Activity and resistance to temocillin in *Pseudomonas aeruginosa* isolated from cystic fibrosis patients

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INTRODUCTION

Temocillin (TMO) is a semi-synthetic 6-α-methoxy derivative of ticarcillin (Fig.1). It is highly stable to most β-lactamases including AmpC-type cephalosporinases and extended-spectrum types (ESBLs) [1]. TMO was developed and first marketed in the UK by Beecham Pharmaceuticals in the 1980s. It shows a good safety profile, and its pharmacokinetic properties are similar to those of most other β-lactams, with however, a prolonged in vivo half-life and high area under the serum concentration curve (AUC).

Abandoned due to lack of activity against Gram-positive organisms, anaerobes and wild strains of *Pseudomonas aeruginosa*, TMO was recently reintroduced because of its activity against ESBL-producing Enterobacteriaceae [2, 3].

Recent studies from our laboratory showed that intrinsic resistance of *P. aeruginosa* to TMO was due to active efflux by the constitutively-expressed transporter MexAB-OprM. Yet, some strains of *P. aeruginosa* isolated from cystic fibrosis patients regain susceptibility to TMO because of natural mutations in the proteins constituting this efflux system [4].

OBJECTIVES

- to evaluate the activity of TMO and antipseudomonal β-lactams against an international collection of *P. aeruginosa* from cystic fibrosis (CF) patients.
- to determine the resistance mechanisms behind the poor susceptibility to TMO.

METHODS

Strains of *P. aeruginosa* from CF patients

- ✓ 99 strains kindly provided by Dr M. Tunney, The Queen's University of Belfast, United Kingdom.
- ✓ 88 strains kindly provided by Drs A. Vergison / O. Denis, Hôpital Erasme, Brussels, Belgium.
- ✓ 80 strains kindly provided by Dr P. Plésiat, Laboratoire de bactériologie, Hôpital Jean Minjoz, Besançon, France.
- ✓ 68 strains kindly provided by Dr B.C. Kahl, University Hospital Münster, Münster, Germany.

Antibiotics used

Temocillin (TMO) : Commercially available temocillin disodium salt, was procured as the clinical form of the corresponding branded product, Negaban® from EUMEDICA S.A., Manage, Belgium. Ticarcillin (TIC): Ticarcillin disodium salt (Sigma-Aldrich, Belgium). Meropenem (MEM): Meropenem trihydrate (Meronem IV, AstraZeneca, United States).

Antibiotic susceptibility testing

Minimal Inhibitory Concentrations MICs were determined by microdilution in cation-adjusted Muller Hinton broth, following CLSI recommendations [5]. Susceptibility was established based on European Committee for Antimicrobial Susceptibility Testing (EUCAST) criteria [6]. The correlation between MICs of TMO and MEM against individual strains was examined using quantile density contour analysis (JMP® versions 10.0.2, SAS Institute Inc, Cary, NC).

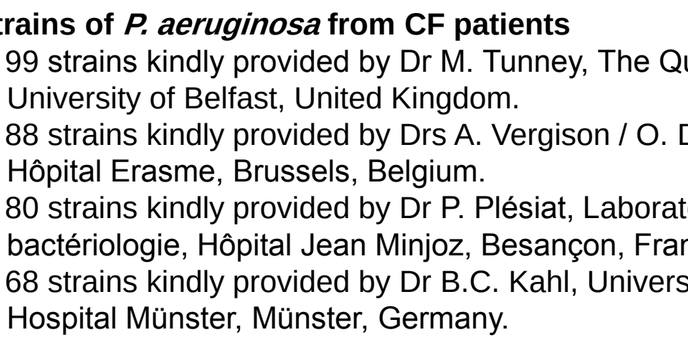
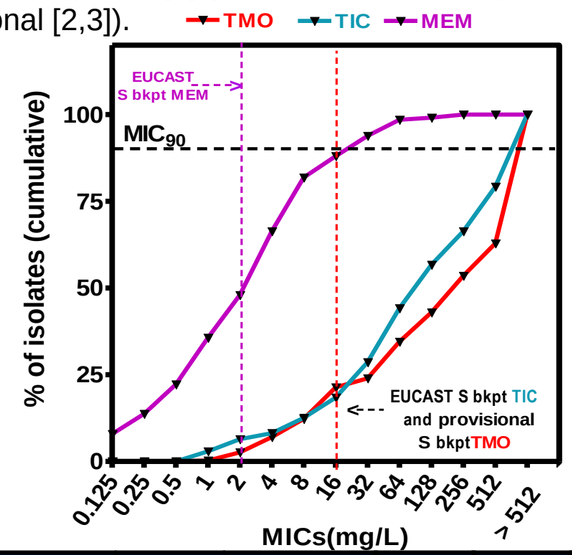


Figure 1 shows the structure of temocillin (6-alpha-methoxy-ticarcillin). The red arrow indicates the methoxy group that plays an important role in resistance to hydrolysis by β-lactamases.

RESULTS

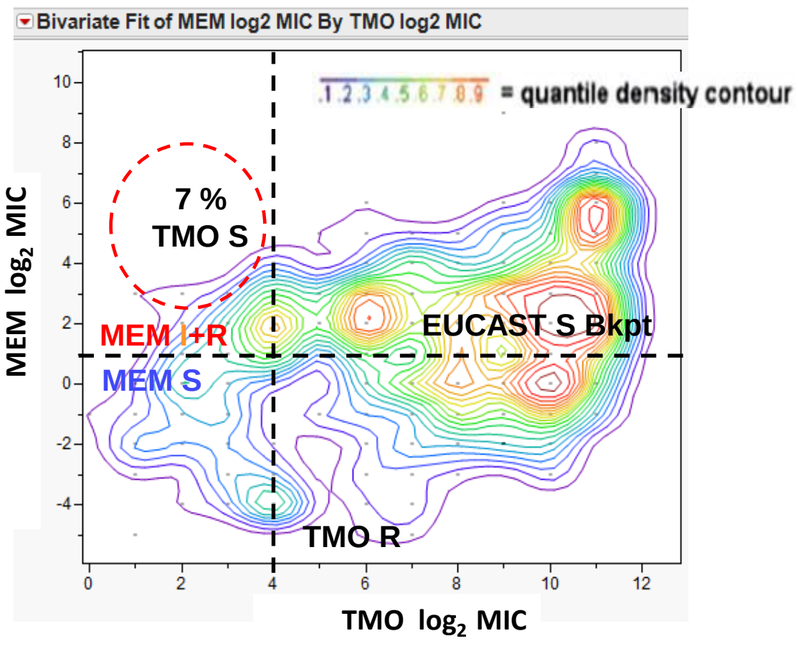
Figure 2 : Cumulative percentages of MIC distribution for TMO vs.TIC and MEM as compared to EUCAST susceptibility breakpoints [6] (note that TMO breakpoint is still provisional [2,3]).



β-lactam	MIC_{90}	MIC Range	% S	% R
Temocillin (TMO)	> 512	1 - >1024	22	78
Ticarcillin (TIC)	> 512	1 - >512	19	81
Meropenem (MEM)	16	0.016-256	48	52

- ➢ TMO was as active as TIC, its parent compound, which is considered as a potential therapeutic option against *P. aeruginosa* , with 22% vs. 19 % of the strains displaying an MIC ≤16 mg/L.
- ➢ MEM shows lower MIC values and higher proportion of susceptible strains.

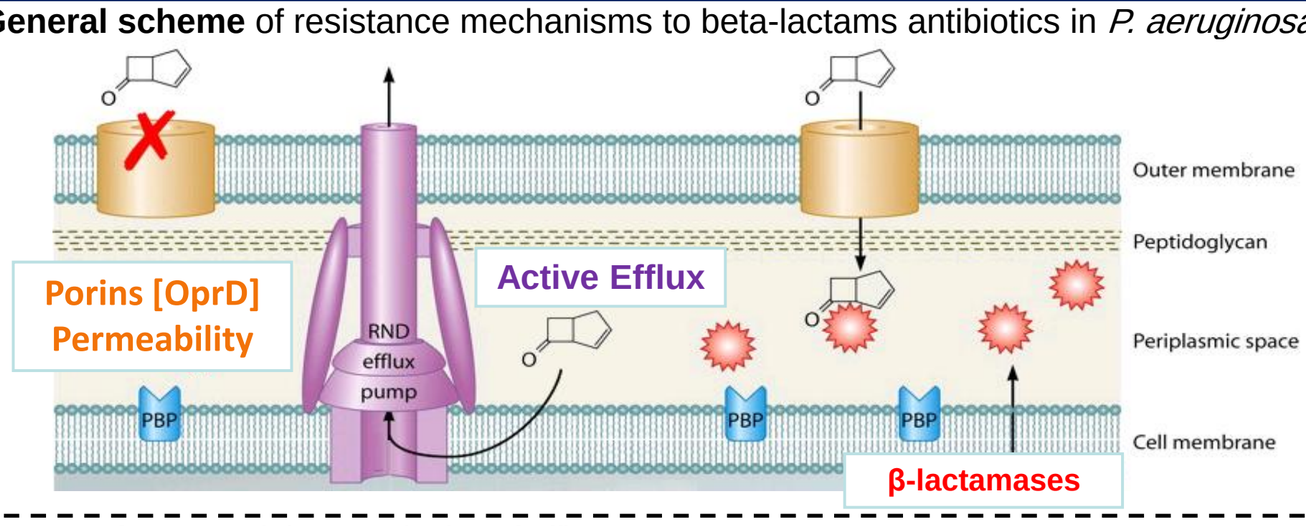
Figure 3 : Correlation between MICs of individual strains for TMO vs. MEM using quantile density contour analysis.



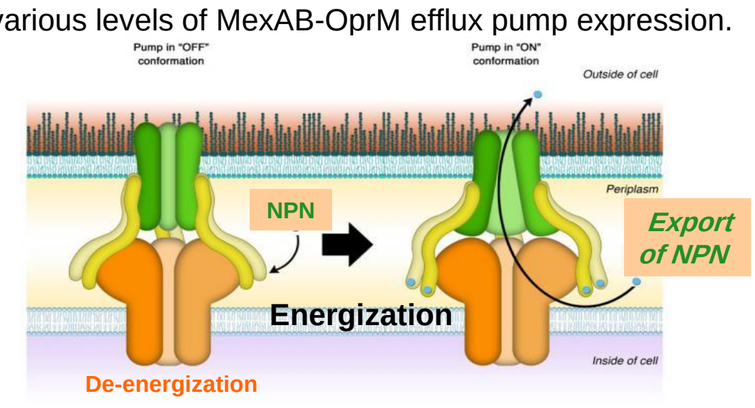
- ➢ **The color gives information on the proportion of strains in each zone of the diagram.**
- ➢ **A small proportion of isolates are more susceptible to TMO than to MEM (7%) , possibly due to the expression of resistance mechanisms that do not affect TMO.**

PERSPECTIVES

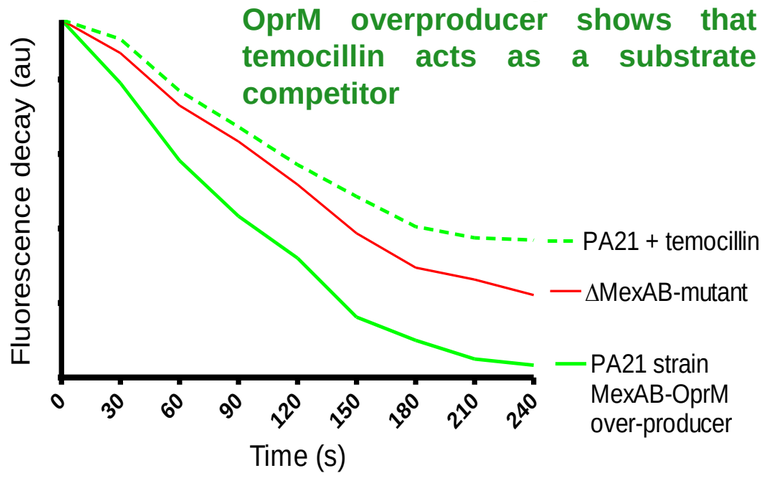
General scheme of resistance mechanisms to beta-lactams antibiotics in *P. aeruginosa* [7].



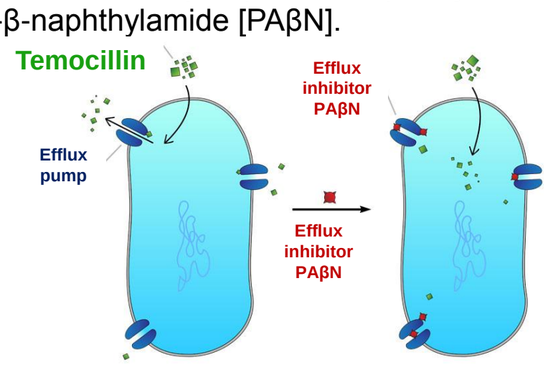
Perspective 1: Real-time monitoring of efflux systems functionality using N-Phenylnaphtylamine (NPN) as a fluorophore in *Pseudomonas* strains displaying various levels of MexAB-OprM efflux pump expression.



➢ **NPN Efflux Assay in a MexAB-OprM overproducer shows that temocillin acts as a substrate competitor**

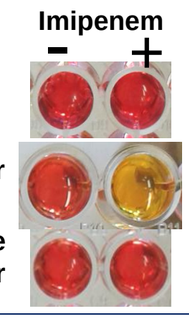


Perspective 2: Temocillin susceptibility pattern in the presence of the efflux pump inhibitor phenylalanine-arginine-β-naphthylamide [PAβN].

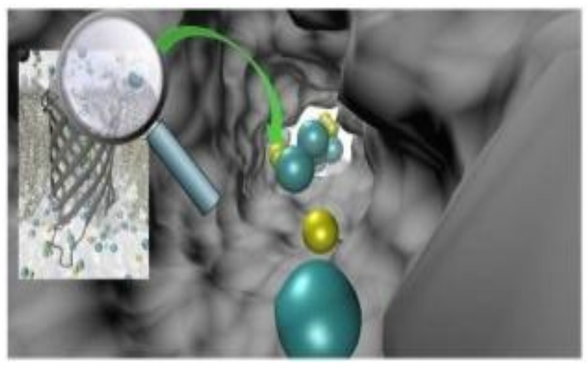


Adapted from Jason Sello , Brown university, 2011

Perspective 3: Genotypic and phenotypic screening of β-Lactamases including carbapenemases (NDM, OXA-48, IMP, KPC, VIM), AmpC-type cephalosporinases and extended-spectrum types (ESBLs) using PCR & the Carba/ESBL NDP tests [8, 9].



Perspective 4: Structure-function relationship of membrane channels OprD at Jacobs University Bremen.
Our cooperation partner Professor M. Winterhalter applies the planar lipid bilayer (PLB) technique which allows to study the influx of temocillin and carbapenems through wild-type or altered OprD porins, by reconstituting porin monomer into planar lipid bilayers then recording the time dependent conductance. The study of ion current noise during the penetration of antibiotics into the channel allows conclusion on the mode of permeation.



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Acknowledgements

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