INTRODUCTION

Pseudomonas aeruginosa is primarily a nosocomial pathogen, responsible for opportunistic infections. These are of great concern, because P. aeruginosa presents intrinsic as well as acquired resistance to a wide variety of antimicrobial agents. In this context, polytopic efflux pumps play a central role in the multiresistance of P. aeruginosa towards both antibiotics and antiseptics.

Seven “Mex-type” efflux pumps able to transport various antimicrobial agents have been characterized in P. aeruginosa, but four of them (MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM) have been shown to contribute to clinically significant resistance levels (Llanes et al., 2004). Inhibitors of Mex efflux pumps have been described, among which Phenyl-Alanine-ß-Naphtylamide restores antibiotic activity (at least in vitro) in strains resistant by overexpression of efflux pumps (Lomovskaya et al., 2001).

In this context, the development of rapid and reliable methods for the early detection of this efflux pumps in clinical isolates may positively assist for the selection of appropriate therapeutic agents in a given patient and also for the screening of resistance mechanisms in epidemiological surveys. These could include both phenotypic (such as changes in MIC) and genotypic characterization (modification in gene expression level).

METHODS

Stains and growth conditions: P. aeruginosa wild type (PAO1), P. aeruginosa overexpressing MexAB-OprM (wild mutant) (Kohler et al., 1997), P. aeruginosa overexpressing MexCD-OprJ (M. Michea-Hamzehpour et al., 1995), P. aeruginosa overexpressing MexEF-OprN (M. Michea-Hamzehpour et al., 1997) and P. aeruginosa overproducing MexXY-OprM (Hoppestad et al., 2003). The strains were grown over-night on Muller-Hinton broth (MHB) at 37°C under aerobic conditions and gentle agitation (100 rpm).

MICs: Minimal inhibitory concentrations (MIC's) were determined by the broth microdilution method according to NCCLS guidelines. Inocula of 5x10⁵ bacteria/ml in exponential growth were used. Measurements were done in the absence and in the presence of 50 mlg Phe-Asn-S-Naphtylamide, an efflux pumps inhibitor (PANA).

RESULTS: phenotypic characterization

<table>
<thead>
<tr>
<th>Strains</th>
<th>PAO1</th>
<th>MexAB-OprM</th>
<th>MexCD-OprJ</th>
<th>MexEF-OprN</th>
<th>MexXY-OprM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin</td>
<td>256(3)</td>
<td>256(3)</td>
<td>&gt;1024(3)</td>
<td>Carbenicillin+</td>
<td></td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>32(3)</td>
<td>128(3)</td>
<td>16(3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The table shows the MIC measured in absence or in presence of P. aeruginosa.

RESULTS: genotypic characterization

Detection of MexE/MexX expression by RT-PCR: MexE and MexX are not expressed in wild-type cells, so that amplification of the corresponding mRNA denotes resistance. We illustrate here RT-PCR amplification products of mexA (mexA, which is constitutively expressed, is used here as positive control for the RT-PCR reaction).

Quantification of mexA/MexX expression by QC-RT-PCR:

<table>
<thead>
<tr>
<th>Strain</th>
<th>PAO1</th>
<th>MexAB-OprM</th>
<th>MexCD-OprJ</th>
<th>MexEF-OprN</th>
<th>MexXY-OprM</th>
</tr>
</thead>
<tbody>
<tr>
<td>mexA</td>
<td>0.5(3)</td>
<td>0.5(3)</td>
<td>0.5(3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mexX</td>
<td>0.5(3)</td>
<td>0.5(3)</td>
<td>0.5(3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The QC-RT-PCR (Quantitative-Competitive-RT-PCR) demonstrates a 6-fold overexpression of MexA and a 8-fold overexpression of MexX.

REFERENCES

Llanes et al., Clinical Strains of Pseudomonas aeruginosa Overproducing MexAB-OprM and MexCD-OprJ are Cross Resistant to Different Quinolones, 2004; AAC, 45(1):105-116

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Phenotypic and genotypic detection of Mex efflux pumps in P. aeruginosa


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