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# Efflux of novel quinolones in contemporary *Streptococcus pneumoniae* isolates from community-acquired pneumonia

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Sir,

Quinolones with enhanced activity against *Streptococcus pneumoniae* are included as a treatment option for community-acquired pneumonia in therapeutic guidelines from both North America and Europe,[1,2] and epidemiological surveys show that resistance to levofloxacin or moxifloxacin remains low even with large usage of these antibiotics.[3] Yet, *S. pneumoniae* harbours efflux transporters for quinolones[4,5] that may reduce the susceptibility of clinical isolates in a manner that will remain undetected if reporting is based only on the interpretative criteria proposed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) or the US CLSI. While efflux in *S. pneumoniae* seems to primarily affect ciprofloxacin and norfloxacin (which are not recommended for treating infections caused by *S. pneumoniae*), much less is known about the susceptibility of novel quinolones to these transporters in current clinical isolates.

In the present study, we collected 183 non-duplicate isolates from patients with confirmed clinical and radiological diagnosis of community-acquired pneumonia during the 2007–09 period. We measured the MICs of ciprofloxacin, levofloxacin, moxifloxacin and the two new quinolones garenoxacin and gemifloxacin for these isolates. We followed exactly the CLSI methodology except that we used 0.5 $\log_2$ concentration increments to reduce the intrinsic 1 $\log_2$ dilution error associated with the conventional methods of MIC determinations, and performed the determinations in the presence or absence of reserpine (10 mg/L; commonly used to detect the efflux-mediated decrease in susceptibility of *S. pneumoniae* to quinolones).[6] The results are shown in the left-hand panels of Figure 1. In the absence of reserpine, median MICs were 1 mg/L of ciprofloxacin, 0.75 mg/L of levofloxacin, 0.125 mg/L of moxifloxacin, 0.047 mg/L of garenoxacin and 0.012 mg/L of gemifloxacin [see Table S1, available as Supplementary data at *JAC* Online, for more numerical data (MIC range, $MIC_{50}$ and $MIC_{90}$)]. All strains should be considered as susceptible to levofloxacin and moxifloxacin (using either the EUCAST or CLSI breakpoints) and also to gemifloxacin for 181/183 strains (using the CLSI breakpoint; no EUCAST breakpoint defined). In the presence of reserpine, the MIC distributions of ciprofloxacin, garenoxacin and gemifloxacin were markedly shifted towards lower values, with median values lowered by 1 $\log_2$ dilution for ciprofloxacin and gemifloxacin, and 0.5 $\log_2$ dilution for garenoxacin. In contrast, only minor shifts in distribution were seen for levofloxacin and moxifloxacin. To get further insight into the impact of efflux on the decrease in bacterial susceptibility to each quinolone, we calculated the MIC change for each isolate (by decrements of 0.5 $\log_2$ dilutions) and present the results as a function of the original MIC (without reserpine) in the right-hand panels of Figure 1. For ciprofloxacin, 93.4% of the strains had an MIC $\geq$0.75 mg/L, with 29.2% of these showing a difference of more than 1 $\log_2$ dilution upon exposure to reserpine. For gemifloxacin, reserpine caused an increase in susceptibility of $\geq$1 $\log_2$ dilution in 65% of the isolates with a basal MIC (in the absence of reserpine) $\geq$0.006 mg/L. For garenoxacin, the susceptibility of 60% of the isolates was increased in the presence of reserpine (this was seen whatever the basal MIC), but the effect rarely exceeded 1 $\log_2$ dilution. For moxifloxacin and levofloxacin, increases in susceptibility were seen for 39% and 45% of the isolates, respectively, but affecting mainly the strains with a corresponding basal MIC $\geq$0.188 mg/L (moxifloxacin) or $\geq$0.75 mg/L (levofloxacin). The shift was $<$1 $\log_2$ dilution in 59% of the isolates for moxifloxacin and in 86% for levofloxacin.

The data strongly suggest that gemifloxacin and ciprofloxacin are both subject to efflux in *S. pneumoniae*. Of interest is the fact that gemifloxacin has so far not been used in Europe and could, therefore, not have triggered its own efflux. Ciprofloxacin has never been included in therapeutic recommendations for treatment of streptococcal infections in Belgium. We may suspect that it is its wide use for other indications that has triggered the emergence of *S. pneumoniae* strains capable of developing efflux-mediated resistance to ciprofloxacin through repeated exposure to subinhibitory concentrations of this antibiotic.[6] It is ironic that this affects gemifloxacin, a not-yet-used but potentially very active antibiotic, even though not all isolates were positive in our assay. Since efflux is known to facilitate the selection of first-step mutants amongst fluoroquinolone-susceptible organisms, our data must be taken as a warning should gemifloxacin be introduced on a wide scale in therapeutics. In a more general context, and based on the observation that strains with efflux may be quite frequent, surveillance studies for the detection of new variants of efflux transporters affecting levofloxacin and moxifloxacin may be warranted. This could have a direct clinical significance if those strains, as recently suggested,[5] were also to show mutations or other low-level mechanism(s) of resistance.

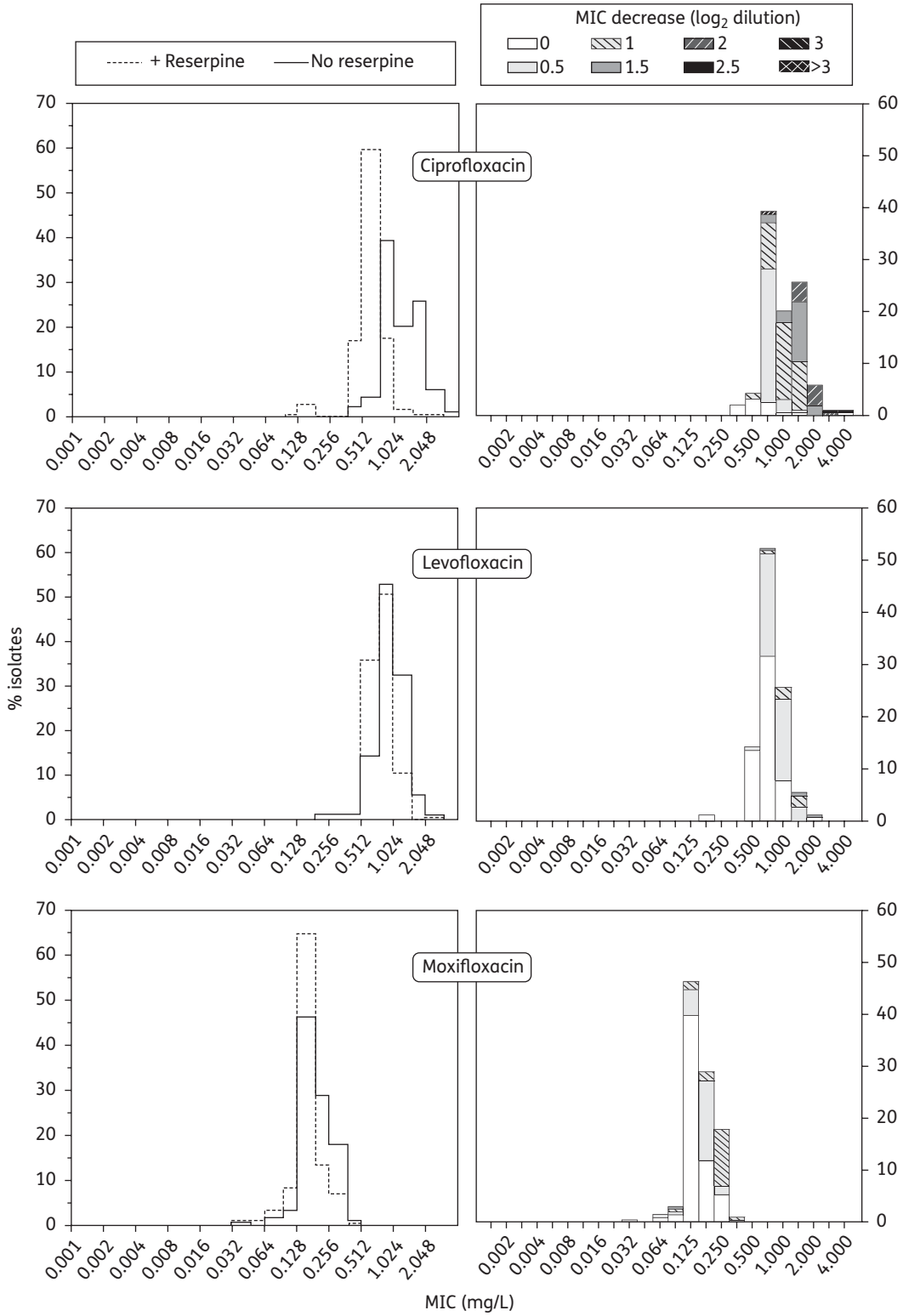


**Figure 1.** MIC distribution of five quinolones for 183 non-duplicate isolates of *S. pneumoniae* obtained from clinically confirmed cases of community-acquired pneumonia collected in Belgium during the 2007–09 period. Left-hand panels: MIC distributions determined in the absence (control; continuous line) or presence (broken line) of 10 mg/L reserpine {statistical analysis: $P<0.0001$ for each quinolone when comparing distributions in the absence and presence of reserpine by two-tailed paired tests [Wilcoxon signed rank test (non-parametric) and by *t*-test (parametric)]. Right-hand panels: reduction of MIC (in blocks of 0.5 $\log_2$ dilutions from 0 to 3 $\log_2$ dilutions) after addition of 10 mg/L reserpine and plotted as a function of the MIC distribution of the isolates in the absence of reserpine.

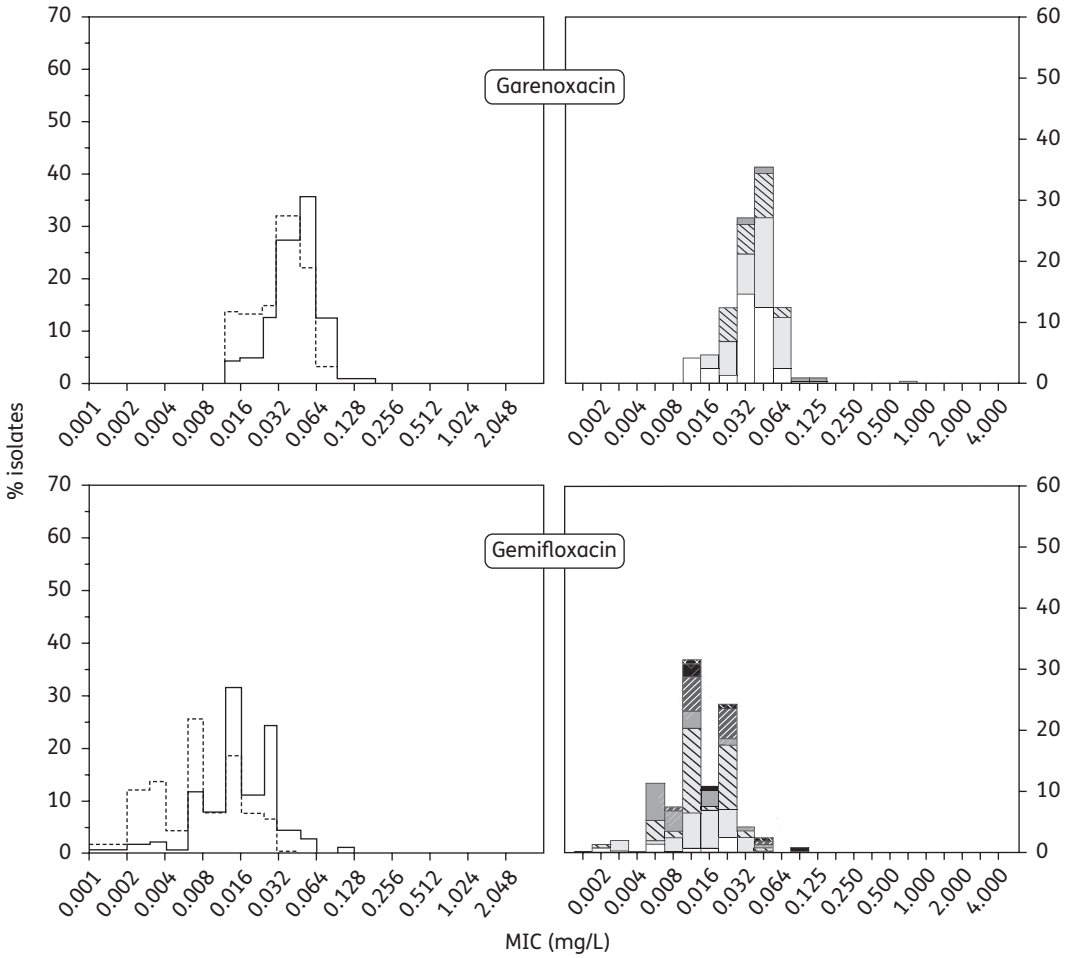


**Figure 1.** (Continued)

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## Transparency declarations

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## Supplementary data

Table S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

## References

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## Supplementary data

**Table S1.** MIC distribution of quinolones for *S. pneumoniae* from clinically confirmed community-acquired pneumonia in the absence (−) or in the presence (+) of 10 mg/L reserpine

| MIC (mg/L) | Ciprofloxacin | | Levofloxacin | | Moxifloxacin | | Garenoxacin | | Gemifloxacin | |
|---|---|---|---|---|---|---|---|---|---|---|
| | − | + | − | + | − | + | − | + | − | + |
| Lowest | 0.375 | 0.094 | 0.188 | 0.188 | 0.032 | 0.032 | 0.012 | 0.012 | 0.001 | <0.001 |
| $MIC_{50}$ | 1 | 0.5 | 0.75 | 0.75 | 0.125 | 0.125 | 0.047 | 0.031 | 0.012 | 0.006 |
| $MIC_{90}$ | 1.5 | 0.75 | 1 | 1 | 0.25 | 0.188 | 0.064 | 0.05 | 0.024 | 0.016 |
| Highest | 4 | 2 | 2 | 2 | 0.375 | 0.375 | 0.75 | 0.75 | 0.094 | 0.032 |

CLSI breakpoints (susceptible ≤/resistant ≥): levofloxacin, 2/8; moxifloxacin, 1/4; gemifloxacin, 0.12/0.5.

EUCAST breakpoints (susceptible ≤/resistant >): ciprofloxacin, 0.12/2; levofloxacin, 2/2; moxifloxacin, 0.5/0.5.