. Telke², Abiola Olumuyiwa Olaitan¹, Serge Morand² and Jean-Marc Rolain¹

Efflux pump overexpression due to a complex mechanism!!

Efflux pump inhibitor

Telke A et al, J Antimicrob Chemother, 2017; Dandachi I et al submitted
Objectif: Evaluate the effect of CCCP on a collection of colistin-resistant strains of Gram negative bacteria.
Role of efflux in Gram negative bacteria

Strains tested:
- 90 Enterobacteriaceae including:
  - 32 K. pneumoniae
  - 24 E. coli
  - 7 Enterobacter sp.
  - 2 Salmonella sp.
  - 13 S. marcescens
  - 3 Morganella morganii
  - 4 Providencia spp.
  - 3 Pseudomonas aeruginosa

[CCCP]= 10 mg/L

CCCP rescue also colistin heteroresistance

Baron S and Rolain JM, J Antimicrob Chemother, 2018
Heteroresistance and antibiotic failure
Does it matter?

• Suggested in *Enterobacter cloacae*
• Not detected with E-test method
• Responsible for treatment failure in mice

Survival % in infection model on mice caused by a susceptible strain (left) and the resistant subpopulation (right)

Antibiotic failure mediated by a resistant subpopulation in *Enterobacter cloacae*

Victor I. Band1,2,†, Emily K. Crispell1,2,†, Brooke A. Napier1,2, Carmen M. Herrera3, Greg K. Tharp4, Kranthi Vavikolanu5, Jan Pohl6, Timothy D. Read5,7,8, Steven E. Bosingar1,2,9, M. Stephen Trent1, Eileen M. Burd5,7,9, and David S. Weiss2,5,7,10,*

Nat. microbiol, 2016
Heteroresistance: does it matter?

Carbapenem-Resistant *Klebsiella pneumoniae* Exhibiting Clinically Undetected Colistin Heteroresistance Leads to Treatment Failure in a Murine Model of Infection

Victor I. Band,1,5,6 Sarah W. Satola,1,5,6 Eileen M. Burd,1,5,6 Monica M. Farley,5,6,7 J oess T. Jacob,5,6 David S. Weiss,5,6,8

A

B

C

D

E

Heteroresistant isolate with both resistant and susceptible subpopulations

![Image of experimental setup and data analysis](image-url)
One hyperviscoumucous KPC-3 isolate (out of 265 isolates)
Colistin Heteteroresistant (CDC98)
Perspectives

• Not new but probably under-diagnosed
• Need to improve the detection of heteroresistance in routine laboratory
• Understanding the real impact of this heteroresistance:
  – Risk of therapeutic failure?
  – Alternatives?

• Management of the use of colistin to treat carbapenem-resistant *K. pneumoniae*
  • Limit its use?
  • Only in combination?
ACKNOWLEDGMENTS

Staff and students from my team
Thank you for your attention

http://www.mediterranee-infection.com