

# *Haemophilus influenzae* og antibiotika Aktuelt om resistens

Dagfinn Skaare  
Mikrobiologisk laboratorium  
Sykehuset i Vestfold HF

[dagfinn.skaare@siv.no](mailto:dagfinn.skaare@siv.no)

# Hvordan unngå det unngåelige?

## Antimicrobial Resistance in *Haemophilus influenzae*: How Can We Prevent the Inevitable? Commentary on Antimicrobial Resistance in *H. influenzae* Based on Data from the TARGETed Surveillance Program

**Robert P. Rennie<sup>1</sup> and Khalid H. Ibrahim<sup>2</sup>**

<sup>1</sup>Medical Microbiology Laboratory, University of Alberta Hospital, Edmonton, Canada; and <sup>2</sup>Global Scientific Affairs, Bayer HealthCare, West Haven, Connecticut

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S234 • CID 2005:41 (Suppl 4) • Rennie and Ibrahim

# Hvordan unngå det uunngåelige?

*..it will be important to follow these organisms in the community to determine if resistance determinants may spread more widely than we have thus far believed.*

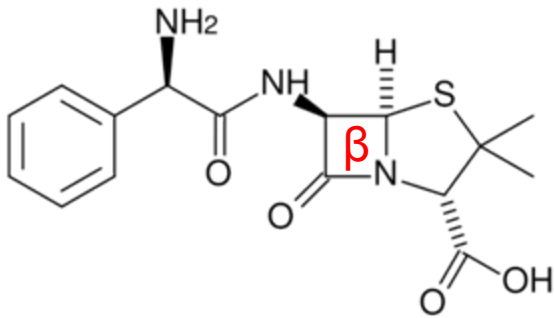
*The implications for treatment, infection prevention and control, and public health should not be underestimated as it has been with other organisms such as *S. pneumoniae* and *Staphylococcus aureus*.*

Rennie and Ibrahim, CID 2005

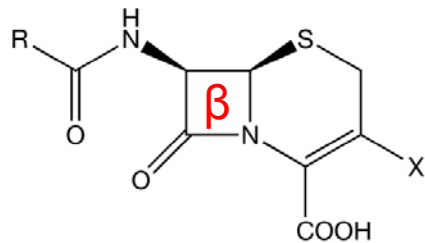
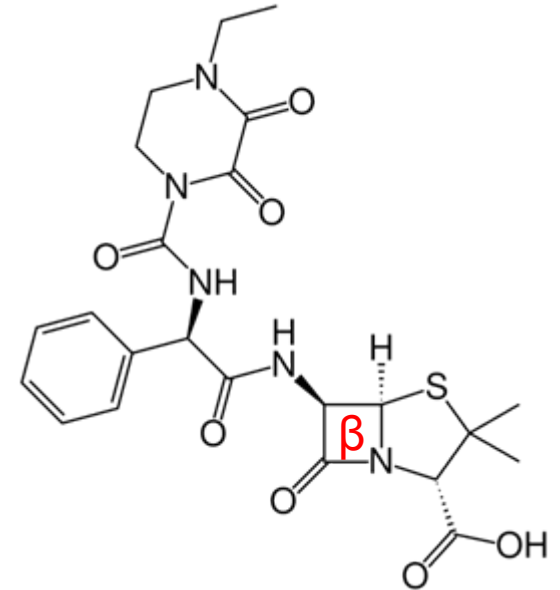
# Aktuelle antibiotika

- Betalaktamer
- Trimetoprim-sulfa
- Kinoloner
- Tetracycliner
- Kloramfenikol
- Makrolider?

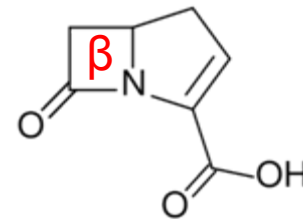
# Betalaktamer



- Penicilliner
  - Aminopenicilliner
  - Piperacillin

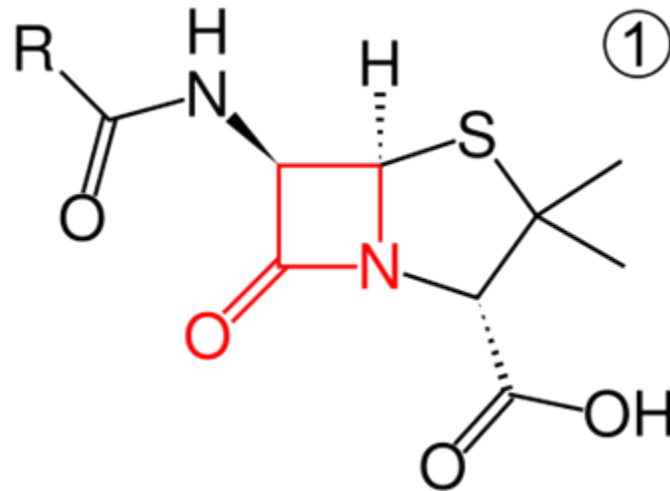


- Cefalosporiner
  - 2.-4. generasjon
- Karbapenemer



# Betalaktamer

- Felles struktur: Betalaktamringen
- Binder seg til og hemmer PBP
- Ulike R-sidekjeder gir ulik PBP-spesifisitet



# Penicillinbindende proteiner (PBP)

- Enzymer i cellemembranen
- Nødvendige for syntese av peptidoglykaner
- PBP hos *H. influenzae*: 1AB, 2, 3AB, 4, 5, 6
- Ulike betalaktamer har ulike PBP-profiler
  - Aminopenicilliner  $4 > 3AB = 1AB$
  - Cefalosporiner  $3AB > 1B > 4$
  - Karbapenemer  $4 > 2 = 1B$

# Betalaktamresistens hos *H.influenzae*

## 1. Betalaktamase

- Plasmidmedierte enzymer
  - TEM-1
  - ROB-1
- Destruerer antibiotikamolekylet
- Resistens kun mot penicilliner
- Hemmes av klavulansyre

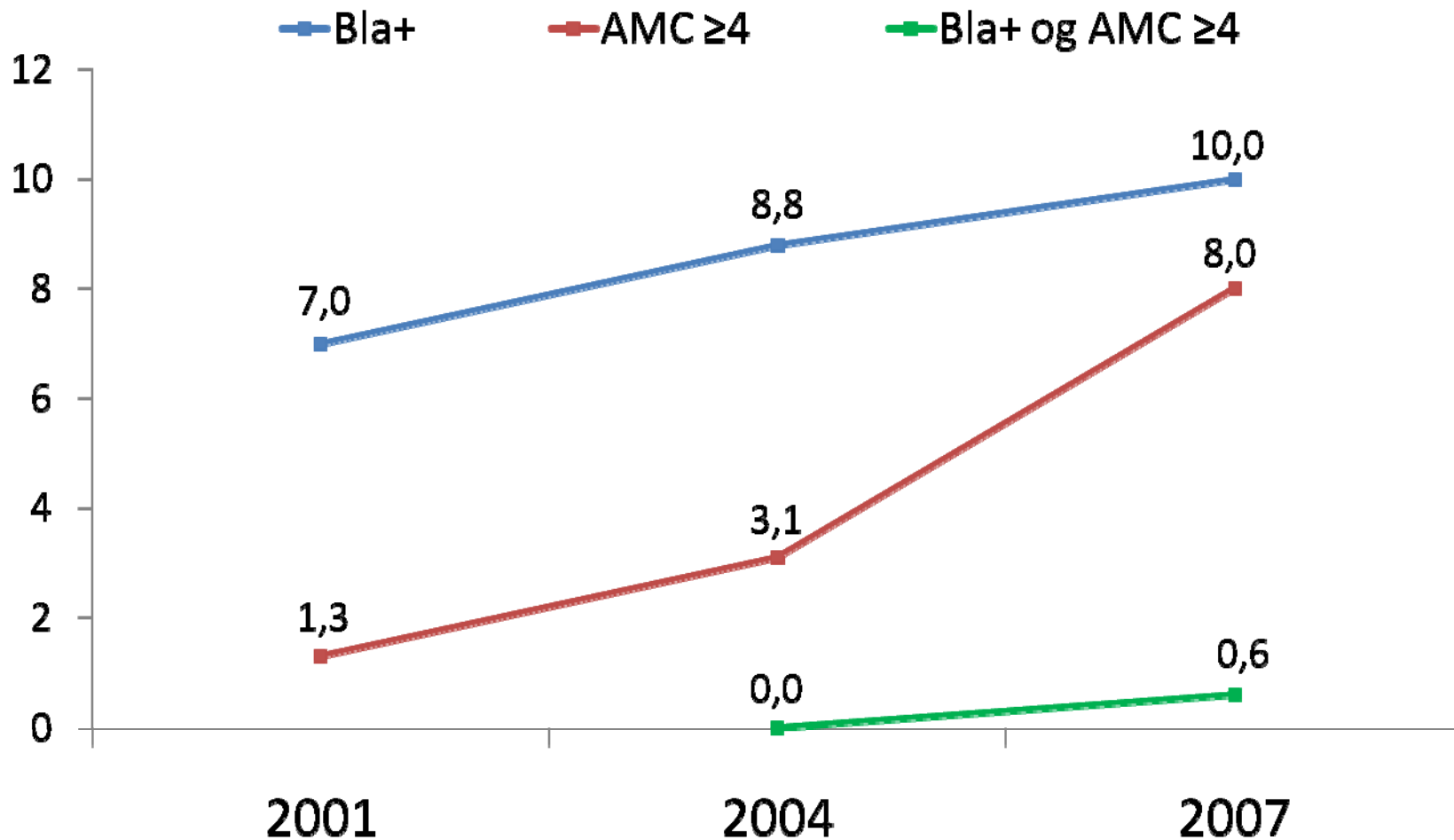
# Betalaktamresistens hos *H.influenzae*

## 2. Endret PBP3

- Mutasjoner i genet *ftsI*
- Redusert affinitet
- Gir varierende grad av resistens
- Resistens mot penicilliner og cefalosporiner
- Hemmes ikke av klavulansyre

# Betalaktamresistens hos *H.influenzae*

Data fra NORM



# Genetisk BLNAR (gBLNAR)

Arg-517→His                      I                      Low-BLNAR

Asn-526→Lys                      II                      Low-BLNAR

Arg-517→His  
+ Ser-385→Thr                      "III-like"                      BLNAR

Asn-526→Lys  
+ Ser-385→Thr                      III                      BLNAR

= gBLNAR

# gBLNAR og resistens

<b>Ampicillin</b>	n	0,002	0,004	0,008	0,016	0,032	0,064	0,125	0,25	0,5	1	2	4	8	16	32
BLNAS	118					■	■	■	■	■						
Low-BLNAR	249						■	■	■	■	■	■	■	■		
BLNAR	78									■	■	■	■	■	■	
<b>Cefotaxim</b>	n	0,002	0,004	0,008	0,016	0,032	0,064	0,125	0,25	0,5	1	2	4	8	16	32
BLNAS	118		■	■	■	■	■									
Low-BLNAR	249				■	■	■	■	■							
BLNAR	78						■	■	■	■	■	■				

Hasegawa et al 2004, Ubukata et al 2001, Osaki et al 2005, Dabernat et al 2002

# Endret PBP3 gir økt virulens



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## An amino acid substitution in PBP-3 in *Haemophilus influenzae* associate with the invasion to bronchial epithelial cells

Tadashi Okabe<sup>a</sup>, Yoshitaka Yamazaki<sup>a,b,\*</sup>, Miho Shiotani<sup>a</sup>,  
Takefumi Suzuki<sup>a</sup>, Mayumi Shiohara<sup>a</sup>, Eriko Kasuga<sup>a</sup>, Shigeyuki Notake<sup>c</sup>,  
Hideji Yanagisawa<sup>c</sup>

<sup>a</sup>Department of Laboratory Medicine, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan

<sup>b</sup>Department of Endoscopy, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan

<sup>c</sup>Miroku Medical Laboratory, Co., 659-2 Innai, Saku 384-2201, Japan

# EUCAST brytningspunkter

Penicillins Click on antibiotic name to see wild type MIC distributions.		Species-related breakpoints (S</R>)									
		<i>Enterobacteriaceae</i> <sup>A</sup>	<i>Pseudomonas</i> <sup>B</sup>	<i>Acinetobacter</i> <sup>C</sup>	<i>Staphylococcus</i> <sup>D</sup>	<i>Enterococcus</i> <sup>E</sup>	<i>Streptococcus</i> A,B,C,G <sup>F</sup>	<i>S.pneumoniae</i> <sup>G</sup>	<i>Other streptococci</i> <sup>H</sup>	<i>H.influenzae</i> <sup>I</sup>	<i>M.catarrhalis</i> <sup>J</sup>
<a href="#">Benzylpenicillin</a>	RD	--	--	--	0.12/0.12	Note <sup>E</sup>	0.25/0.25	0.06/2	0.25/2	IE	--
<a href="#">Ampicillin</a> <sup>N</sup>	RD	Note <sup>A</sup> /8	--	--	Note <sup>D</sup>	4/8	Note <sup>F</sup>	0.5/2	0.5/2	1/1	1/1
<a href="#">Ampicillin/sulbactam</a> <sup>O</sup>	RD	Note <sup>A</sup> /8	--	IE	Note <sup>D</sup>	4/8	Note <sup>F</sup>	Note <sup>G</sup>	Note <sup>H</sup>	1/1	1/1
<a href="#">Amoxicillin</a>	RD	Note <sup>A</sup> /8	--	--	Note <sup>D</sup>	4/8	Note <sup>F</sup>	Note <sup>G</sup>	0.5/2	1/1	1/1
<a href="#">Amoxicillin/clavulanate</a> <sup>O</sup>	RD	Note <sup>A</sup> /8	--	--	Note <sup>D</sup>	4/8	Note <sup>F</sup>	Note <sup>G</sup>	Note <sup>H</sup>	1/1	1/1
<a href="#">Piperacillin</a>	RD	8/16	16/16	IE	Note <sup>D</sup>	Note <sup>E</sup>	Note <sup>F</sup>	Note <sup>G</sup>	Note <sup>H</sup>	Note <sup>I</sup>	Note <sup>J</sup>
<a href="#">Piperacillin/tazobactam</a> <sup>O</sup>	RD	8/16	16/16	IE	Note <sup>D</sup>	Note <sup>E</sup>	Note <sup>F</sup>	Note <sup>G</sup>	Note <sup>H</sup>	Note <sup>I</sup>	Note <sup>J</sup>
<a href="#">Ticarcillin</a>	RD	8/16	16/16	IE	Note <sup>D</sup>	Note <sup>E</sup>	--	--	IE	IE	IE
<a href="#">Ticarcillin/clavulanate</a> <sup>O</sup>	RD	8/16	16/16	IE	Note <sup>D</sup>	Note <sup>E</sup>	--	--	IE	IE	IE
<a href="#">Phenoxymethylpenicillin</a>	RD	--	--	--	Note <sup>D</sup>	--	Note <sup>F</sup>	Note <sup>G</sup>	IE	IE	--

**I. *Haemophilus influenzae*:** Always test for beta-lactamase and report positive strains resistant to penicillins without beta-lactamase inhibitors. Breakpoints relate only to beta-lactamase negative strains. Strains may be resistant to penicillins, aminopenicillins and/or cephalosporins due to changes in PBP's (BLNAR, Betalactamase negative ampicillin resistant) and a few strains have both resistance mechanisms (BLPACR, betalactamase positive amoxicillin/clavulanate resistant). Isolates susceptible to ampicillin and amoxicillin are also susceptible to piperacillin and piperacillin/tazobactam and isolates susceptible to amoxicillin/clavulanate to piperacillin/tazobactam.

# EUCAST brytningspunkter - parenterale cefalosporiner

Cephalosporins		Species-related breakpoints (S<=I/R>)							
		<i>Enterobacteriaceae</i> <sup>2</sup>	<i>Pseudo-</i> <i>monas</i> <sup>3</sup>	<i>Acineto-</i> <i>bacter</i>	<i>Staphylo-</i> <i>coccus</i> <sup>4</sup>	<i>Entero-</i> <i>coccus</i>	<i>Strepto-</i> <i>coccus</i> A,B,C,G	<i>S.pneu-</i> <i>-</i> <i>moniae</i>	<i>H.influenzae</i> <i>M.catarrhalis</i>
<a href="#">Cefazolin</a>	RD	--	--	--	note <sup>4</sup>	--	--	--	--
<a href="#">Cefepime</a>	RD	1/8	8/8	--	note <sup>4</sup>	--	0.5/0.5 <sup>6</sup>	1/2	0.25/0.25 <sup>6</sup>
<a href="#">Cefotaxime</a>	RD	1/2	--	--	note <sup>4</sup>	--	0.5/0.5 <sup>6</sup>	0.5/2 <sup>6</sup>	0.12/0.12 <sup>6</sup>
<a href="#">Ceftazidime</a>	RD	1/8	8/8	--	--	--	--	--	--
<a href="#">Ceftriaxone</a>	RD	1/2	--	--	note <sup>4</sup>	--	0.5/0.5 <sup>6</sup>	0.5/2 <sup>6</sup>	0.12/0.12 <sup>6</sup>
<a href="#">Cefuroxime</a>	RD	8/8 <sup>5</sup>	--	--	note <sup>4</sup>	--	0.5/0.5 <sup>6</sup>	0.5/1	1/2

6. Strains with MIC values above the S/I breakpoint are very rare or not yet reported. The identification and antimicrobial susceptibility tests on any such isolate must be repeated and if the result is confirmed the isolate sent to a reference laboratory. Until there is evidence regarding clinical response for confirmed isolates with MIC above the current resistant breakpoint (in italics) they should be reported resistant.

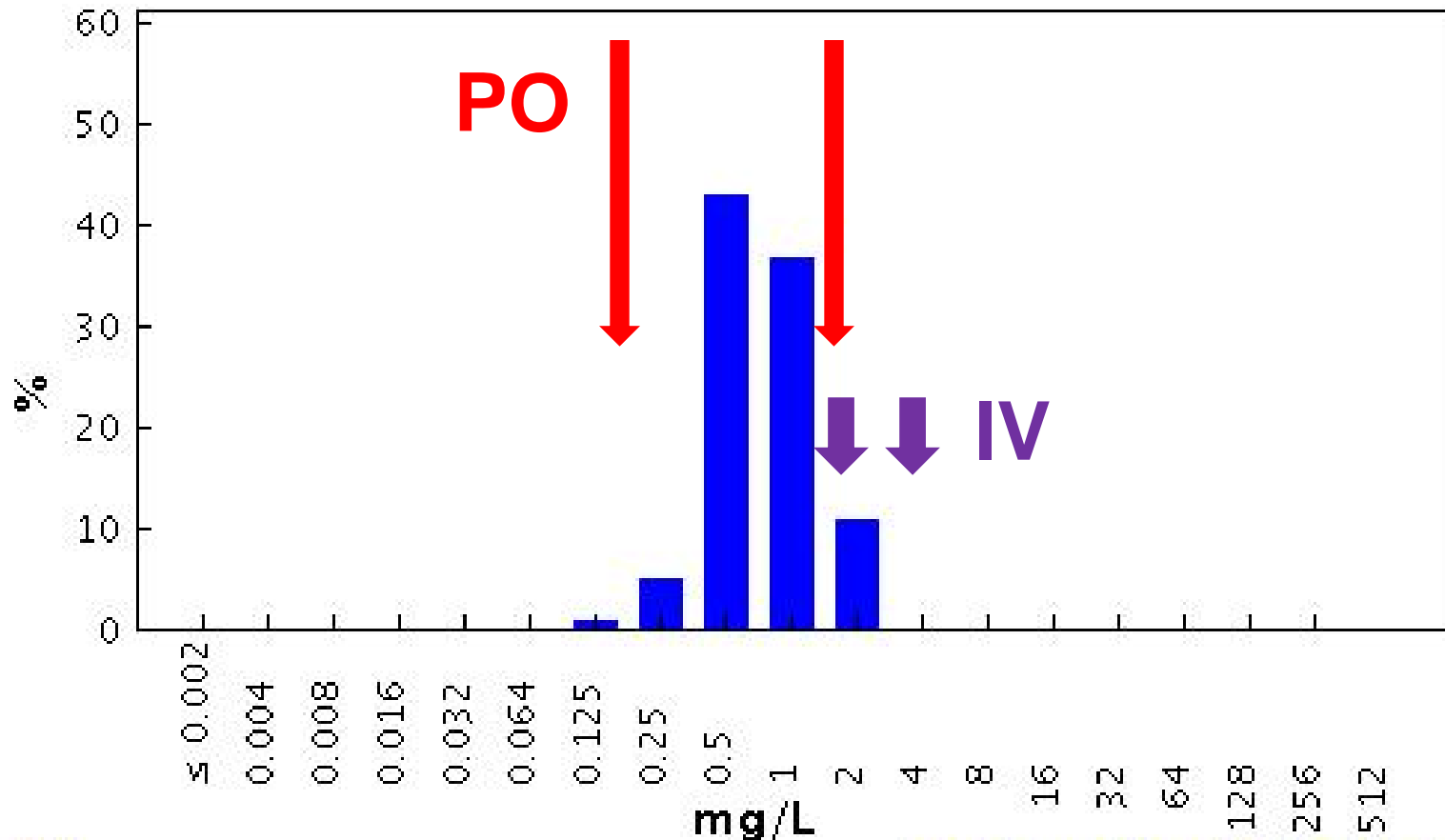
# EUCAST brytningspunkter - perorale cefalosporiner

Cephalosporins		Species-related breakpoints (S≤/R>)									
		<i>Enterobacteriaceae</i> <sup>B</sup>	<i>Pseudomonas</i>	<i>Acinetobacter</i>	<i>Staphylococcus</i> <sup>D</sup>	<i>Enterococcus</i>	<i>Streptococcus</i> A,B,C,G	Other streptococci	<i>S.pneumoniae</i>	<i>H.influenzae</i>	<i>M.catarrhalis</i>
<a href="#">Cefaclor</a>	RD	--	--	--	Note <sup>D</sup>	--	Note <sup>E</sup>	--	0.03/0.5	0.5/0.5	0.5/0.5
<a href="#">Cefadroxil</a>	RD	16/16 <sup>C</sup>	--	--	Note <sup>D</sup>	--	Note <sup>E</sup>	--	--	--	-
<a href="#">Cefalexin</a>	RD	16/16 <sup>C</sup>	--	--	Note <sup>D</sup>	--	Note <sup>E</sup>	--	--	--	-
<a href="#">Cefixime</a>	RD	1/1 <sup>C</sup>	--	--	--	--	--	--	--	0.12/0.12	0.5/1
<a href="#">Cefpodoxime</a>	RD	1/1 <sup>C</sup>	--	--	Note <sup>D</sup>	--	Note <sup>E</sup>	--	0.25/0.5	0.25/0.5	0.25/0.5
<a href="#">Ceftibuten</a>	RD	1/1 <sup>C</sup>	--	--	IE	--	Note <sup>E</sup>	--	IE	1/1	1/1
<a href="#">Cefuroxime-axetil</a>	RD	8/8 <sup>C</sup>	--	--	Note <sup>D</sup>	--	Note <sup>E</sup>	--	0.25/0.5	0.12/1	0.12/2

# Cefuroxime / Haemophilus influenzae

Antimicrobial wild type distributions of microorganisms - reference database

## EUCAST MIC Distribution



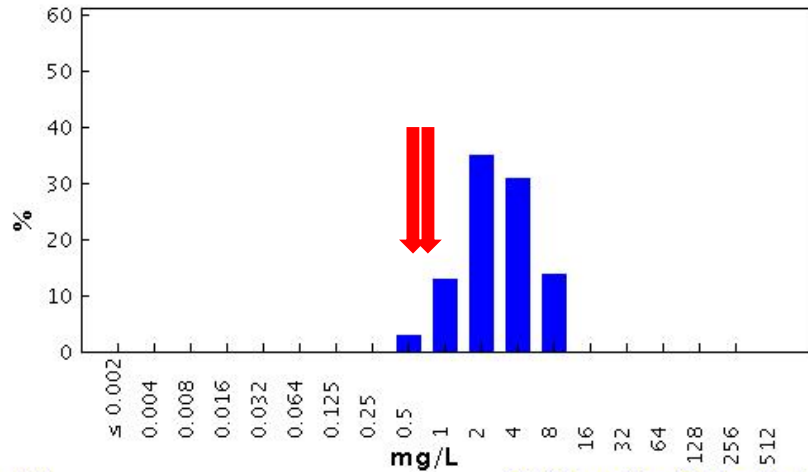
MIC  
Epidemiological cut-off: WT ≤ 2 mg/L

80273 observations (31 data sources)  
Clinical breakpoints: S ≤ 1 mg/L, R > 2 mg/L

### Cefaclor / Haemophilus influenzae

Antimicrobial wild type distributions of microorganisms - reference database

EUCAST MIC Distribution

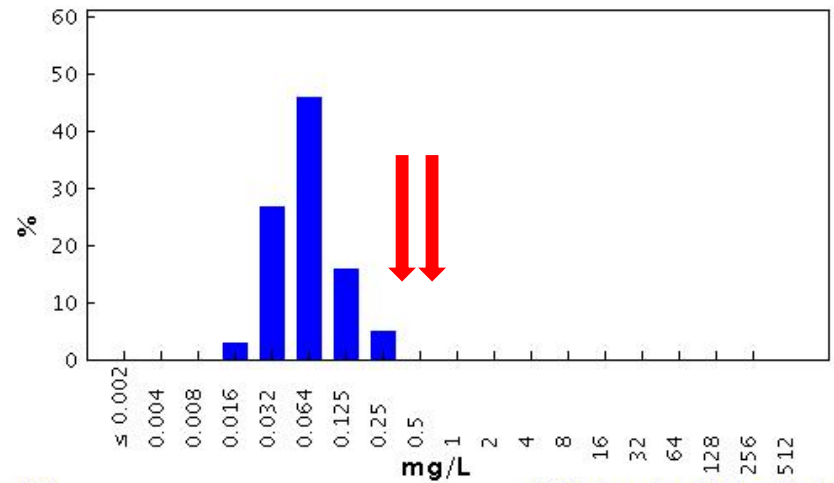


MIC 26240 observations (14 data sources)  
Epidemiological cut-off: WT ≤ 8 mg/L Clinical breakpoints: S ≤ 0.5 mg/L, R > 0.5 mg/L

### Cefpodoxime / Haemophilus influenzae

Antimicrobial wild type distributions of microorganisms - reference database

EUCAST MIC Distribution

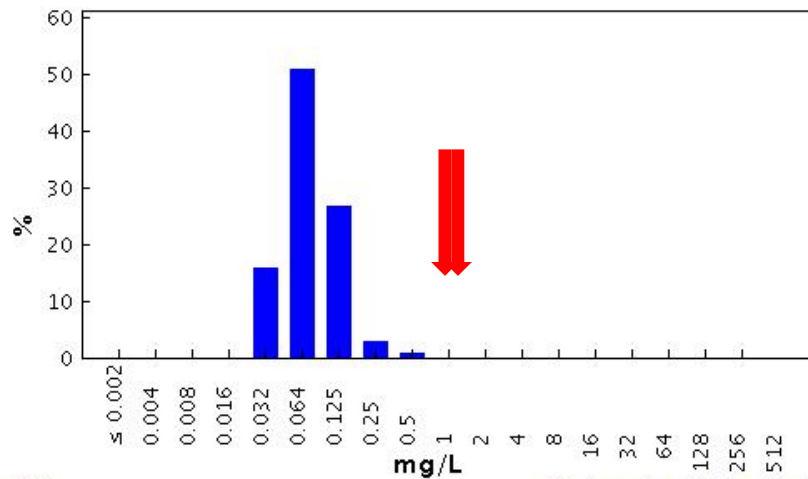


MIC 17394 observations (5 data sources)  
Epidemiological cut-off: WT ≤ 0.25 mg/L Clinical breakpoints: S ≤ 0.25 mg/L, R > 0.25 mg/L

### Ceftibuten / Haemophilus influenzae

Antimicrobial wild type distributions of microorganisms - reference database

EUCAST MIC Distribution

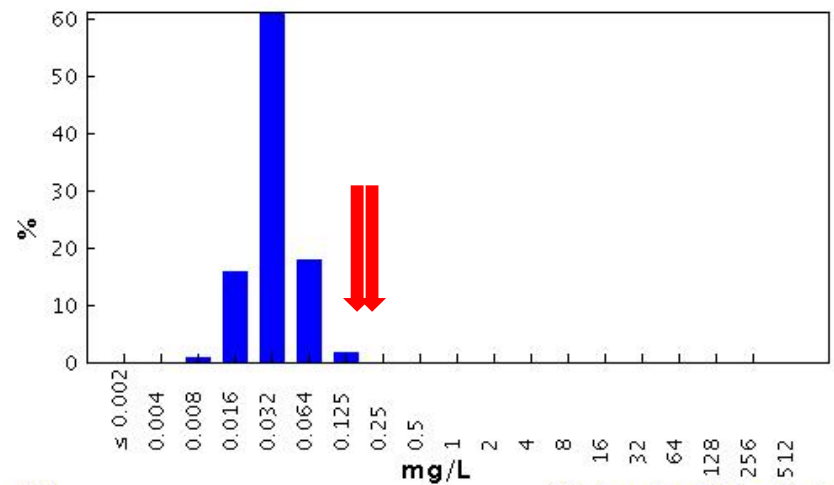


MIC 292 observations (2 data sources)  
Epidemiological cut-off: WT ≤ 0.5 mg/L Clinical breakpoints: S ≤ 1 mg/L, R > 1 mg/L

### Cefixime / Haemophilus influenzae

Antimicrobial wild type distributions of microorganisms - reference database

EUCAST MIC Distribution



MIC 7112 observations (7 data sources)  
Epidemiological cut-off: WT ≤ 0.125 mg/L Clinical breakpoints: S ≤ 0.125 mg/L, R > 0.125 mg/L

# Orale cefalosporiner selekterer for PBP-mediert resistens

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2008, p. 1880–1883  
0066-4804/08/\$08.00+0 doi:10.1128/AAC.00936-07  
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## Comparison of the Efficacies of Oral $\beta$ -Lactams in Selection of *Haemophilus influenzae* Transformants with Mutated *ftsI* Genes<sup>∇</sup>

Sho Takahata,\* Yoshihisa Kato, Yumiko Sanbongi, Kazunori Maebashi, and Takashi Ida  
*Pharmaceutical Research Center, Meiji Seika Kaisha, Ltd., 760 Morooka-cho, Kohoku-ku, Yokohama 222-8567, Japan*

Received 20 July 2007/Returned for modification 14 October 2007/Accepted 5 March 2008

**Horizontal transfer of the mutated *ftsI* gene from  $\beta$ -lactamase-nonproducing ampicillin-resistant (BLNAR) *Haemophilus influenzae* to a susceptible strain was examined in vitro under selection with nine oral  $\beta$ -lactams (ampicillin, amoxicillin, cefprozil, cefuroxime, cefpodoxime, cefdinir, cefcapene, cefditoren, and tebipenem). Compared to the penicillins and the carbapenem, the cephalosporins showed a wide selection window for the genetic transfer.**

# Orale cefalosporiner selekterer for PBP-mediert resistens

*Cephalosporins [...] showed broad concentration ranges for selecting the BLNAR variants [...].*

*Peak serum concentrations (about 1 to 2 g/ml) of most of the expanded-spectrum oral cephalosporins tested generally fall within or slightly above the selection window.*

*Therefore, most cephalosporins are likely to select BLNAR strains.*

Takahata et al. AAC 2008

# Hva med ESBL?

*Journal of Antimicrobial Chemotherapy* (2008) **61**, 509–514

doi:10.1093/jac/dkm523

Advance Access publication 1 February 2008

JAC

## Characterization of extended-spectrum $\beta$ -lactamase-producing isolates of *Haemophilus parainfluenzae*

Stephen G. Tristram<sup>1\*</sup>, Marthinus J. Pitout<sup>2</sup>, Karen Forward<sup>3</sup>, Sarah Campbell<sup>4</sup>,  
Scott Nichols<sup>1</sup> and Ross J. Davidson<sup>3,4</sup>

<sup>1</sup>*School of Human Life Sciences, University of Tasmania, Launceston, Tasmania 7250, Australia;* <sup>2</sup>*University of Pretoria, Pretoria, South Africa;* <sup>3</sup>*Dalhousie University, Halifax, Canada;* <sup>4</sup>*Queen Elizabeth II Health Sciences Centre, Halifax, Canada*

# Hva med ESBL?

*Given the high prevalence of TEM-1 beta-lactamase-positive strains of H. influenzae and H. parainfluenzae, and the widespread use of cephalosporins, it is surprising that TEM-derived extended-spectrum beta-lactamases (ESBLs) and associated extended-spectrum cephalosporin resistance have not emerged as they have in Enterobacteriaceae.*

Tristram et al, JAC 2008

# Hva med ESBL?

*It is possible that they have emerged and gone undetected, and without access to molecular methods, a routine laboratory could easily interpret the susceptibility profile of an IRT producing H. influenzae as a BLNAR or BLPACR strain.*

Tristram et al, CMR 2007

**Table 1.** MICs (mg/L) of cefotaxime for recombinant strains of *H. influenzae* Rd

Strain	Additional resistance mechanism				
	nil	TEM-1 <sup>a</sup>	TEM-3 <sup>a</sup>	TEM-4 <sup>a</sup>	TEM-5 <sup>a</sup>
Rd	0.06	0.06	1.0	0.5	0.12
BLNAR1 <sup>b</sup>	0.5	0.5	<b>4.0</b>	<b>4.0</b>	2.0
BLPACR4 <sup>b</sup>	1.0	1.0	<b>8.0</b>	<b>8.0</b>	2.0
BLNAR5 <sup>b</sup>	0.5	0.5	<b>4.0</b>	<b>4.0</b>	2.0
BLPACR7 <sup>b</sup>	0.25	0.25	<b>4.0</b>	<b>4.0</b>	1.0

CLSI susceptibility breakpoint for cefotaxime is  $\leq 2.0$  mg/L.

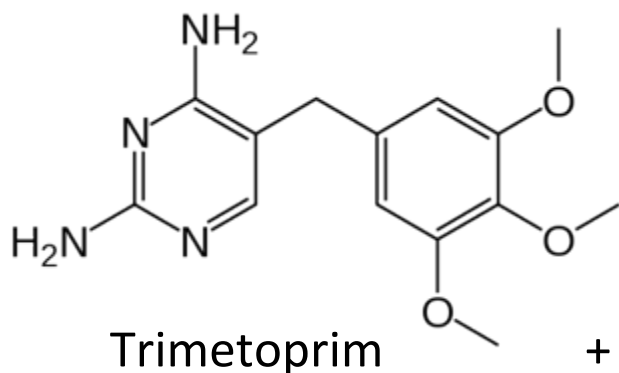
Strains categorized as resistant are in bold.

<sup>a</sup>As described by Tristram.<sup>5</sup>

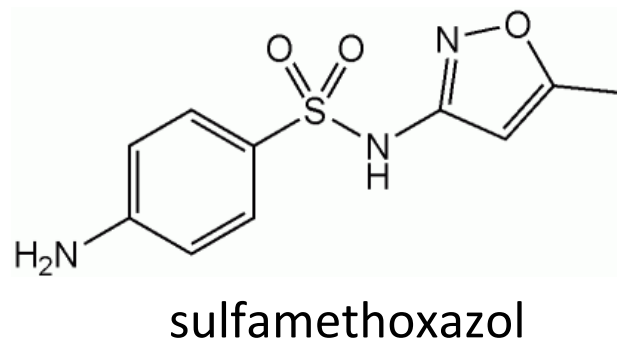
<sup>b</sup>As described by Matic *et al.*<sup>7</sup>

# Trimetoprim-sulfa (TMS)

- To midler som hemmer ulike trinn i folsyresyntesen



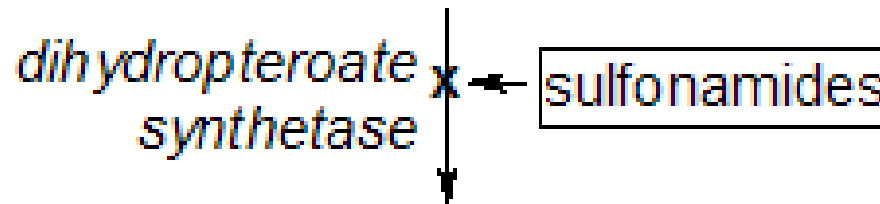
+



- Bakteriostatisk effekt
- Resistens oftest rettet mot trimetoprim
- Kromosomalt betinget overproduksjon av endret dihydrofolat reductase (DHFR)

# TMS - virkningsmekanisme

**dihydropteroate diphosphate + p-aminobenzoic acid (PABA)**

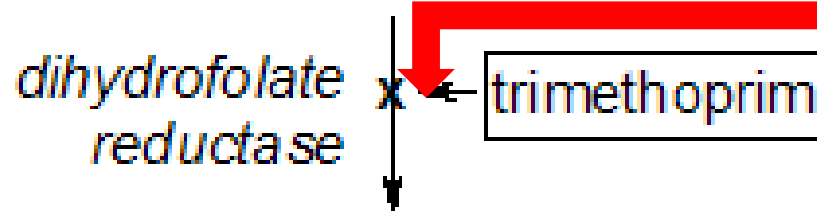


**dihydropteroic acid**



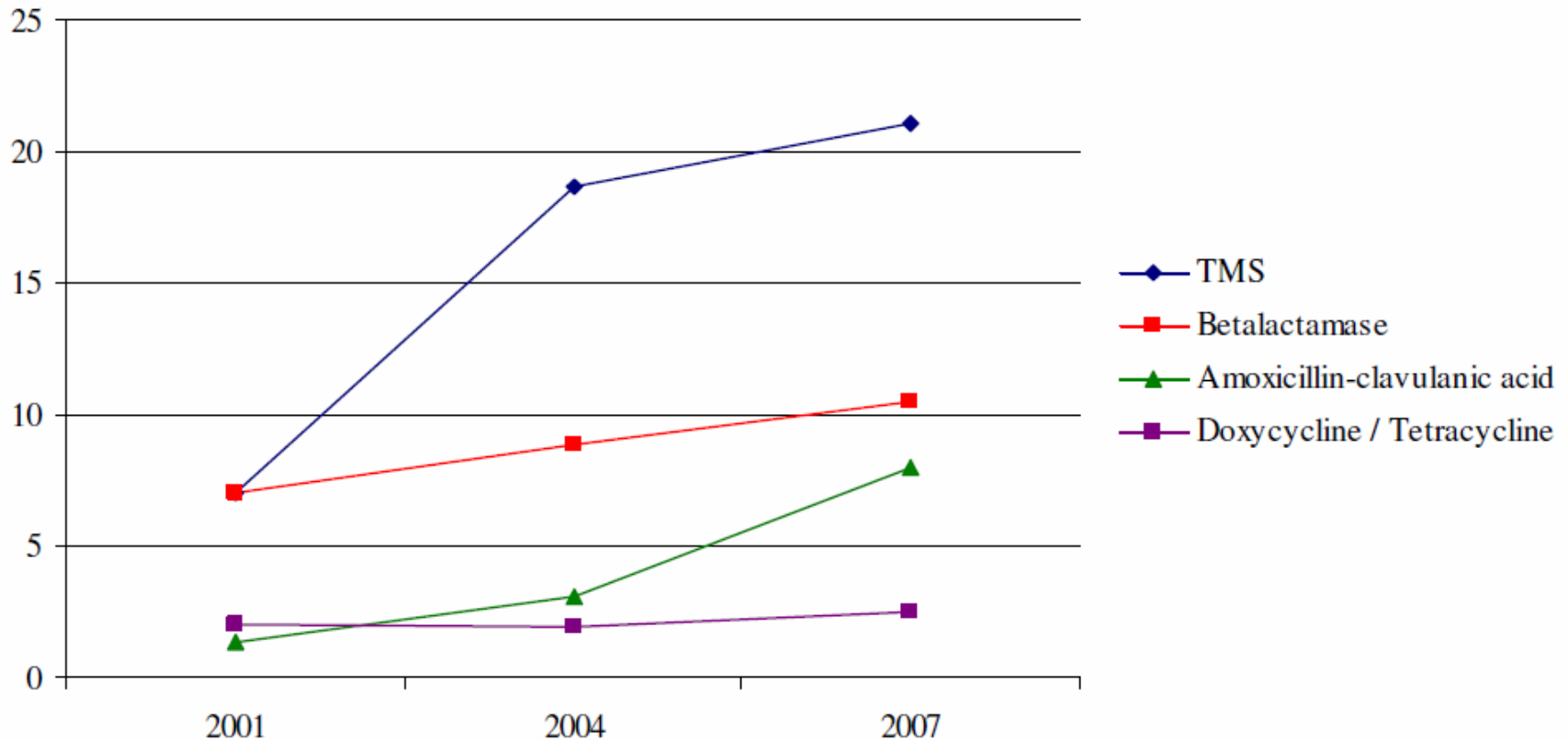
**dihydrofolic acid**

**Endret DHFR**



**tetrahydrofolic acid**

# NORM 2001 - 2007



**FIGURE 32.** Prevalence of non-susceptibility to various antimicrobials in *Haemophilus influenzae* respiratory tract isolates 2001-2007. Susceptibility testing was performed on PDM II agar in 2001 / 2004 and MH II agar in 2007, both supplemented with 1% haemoglobin and 1% IsoVitalex. Doxycycline ( $S \leq 4$  mg/L and  $R > 4$  mg/L) was substituted by tetracycline ( $S \leq 2$  mg/L and  $R > 2$  mg/L) in 2007.

# Kombinasjon med endret PBP3

NORM 2007:

*[...] the prevalence of non-susceptibility to trimethoprim-sulfamethoxazole was significantly higher among isolates with cefuroxime MICs > 4 mg/L (41.2%) than among isolates with cefuroxime MICs ≤ 4 mg/L (18.5%).*

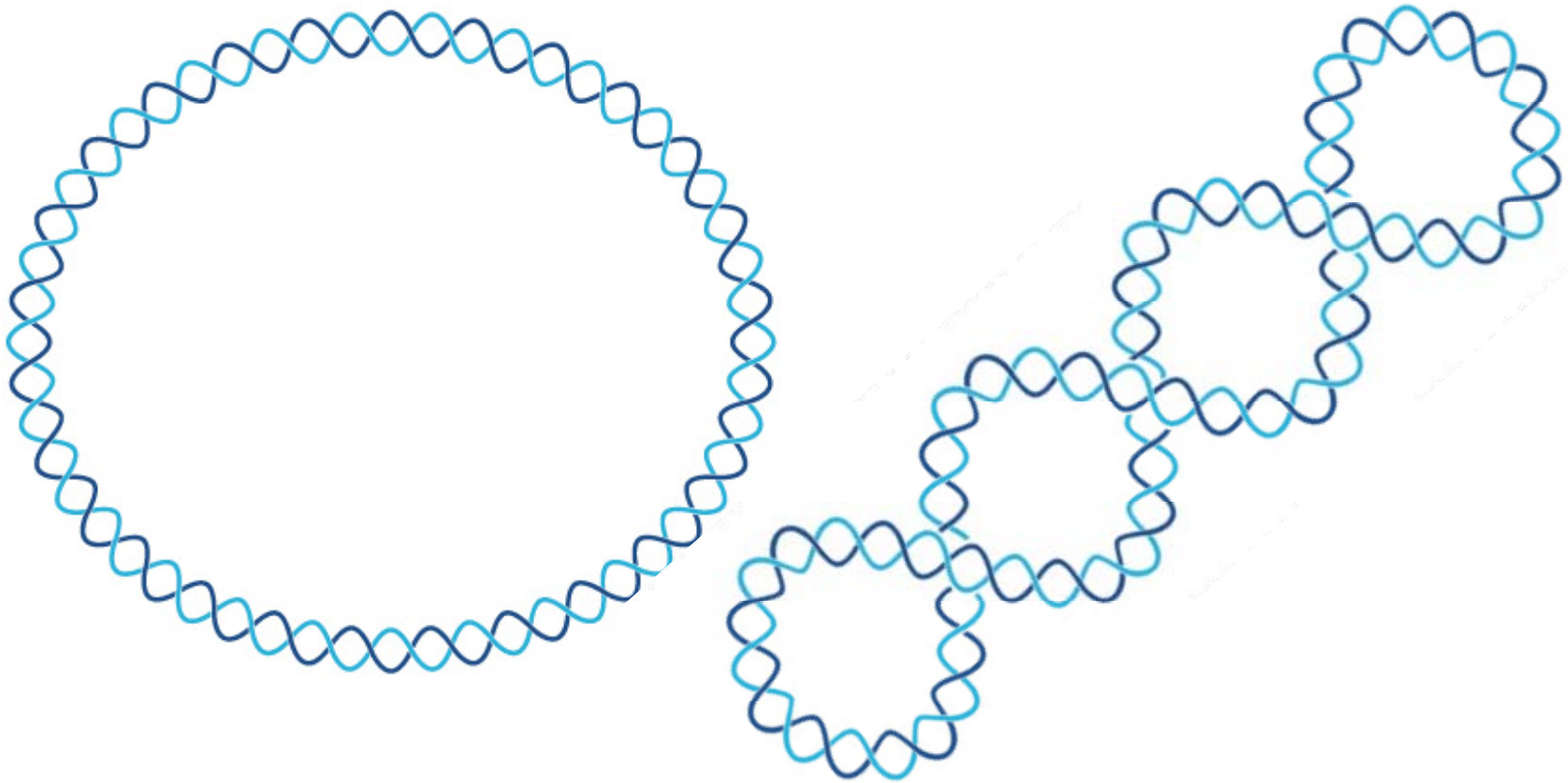
# Kinoloner

- Bredt antibakterielt spekter
- Kan gis peroralt og parenteralt
- Inndeles i generasjoner:
  - 1.gen: Nalidixin
  - 2.gen: Ciprofloxacin, ofloxacin, norfloxacin
  - 3.gen: Levofloxacin, moxifloxacin, gatifloxacin
  - 4.gen: Gemifloxacin
- Bactericid effekt
- Topoisomerasehemmere

# Topoisomeraser

- Enzymer som håndterer DNA supercoiling
- Nødvendige for DNA-syntese og –transkripsjon
- Kinoloner hemmer type II-topoisomeraser
  - DNA gyrase
  - Topoisomerase IV

# DNA supercoiling



# Kinolonresistens hos *H. influenzae*

- Endret DNA gyrase og topoisomerase IV
- Punktmutasjoner i QRDR (quinolone resistance determining region)
  - GyrA (Ser-84, Asp-88)
  - ParC (Ser-84, Glu-88)
- Resistens oppstår trinnvis og kan utvikles under pågående behandling

## Ciprofloxacin-Resistant *Haemophilus influenzae* Strains Possess Mutations in Analogous Positions of GyrA and ParC

MARIOS GEORGIU,<sup>1</sup> ROSARIO MUÑOZ,<sup>2</sup> FEDERICO ROMÁN,<sup>1</sup> RAFAEL CANTÓN,<sup>3</sup>  
 RAFAEL GÓMEZ-LUS,<sup>4</sup> JOSÉ CAMPOS,<sup>1\*</sup> AND ADELA G. DE LA CAMPA<sup>2</sup>

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H93/25	0.01	None	None
MAP	0.10	<sup>88</sup> D→Y (GAT→TAT)	None
24194	2.00	<sup>88</sup> D→N (GAT→AAT)	<sup>84</sup> S→I (AGT→ATT)
19594	2.00	None	None
0694	4.00	<sup>84</sup> S→L (TCC→TTA)	<sup>84</sup> S→I (AGT→ATC)
2495B	16.00	<sup>88</sup> D→N (GAT→AAT)	<sup>88</sup> E→K (GAA→AAA)
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R1 <sup>2495B-A</sup>	0.80	<sup>88</sup> D→N (GAT→AAT)	
R1 <sup>24194-C</sup>	2.00	<sup>88</sup> D→Y (GAT→TAT)	<sup>84</sup> S→I (AGT→ATT)
R2 <sup>0694-A/-C</sup>	4.00	<sup>84</sup> S→L (TCC→TTA)	<sup>84</sup> S→I (AGT→ATC)
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MAP	0.10	<sup>88</sup> D→Y (GAT→TAT)	None
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MAP	0.10	<sup>88</sup> D→Y (GAT→TAT)	None
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**2 GyrA- og  
 1 ParC-  
 substitusjon:  
 MIC = 32**

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# EUCAST brytningspunkter - kinoloner

Fluoroquinolone <sup>B</sup>		Species-related breakpoints (S</R>)									
		Entero- bacteriaceae <sup>C</sup>	Pseudo- monas/	Acineto- bacter	Staphylo- coccus	Entero- coccus	Strepto- coccus A,B,C,G	S.pneu- moniae <sup>F</sup>	Other strepto- cocci	<i>H.influenzae</i> <i>M.catarrhalis</i>	<i>N.gonorr- hoeae</i>
<a href="#">Ciprofloxacin</a>	<a href="#">RD</a>	0.5/1	0.5/1	1/1 <sup>D</sup>	1/1 <sup>E</sup>	--	--	0.125/2	--	0.5/0.5 <sup>G</sup>	0.03/0.06
<a href="#">Levofloxacin</a>	<a href="#">RD</a>	1/2	1/2	1/2	1/2	--	1/2	2/2	IE	1/1 <sup>G</sup>	IE
<a href="#">Moxifloxacin</a>	<a href="#">RD</a>	0.5/1	--	--	0.5/1	--	0.5/1	0.5/0.5	IE	0.5/0.5 <sup>G</sup>	IE
<a href="#">Norfloxacin</a>	<a href="#">RD</a>	0.5/1	--	--	--	--	--	--	--	--	IE
<a href="#">Ofloxacin</a>	<a href="#">RD</a>	0.5/1	--	--	1/1 <sup>E</sup>	--	--	0.125/4	--	0.5/0.5 <sup>G</sup>	0.12/0.25

G. Strains with MIC values above the S/I breakpoint are very rare or not yet reported. The identification and antimicrobial susceptibility tests on any such isolate must be repeated and if the result is confirmed the isolate sent to a reference laboratory. Until there is evidence regarding clinical response for confirmed isolates with MIC above the current resistant breakpoint (in italics) they should be reported resistant. *Haemophilus/Moraxella* - fluoroquinolone low-level resistance (ciprofloxacin MIC:s of 0.125 - 0.5 mg/L) may occur in *H.influenzae*. There is no evidence that low-level resistance is of clinical importance in respiratory tract infections with *H.influenzae*.

# Hypermutable og resistens

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0066-4804/07/\$08.00+0 doi:10.1128/AAC.01437-06  
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Vol. 51, No. 4

## Fluoroquinolone Resistance in *Haemophilus influenzae* Is Associated with Hypermutable<sup>∇</sup>

María Pérez-Vázquez,<sup>1</sup> Federico Román,<sup>1</sup> Silvia García-Cobos,<sup>1</sup> and José Campos<sup>1,2\*</sup>

*Antibiotic Laboratory, Bacteriology Service, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain,<sup>1</sup> and Consejo Superior de Investigaciones Científicas, Madrid, Spain<sup>2</sup>*

Received 17 November 2006/Returned for modification 22 December 2006/Accepted 26 January 2007

**Forty-three percent (12/28) of ciprofloxacin (CIP)-nonsusceptible respiratory isolates of *Haemophilus influenzae* were hypermutable, compared with 8.5% (3/35) in the CIP-susceptible control group ( $P = 0.002$ ). CIP-nonsusceptible mutants were obtained with hypermutable strains only; these mutants developed three resistance mechanisms in a step-by-step process: target modifications, loss of a porin protein, and increased efflux.**

# Hypermutableitet og resistens

*[...] hypermutability is a risk condition for the development of fluoroquinolone resistance in *H. influenzae*.*

*Since hypermutable isolates are particularly frequent in chronic respiratory infections, precautions are advised when treating these patients with fluoroquinolones.*

Pérez-Vazquez et al. AAC 2007

# Assosiert med endret PBP

- Corkill et al. JAC 1994:
  - Luftveisisolater av *H. influenzae* med nedsatt følsomhet for kinoloner (n=5)
  - Alle var betalaktamase negative og hadde nedsatt følsomhet for amoxicillin, lorakarbef og cefuroxim

# Screening med nalidixinsyre

**Table 1.** MIC, disc diffusion zone diameters and QRDR amino acid substitutions of *Haemophilus influenzae* NCTC 11931 and two clinical isolates

Isolate	MIC (mg/L)			Disc susceptibility zone diameter (mm)		Predicted QRDR amino acid changes	
	CIP	LVX	MOX	CIP (1 µg)	NAL (30 µg)	GyrA	ParC
NCTC 11931	0.015	0.015	0.015	35	31	<sup>80</sup> PHGDSAVYDTIVR <sup>92</sup>	<sup>80</sup> PHGDSACYEAMVL <sup>92</sup>
A1405	0.06	0.06	0.06	29	no zone	<sup>84</sup> S → F	none
A1012	2.0	2.0	1.0	13	no zone	<sup>84</sup> S → F	<sup>84</sup> S → R

CIP = ciprofloxacin; LVX = levofloxacin; MOX = moxifloxacin; NAL = nalidixic acid; S = serine; F = phenylalanine; R = arginine.

Brenwald et al, JAC 2003

# EUCAST Expert rules

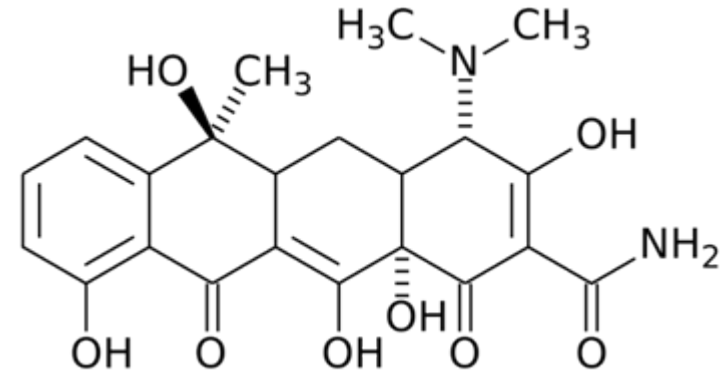
EUCAST Expert rules in antimicrobial susceptibility testing, version 1, April 2008

Table 5: Exceptional phenotypes of Gram-negative bacteria.

Rule no.	Organisms	Exceptional phenotypes
5.1	Any Enterobacteriaceae	Resistant to ertapenem, meropenem, imipenem (except <i>Proteus</i> spp.).
5.2	<i>Pseudomonas aeruginosa</i> and <i>Acinetobacter</i> spp.	Resistant to colistin.
5.3	<i>Haemophilus influenzae</i>	Resistant to any third-generation cephalosporin, carbapenems, fluoroquinolones.
5.4	<i>Moraxella catarrhalis</i>	Resistant to ciprofloxacin, any third-generation cephalosporin.
5.5	<i>Neisseria meningitidis</i>	Resistant to penicillin (MIC >1 mg/L), third generation cephalosporins, ciprofloxacin.
5.6	<i>Neisseria gonorrhoeae</i>	Resistant to third-generation cephalosporins, spectinomycin.

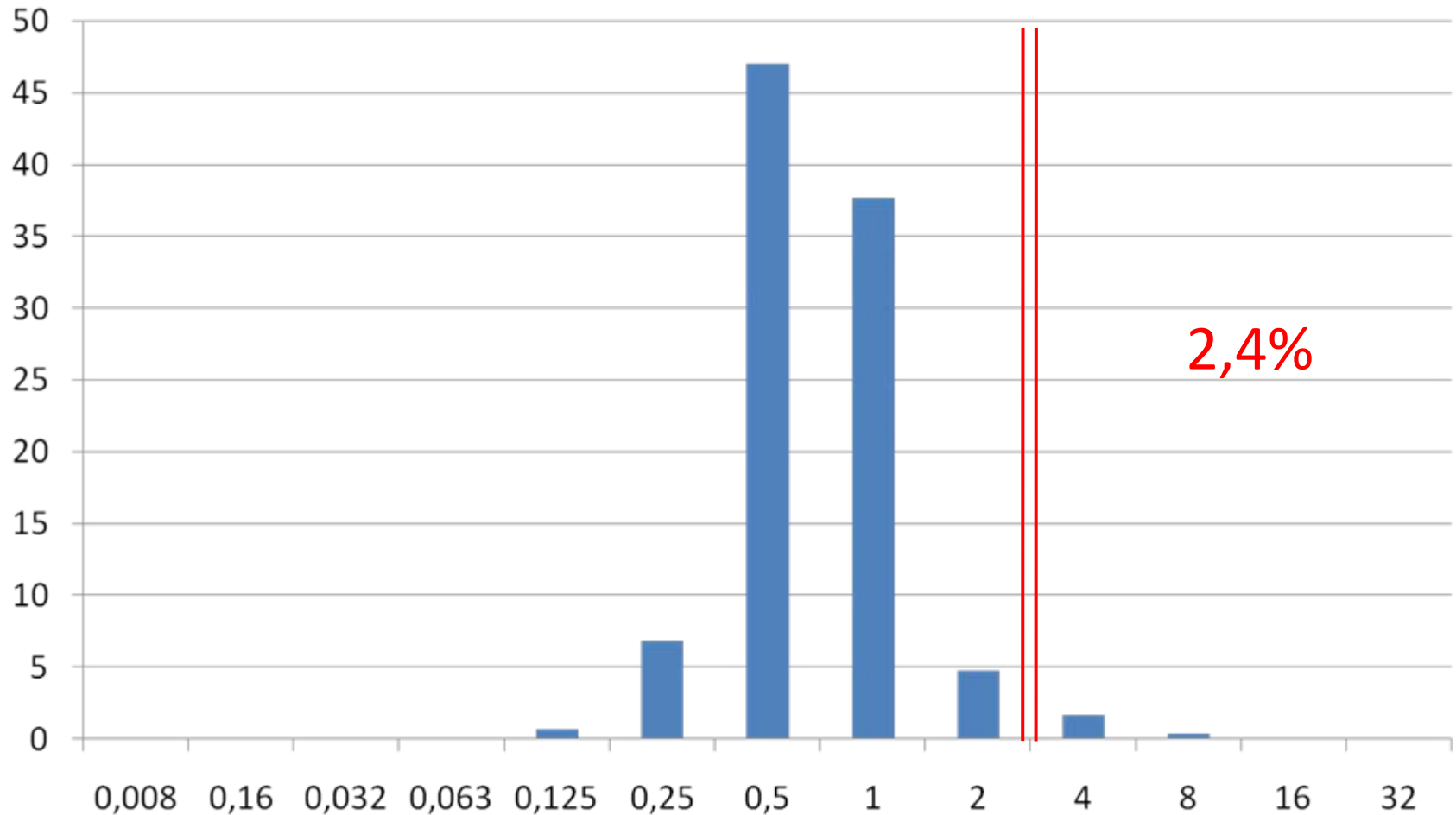
# Tetracycliner

- Fire hydrokarbonringer
- Bredt antibakterielt spekter
- Kan gis peroralt og parenteralt
- Proteinsyntesehemmere med bakteriostatisk effekt
- Resistens hos *H. influenzae* oftest effluksmediert
- Resistensgenet *tet(B)* overføres med konjugative plasmider



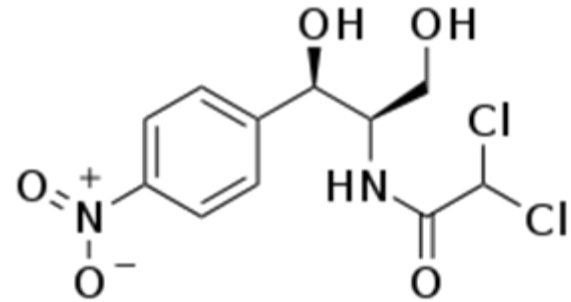
# Tetracyclin MIC-distribusjon

NORM 2007



# Kloramfenikol

- Lokalbehandling av øyeinfeksjoner
- Systemisk ved meningitt
- Proteinsyntesehemmer med bakteriostatisk effekt
- Resistens skyldes oftest inaktivering av kloramfenikol
- Genet *cat* overføres med et konjugativt plasmid
- Samme plasmid kan også overføre resistensgener mot tetracycliner og penicilliner



# Kloramfenikolresistens hos *H.i.*

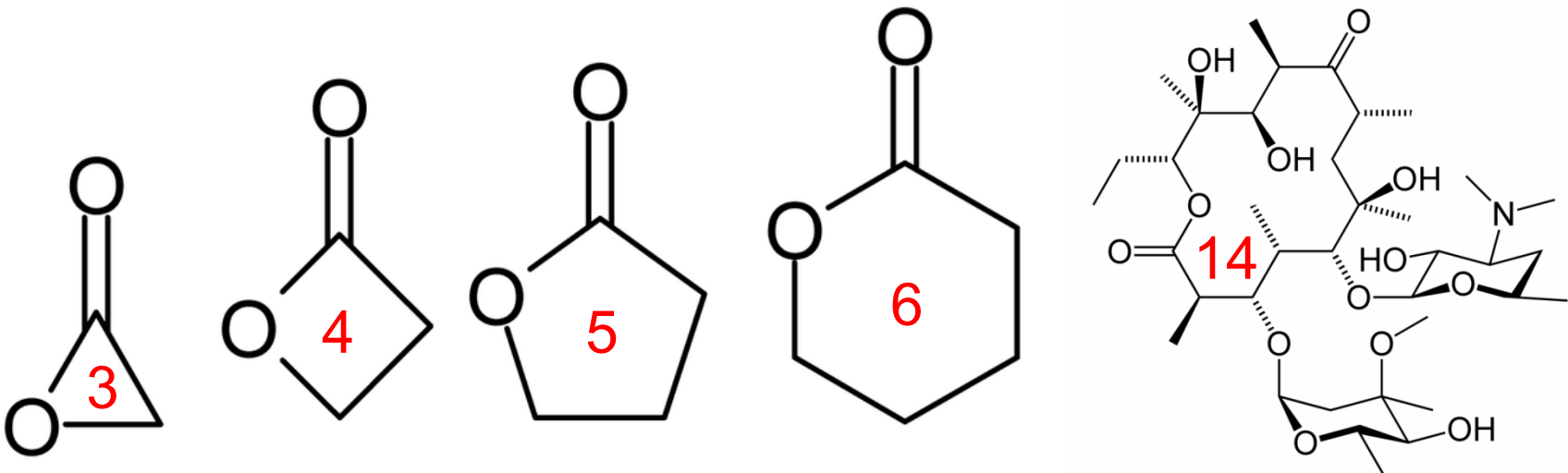
- Sykehuset i Vestfold 2008:
  - Totalt 48 *H. influenzae* isolater (hovedsakelig fra øyeseekret)
  - 1/48 kloramfenikol R
  - Resistent isolat hentet fra et barn med rørotitt
  - Også betalaktamase positiv, tetracyclin R og trim-sulfa R
  - Bærer av ACCoT-plasmid??  
**ACCoT**: Konjugativt plasmid kjent hos *Enterobacteriaceae* med resistens-gener mot **A**mpicillin, **C**hloramphenicol, **Co**-trimoxazol og **T**tetracyclin

# Kloramfenikolresistens hos *H.i.*

- Ladhani et al, JAC 2008:
  - Alle invasive *H. influenzae* i England and Wales 1985 – 2004 (n=6805; blod 72.5%, CSF 27.5%)
  - 54.9% Hib, 38.9% non-, 6.2% andre serotyper
  - 1.2% av isolatene kloramfenikol R
- Skoe et al, Tidsskr Nor Legeforen 2009:
  - 3.3% av Hib meningittisolater fra Sør-Trøndelag 1988-2007 (n=31) kloramfenikol R
- K Wendelbo, FHI, Årskonferansen 2007:
  - Systemiske isolater undersøkt ved FHI (n=61)
  - 22% (!) av invasive Hib isolert etter 1997 kloramfenikol R

# Makrolider

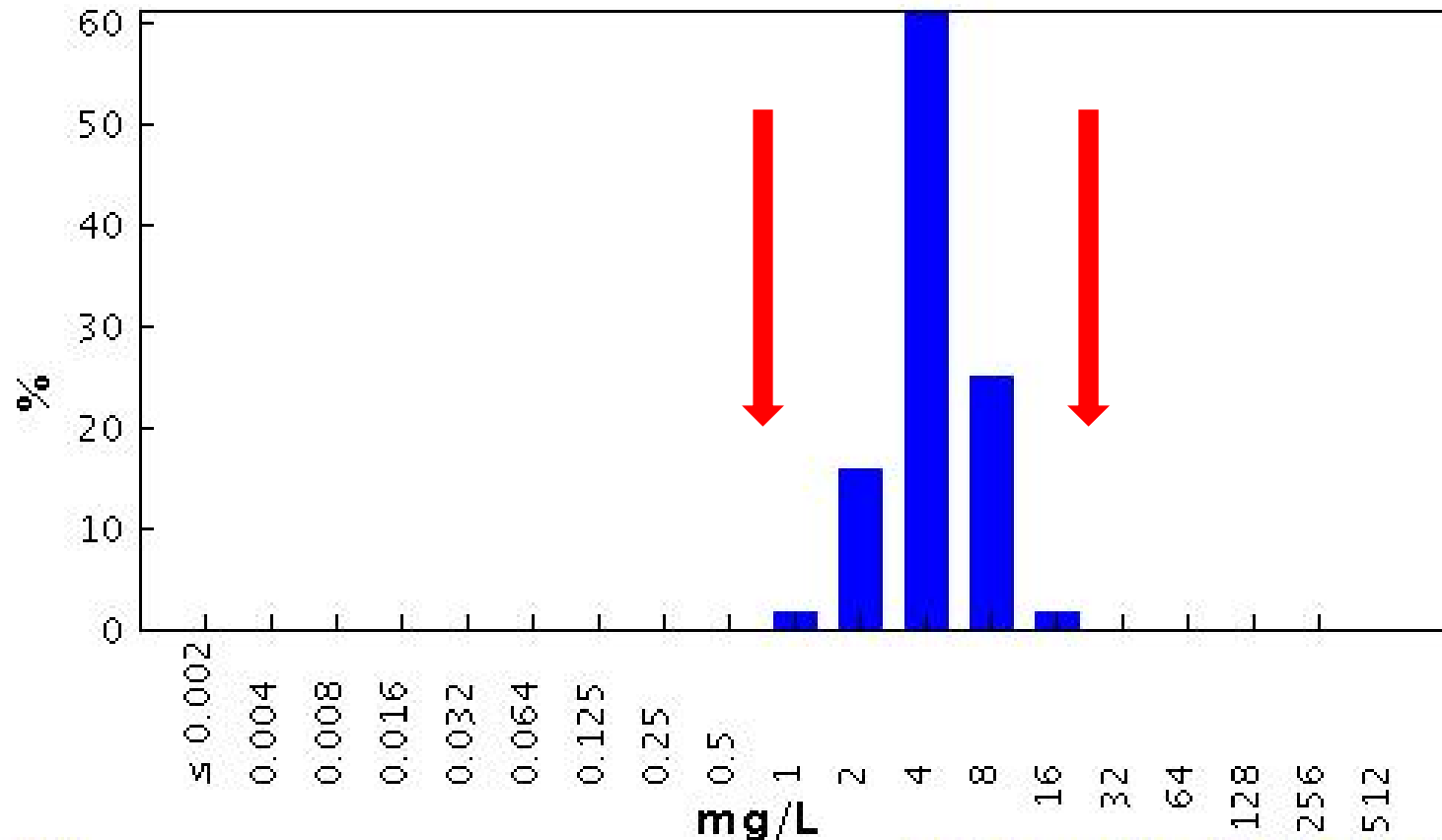
- Makrolider, azalider og ketolider
- Proteinsyntesehemmere med bakteriostatisk effekt
- *H. influenzae* naturlig resistent pga effluks
- Felles struktur: Laktonring (14-, 15- eller 16-)



# Erythromycin / Haemophilus influenzae

Antimicrobial wild type distributions of microorganisms - reference database

## EUCAST MIC Distribution



MIC  
Epidemiological cut-off: WT ≤ 16 mg/L

26246 observations (12 data sources)  
Clinical breakpoints: S ≤ 0.5 mg/L, R > 16 mg/L

# EUCAST Expert rules

EUCAST Expert rules in antimicrobial susceptibility testing, version 1, April 2008

**Table 3: Intrinsic resistance (R) in other Gram-negative bacteria**

These bacteria are also intrinsically resistant to glycopeptides, lincosamides, daptomycin and linezolid.

Rule no.	Organisms	Macrolides	Fusidic acid	Streptogramins	Trimethoprim	Nalidixic acid
3.1	<i>Haemophilus influenzae</i>	R	R			
3.2	<i>Moraxella catarrhalis</i>				R	
3.3	<i>Neisseria</i> spp.				R	
3.4	<i>Campylobacter fetus</i>		R	R	R	R
3.5	<i>Campylobacter jejuni/coli</i>		R	R	R	

# Oppsummering

- PBP-mediert betalaktamresistens øker både i omfang og grad. Orale cefalosporiner selekterer for resistens
- Resistens mot trimetoprim-sulfa øker. Forekommer hyppig i kombinasjon med endret PBP
- Resistens mot kinoloner er foreløpig sjelden. Trolig assosiert med PBP-mediert betalaktamresistens
- Plasmidmediert multiresistens (betalaktamer, tetracycliner, trim-sulfa, kloramfenikol) forekommer