Antimicrobial resistance in *Streptococcus pneumoniae* isolates from Belgian community acquired pneumonia, with special reference to efflux mechanism.

Ann Lismond

Pharmacologie cellulaire et moléculaire
Promoteurs: Pr. F. Van Bambeke, Pr. P. Tulkens
Why pneumonia?

Causes of death - standardised death rate, EU-27, 2010
(per 100 000 inhabitants)

Community-Acquired Pneumonia

• Leading cause of morbidity & mortality worldwide

• Mortality: <1% to ~48% associated to severity & risk factors

• Incidence varies depending on period of the year & age

• Long-term prognosis worst for pneumococcal CAP
Causative organisms:
Streptococcus pneumoniae

- Gram + cocci, non motile

- Polysaccharidic capsule (>90 serotypes) → target of current vaccine

- Extremely adaptive: naturally competent (transformation) & recombination

- Upper respiratory tract commensal (20-70% adults)
• **Cause of**
  - Mucosal infections: AOM, CAP, sinusitis, AECB…
  - Invasive infections: bacteraemia, sepsis, meningitis,…
Main antibiotic classes used against *S. pneumoniae*

*S. pneumoniae* resistance mechanisms:

- **β-Lactams**
  - Decreased affinity of PBP (mosaic genes)

- **Macrolides**
  - Ribosomal alteration:
    - Ribosomal methylation (23S)
    - Ribosomal mutation (23S domain V)
    - Mutation in riboproteins L4 or L22
  - Efflux: pumps MefA/E/I

- **Fluoroquinolones**
  - Mutations in Topoisomerase IV / DNA Gyrase
  - Efflux: pumps PmrA, PatA, PatB
Comparison of antibiotic resistance rates of *S. pneumoniae* in various countries
Comparison of antibiotic resistance rates of *S. pneumoniae* in various countries
Comparison of antibiotic resistance rates of *S. pneumoniae* in various countries
Belgium guidelines for initial oral empiric antibiotic therapy for outpatients with CAP (2008)

No risk factor:

- Allergy to Penicillin?
  - With risk factors: yes
  - Children: -CFX-axetil or -MXF

- AMX
  - + amoxiclav

If no improvement after 48h: + Macrolide

BAPCOC: Belgian Antibiotic Policy Coordination Committee (Belgium)
OBJECTIVES

• Pneumonia treatment optimal?
  – Antibiotics recommended ~ resistance ?
  – Vaccine coverage ~ prevalent serotypes ?
  – Interest of new molecules in development ?

• Fluoroquinolones active efflux:
  – Prevalence & clinical relevance?
  – Identification of transporter & Substrate specificity?
  – Expression & Inducibility?
Belgian collection of S. *pneumoniae*

Antimicrobial susceptibility of *Streptococcus pneumoniae* isolates from vaccinated and non-vaccinated patients with a clinically confirmed diagnosis of community-acquired pneumonia in Belgium

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f Clinique des maladies infectieuses, Hôpital Erasme, Brussels, Belgium
g Laboratoire de microbiologie, CHU Saint-Pierre, Brussels, Belgium
h Service des urgences, Cliniques universitaires Saint-Luc, Brussels, Belgium
i Laboratoire de microbiologie, Cliniques universitaires Saint-Luc, Brussels, Belgium
j Laboratoire de microbiologie, CHU Mont-Godinne, Yvoir, Belgium
General protocol

Patient with suspicion of pneumonia

Sampling for microbiology  Clinical examination, X-ray

Isolation of SP  CAP diagnostic

**Microbiology** (A. Lismond)

Analysis of the case
- Symptoms, severity
- X-ray
- AB: previous, current
- Contact of GP
- Reason(s) of referral to hospital

**Clinic:** clinical file (Dr. Carbonnelle)

Microbiological & clinical data are assembled (anonymous)

for 249 isolates, collected between 04/2007 and 03/2009
European Committee on Antimicrobial Susceptibility Testing (EUCAST)

MIC = Minimal Inhibitory Concentration

**Wild type (WT)**: organism characterized by the absence of acquired and mutational resistance mechanisms to the drug.

Wild type micro-organisms may or may not respond clinically to antimicrobial treatment.
Clinical resistance (EUCAST)

A micro-organism is defined as

• **Susceptible (S)** by a level of antimicrobial activity associated with a high likelihood of therapeutic success

• **Intermediate (I)** by a level of antimicrobial agent activity associated with uncertain therapeutic effect

• **Resistant (R)** by a level of antimicrobial activity associated with a high likelihood of therapeutic failure
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Antibiotics susceptibility

CLSI = Clinical and Laboratory Standards Institute
Susceptibility to β-Lactams:

- **Amoxicillin**: 3.2% R = ok
- **Cefuroxime (oral)**: 6.8% R = caution

Clinical breakpoint: EUCAST CLSI

Wild-type population (EUCAST)
Susceptibility to Macrolides:

23.7% R = NO

0.8% R = ok
Susceptibility to Fluoroquinolones:

100% S = Ok!
Belgium guidelines for initial oral empiric antibiotic therapy for outpatients with CAP (2008)

Allergy to Penicillin?

- With risk factors:
  - yes
    - - CFX-axetil or - MXF
      - + Macrolide

- no
  - + amoxiclav
    - High dose for AMX

If no improvement after 48h:

- + Macrolide
  - better than LVX
  - LVX: high dose!

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Pneumococcal vaccines

- >90 ST → 24 covered by vaccines

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<tr>
<td>23F</td>
<td>x</td>
</tr>
<tr>
<td>33F</td>
<td>x</td>
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</table>
Serogroups prevalence in collection

Total:
- 19
- 3
- 1
- 7
- 12
- 6
- 5
- 9
- 23
- 4
- 8

≥60y:
- 19
- 3
- 7
- 12
- 1
- 6
- 9
- 11
- 14
- 18
- 23
- 31
- 33

20-59y:
- 1
- 12
- 5
- 3
- 7
- 6
- 4
- 8
- 19
- 9
- 23
- 10

<5y:
- 19
- 7
- 1
- 6
- 3
- 5
- 22
- 23
- 33
- 9

SG frequencies (%)
Vaccines for children

N=29

<table>
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<th>Serotypes</th>
<th>PCV7</th>
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<tr>
<td>23F</td>
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Apparent coverage of SG: ~45%

PCV-7 theoretical coverage in <5y → ~17% of isolates

Vaccination status: ~60% yes, ~38% no

PCV-13 theoretical coverage in <5y → ~93% of isolates
Vaccines for adults

N=132

PPV-23 theoretical coverage in ≥60y → 58-87%

Vaccination status: ~20% yes, ~60% no, ~20% unknown

PCV-13 theoretical coverage in ≥60y → 55-67%

→ clinical trials to combine PCV13 & PPV23
Anibiotic non-susceptibility is mainly in SG-19, -1, -6, -14!
Percentage of non-susceptible strains in specific serogroups:

→ included in PPV23 and PCV13!
→ Protection from antibiotic resistant strains!

→ Strong association between serogroup & antibiotic non-susceptibility!
OBJECTIVES

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Interest of new molecules for CAP?

Macrolides:

Ketolides:

Telithromycin

Solithromycin

Interest of new molecules for CAP?

- TEL-R
- TEL-I
- TEL-S

**Efficacy and Safety Study of Intravenous to Oral Solithromycin (CEM-101) Compared to Intravenous to Oral Moxifloxacin in Treatment of Patients With Community-Acquired Bacterial Pneumonia**

- **Condition:** Community-acquired Bacterial Pneumonia
- **Interventions:** Drug: Solithromycin; Drug: Moxifloxacin

**Efficacy and Safety Study of Oral Solithromycin (CEM-101) Compared to Oral Moxifloxacin in Treatment of Patients With Community-Acquired Bacterial Pneumonia**

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Efflux pumps

- Transmembrane transporter proteins
- Ubiquitous mechanism
- **Purpose**: expulse toxic substrates out of the cell
  \[\rightarrow AB \text{ can be recognized as substrate} \]
- **Effect**: decrease AB concentration within cell
  \[\rightarrow \text{Low level resistance} \]

\[\text{less AB reach target}\]

\[\text{[PmrA]}\]

\[\text{[PatA/PatB]}\]
Efflux pumps

• Every bacterial genome has various pumps
• Same AB can be substrate of different pumps
• Narrow spectrum (Gram +) : 1 pump can recognize 1 AB class
• Substrate specificity varies within 1 AB class
Fluoroquinolones active efflux in *S. pneumoniae*

3 FQ transporters described:
- PmrA  (Gill *et al*. 1999)
- PatA   (Marrer *et al*. 2006)
- PatB   

Reserpine
Prevalence & clinical relevance?

N=183

Presence: 91%
Strong: 10%
Prevalence & clinical relevance?

N=183

Presence: 45%
Strong: 0 %
Prevalence & clinical relevance?

N=183

Presence: 39%  
Strong: 0 %
Prevalence & clinical relevance?

N=183

Gemifloxacin

Presence: 91%
Strong: 17%
Prevalence & clinical relevance?

Efflux presence? → YES!

Prevalence? → 39 to 91% depending on fluoroquinolone

Effect? → Modest << Strong: CIP (10%) & GEM (17%)

Clinical significance? → None for anti-pneumococcal fluoroquinolones (MXF, LVX & GEM) → MIC < bkpt
→ Risk of selection of resistant strains
OBJECTIVES

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  – Identification of transporter & Substrate specificity?
  – Expression & Inducibility?
Identification of transporter & substrate specificity

Pump? → PatA/PatB >> PmrA heterodimer

Specificity?

NOR >> GEM > LVX > MXF
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  – Expression & Inducibility?
Expression & Inducibility?

Over-expression?

→ Impact on MICs
→ Also in clinical isolates

<table>
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<tr>
<th>CIP</th>
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<th>SP-295</th>
<th>SP-13</th>
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<td>32</td>
<td>2</td>
<td>16</td>
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Impact on MICs

- Also in clinical isolates

PmrA PatA/B

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<tr>
<th>wt</th>
<th>wt</th>
<th>+</th>
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<tbody>
<tr>
<td>ATCC</td>
<td>lab mutants</td>
<td>clinical isolates</td>
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</tbody>
</table>
Expression & Inducibility?

Induction? → Only patA & patB
   → By all FQ tested, even if not substrates
   → In lab strains & in clinical isolates
      → Reversible
      → Time & dose dependant
CONCLUSIONS & PERSPECTIVES

• Pneumonia treatment
  – Continuing surveillance of antibiotics susceptibilities and serotypes distribution!
    → local & up-to-date epidemiology
  – Guidelines to review regularly:
    • Antibiotic resistance rate (CFX)
    • Availability of new molecules (SOL)
  – Vaccines:
    • Formulation to update regularly
    • Excellent coverage for children (PCV13)
    • PCV13 is ‘accepted’ for adults while should be ‘recommended’
    • PCV13 given earlier: from 50y
    • New vaccine independent from capsular polysaccharides?
CONCLUSIONS & PERSPECTIVES

• Fluoroquinolones active efflux:
  – Present but not clinically relevant (MXF, LVX)
  – Transporter = heterodimeric PatA/PatB (> PmrA)
  – Over-expression impacts MIC
  – Even non-substrates can induce over-expression
    → important for design of new molecules:
    non-substrates + non-inducers!

  – Can non-antibiotics induce over-expression?
  – In the clinics: can efflux be triggered by previous antibiotic treatment?
    → new collection from AECB
Thank you !