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MIC distribution and efflux of 4 respiratory fluoroquinolones (GEM, GRN, MXF, LVX) towards *Streptococcus pneumoniae* isolated from patients with confirmed CAP in a country with large fluoroquinolone use (Belgium).

A. Lismond, S. Carbonnelle, F. Van Bambeke, P.M. Tulkens - Unité de Pharmacologie cellulaire et moléculaire, Université catholique de Louvain, Bruxelles, Belgium

Mailing address:
P. M. Tulkens
UCL 73.70 av. Mounier 73
1200 Brussels - Belgium
tulkens@facm.ucl.ac.be

ABSTRACT

Background: Belgium is a country with one the largest fluoroquinolones use in Europe (2.36 DDD per 1,000 inhabitants and per day in ambulatory care, based on 2006 ESAC data [http://www.esac.ua.ac.be]). We have examined the MIC distribution and the influence of reserpine (efflux inhibitor) for MXF and LVX (in clinical use since > 6 years in comparison with GEM and GRN (not yet approved) in a collection of *S. pneumoniae* (SP) isolated from patient with confirmed CAP.

Methods: 134 SP first isolates were collected over the 2004-2008 period from patients received in 6 hospitals and for whom clinical data were consistent with a diagnostic of CAP (ATS criteria). MICs were determined by semi-geometric microdilution in CAMH broth + 2.5% lysed horse blood, with or without reserpine (R; 10 mg/L). Susceptibility was assessed according to EUCAST breakpoints for MXF and LVX.

Results:

FQ	MIC distribution geom. mean -R	MIC distribution geom. mean +R	MIC 90 -R	MIC 90 +R	EUCAST Bkpt	EUCAST % S -R	EUCAST % S +R	Efflux max (dil x)	% Efflux for 1 dil°	% Efflux for ≥ 2 dil°
LVX	0.83	0.71	1	1	2	97.8	98.5	1	3.7	0
MXF	0.16	0.14	0.25	0.25	0.5	98.5	98.5	1	27.6	0
GRN	0.04	0.03	0.06	0.06				2	19.4	0.7
GEM	0.03	0.01	0.06	0.03				3	83.6	17.2

MIC50 and MIC90 of GEM and GRN were much lower than those of MXF and LVX. Efflux (as detected by R) was demonstrated for GEM only (and for a small proportion of isolates) if using a 2-fold dilution decrease criterion, but also MXF, GRN and for almost all isolates for GEM if using a 1-fold dilution increase criterion.

Conclusions: The MICs of LVX support the "high dose" recommendation on which its current EUCAST breakpoint is based, and are at least 3-fold larger than those seen in the original study that established the usefulness of the 500 mg dose (geometric mean: 0.25 mg/L; Preston et al., JAMA 1998;279:125-9). MXF MICs remain under EUCAST breakpoint. Efflux is evident for GEM (in the absence of specific selection pressure) and likely for MXF and GRN but with minor impact on MIC values.

Background and Aim

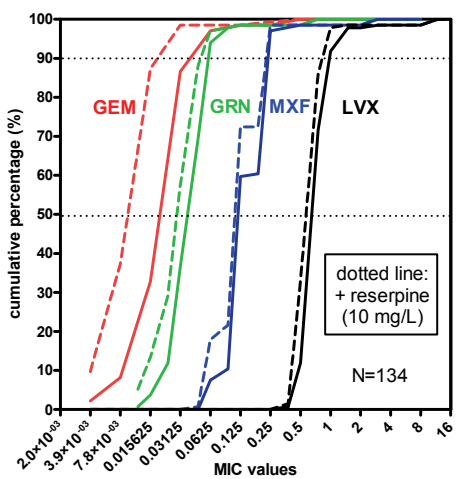
- *Streptococcus pneumoniae* is the most frequent pathogen associated with respiratory tract infections, including community-acquired pneumonia (CAP). The incidence of *S. pneumoniae* isolates with reduced susceptibility to antibiotics is globally increasing worldwide.
- Belgium is a country with one the largest fluoroquinolones use in Europe (2.36 DDD per 1,000 inhabitants and per day in ambulatory care), based on 2006 ESAC data,[1] with levofloxacin (LVX) and moxifloxacin (MXF) being in clinical use for more than 6 years for respiratory tract infections.
- An often raised question is, therefore, whether this use large use of LVX and MXF has been associated with significant emergence of resistance of *S. pneumoniae* to these fluoroquinolones and to others not yet in clinical use such as garenoxacin (GRN) or gemifloxacin (GEM) (neither approved in EU).
- Resistance to quinolones may be due not only to mutation in the topoisomerase genes but also to efflux (often neglected but resulting in suboptimal therapies).[2]

Our aim was

- to evaluate the current level of susceptibility of *S. pneumoniae* isolated from CAP patients towards LVX, MXF (in current use) and GRN and GEM (not used);
- to establish the prevalence of efflux-mediated resistance in the community.

Results

A Cumulative MIC distribution for 134 isolates of *S. pneumoniae* towards fluoroquinolones with and without reserpine



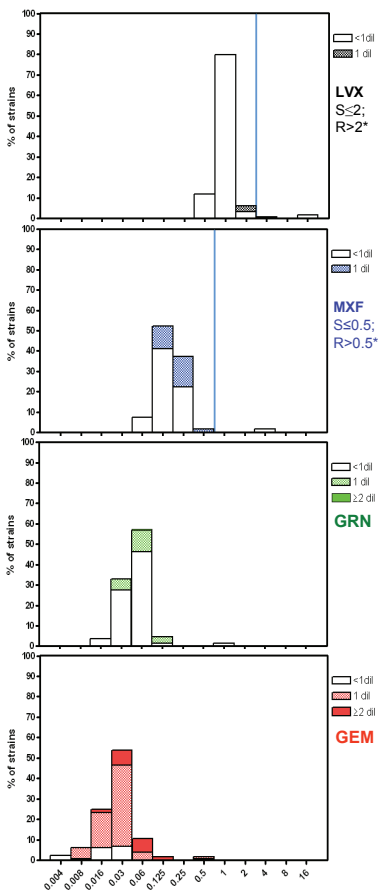
	LVX Res (-)	LVX (+)	MXF (-)	MXF (+)	GRN (-)	GRN (+)	GEM (-)	GEM (+)
MIC_{50} (mg/L)	0,75	0,75	0,125	0,125	0,047	0,031	0,031	0,016
MIC_{90} (mg/L)	1	1	0,25	0,25	0,063	0,063	0,063	0,031
max (mg/L)	12	12	3	3	0,75	0,75	0,5	0,5
EUCAST bkpts S ≤	2		0,5					
EUCAST bkpts R >	2		0,5					
S:	97,8 %	98,5 %	98,5 %	98,5 %				
R:	2,2 %	1,5 %	1,5 %	1,5 %				

Main observations:

- MIC_{50} and MIC_{90} are GEM < GRN < MXF < LVX (below current EUCAST breakpoint for MXF and LVX)
- Efflux can be demonstrated for almost all isolates for GEM if using a 1-fold dilution increase criterion
- efflux is infrequent for GRN and MXF, and exceptional for LVX.

B Prevalence of efflux according to MIC distribution

(blue vertical line: EUCAST bkpt)



Methods

Bacteria: 134 strains of *S. pneumoniae* isolated over the 2004-2008 period from patients admitted in 6 Belgian hospitals, with a diagnosis of CAP (confirmed by an in-depth analysis of the medical file).

Susceptibility testing: MICs determined by microdilution following CLSI recommendations, with *S. pneumoniae* ATCC 49619 used as a quality control. Susceptibility assessed according to EUCAST breakpoints for LVX and MXF.

Resistance due to active efflux: evidenced by determining the effect of an efflux pump inhibitor (reserpine, 10mg/L) on the MICs of LVX, MXF, GRN and GEM.[4]

Conclusions

- The current MICs of LVX support the "high dose" recommendation on which its current EUCAST breakpoint is based, and are at least 3-fold larger than those seen in the original study that established the usefulness of the 500 mg dose (geometric mean: 0.25 mg/L).[5]
- For MXF, MICs remain under EUCAST breakpoint to 98.5 % (based on a dosage of 400 mg/day).
- GRN and GEM MICs are very low (max. 0.75 and 0.5 mg/L, respectively).
- Efflux is evident for GEM (in the absence of specific selection pressure since the drug is not used) and likely for MXF and GRN, but with minor impact on MIC values.

References

1. 2006 ESAC data [http://www.esac.ua.ac.be]
2. Adam *et al.* Molecular characterization of increasing fluoroquinolone resistance in Streptococcus pneumoniae isolates in Canada, 1997 to 2005. Antimicrob Agents Chemother. 2007; 51:198-207
3. Lismond *et al.* Epidemiological survey of susceptibility to β-lactams (AMX, CFX, CRO), macrolides (CLR, TEL), and fluoroquinolones (LVX, MXF) in a Belgian collection of CAP isolates of Streptococcus pneumoniae (SP). 18th ECCMID 19-22 April 2008; P-1747.
4. Piddock *et al.* Activities of new fluoroquinolones against fluoroquinolone-resistant pathogens of the lower respiratory tract. Antimicrob. Agents Chemother. 1998; 42:2956–2960.
5. Preston *et al.* Pharmacodynamics of levofloxacin: a new paradigm for early clinical trials. JAMA. 1998 Jan 14;279(2):125-9.

A copy of this poster will be made available after the meeting at http://www.facm.ucl.ac.be/posters.htm