MDRAB strains were negative for qnrA, qnrB and qnrS and other studies have shown that qnr in A. baumannii is not widespread.1

Four of the eight qnr genes were found in ciprofloxacin-resistant isolates and three in isolates with intermediate susceptibility. C. freundii harbour qnrB8 and was ciprofloxacin-susceptible. Six of the eight (75%) qnr-positive isolates were ESBL-producing strains representing 11% of all the ESBL-positive isolates (n=53). χ² analysis showed that qnr was associated with ESBL-producing strains (P<0.01). Quinolone conjugation studies have revealed that the presence of qnr does not confer high-level resistance, although it has been shown that the qnr gene is able to co-localize with other antibiotic resistance determinants on a plasmid.2 This implies that although they may not be the cause of resistance, qnr genes are associated with isolates that have reduced susceptibility.

Each ESBL-positive isolate harboured only one type of qnr gene. The sequences of qnrA and qnrS genes detected in this study were 100% identical to the published sequences. Within qnrB, however, there were mutations noted in all three of the sequences resulting in two amino acid changes. The change in the amino acids was at the same position irrespective of the qnrB subtype or bacterial species harbouring the gene. Six variants of qnrA have been identified worldwide compared with 19 variants of the qnrB gene and 3 variants of qnrS.6

This study describes for the first time the prevalence of qnrB8 in the UK. Although the full clinical significance of qnr-mediated quinolone resistance remains unclear, this relatively new resistance genotype is clearly expanding in prevalence and repertoire.

Acknowledgements

We would like to thank Dr Neil Woodford from the Antibiotic Resistance Monitoring and Reference Laboratory, Centre for Infections, Health Protection Agency, London, for kindly donating the control strains for this study.

Funding

This work was supported by internal funding and the Clothworkers Foundation who sponsored S. M. N. during this research project.

Transparency declarations

None to declare.

References


Comparison of four commercial methods for determining temocillin susceptibility of Escherichia coli

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Sir,

Temocillin is a semi-synthetic 6-α-methoxyphenicillin derivate of ticarcillin. Its stability to bacterial β-lactamases was confirmed against the majority of extended-spectrum β-lactamases (ESBLs) including CTX-M enzymes.1,2 In vitro susceptibility testing for temocillin can be performed by broth dilution, disc diffusion method or Etest (AB Biodisk, Solna, Sweden). The Vitek 2 system (bioMérieux, France) has developed new Gram-negative antibiotic susceptibility testing cards that include temocillin. Limited data have been published concerning the accuracy of these different susceptibility test methods for this antibiotic. We compare the accuracy of the Vitek 2 system, Etest and two disc diffusion methods (30 μg paper discs from Becton Dickinson, USA and Neo-Sensitabs from Rosco, Denmark) to determine the susceptibility of Escherichia coli strains to temocillin. Strains were recovered from urine specimens (57%), screening rectal swabs (25%), respiratory samples (7%), wound swabs (4%) and other samples (7%). Isolates included ESBL-producing strains (n=88) harbouring CTX-M enzymes in 70 isolates (CTX-M-2, CTX-M-9, CTX-M-15), TEM (TEM-24, TEM-30, TEM-52) in 12 isolates and SHV-12 enzymes in 6 isolates. Non-ESBL isolates (n=68) included 15 AmpC-hyperproducing strains, 25 with wild-type phenotype and 28 with other antibiotic resistance mechanisms.

Production of ESBL was detected by the combined disc method (Bio-Rad, France). The genotypic characterization of the ESBLs was performed by PCR to detect blaTEM, blaSHV and blaCTX-M genes in all isolates, PCR for CTX-M groups in CTX-M-producing strains and DNA sequencing (n=25) as described previously.3
Strains were tested by disc diffusion (paper discs and Neo-Sensitabs) and MIC determination by Etest (AB Biodisk) methods and were inoculated at the same time in AST-N045 and AST-N046 cards for Vitek 2. The agar dilution method was performed according to the CLSI recommendations, as the gold standard method.\(^3\) Susceptibility to temocillin was determined according to breakpoints provided by Fuchs et al.\(^4\) (susceptible, ≤16 mg/L; resistant, ≥32 mg/L) and to BSAC breakpoints for urinary tract or systemic samples (MIC≤32 mg/L and MIC≤8 mg/L, respectively).\(^5\) Interpretation criteria for temocillin paper discs were those published by Fuchs et al.\(^4\) (susceptible, >19 mm; intermediate, 16–18 mm; resistant, <16 mm) and for Neo-Sensitabs, those recommended by the manufacturer (susceptible, ≥17 mm; intermediate, 15–17 mm; resistant, <15 mm). \(E.\) coli ATCC 25922 was used as a reference strain for quality control in each series. Discrepancies were classified as follows: a minor or major error when the reference method showed a susceptible result and the experimental method showed an intermediate or resistant result, respectively; a very major error when the gold standard method classified an isolate as resistant and the experimental method classified the isolate as susceptible.

No differences were observed between AST-N045 and AST-N046 cards. MICs obtained by Etest and Vitek 2 methods were within one dilution of those obtained by agar dilution in 99% and 100% of isolates, respectively. No significant difference in MIC distribution was observed for Etest and Vitek 2, with MIC\(_{90}\)s of 99% and 100% of isolates, respectively. No significant difference within one dilution of those obtained by agar dilution in AST-N046 cards. MICs obtained by Etest and Vitek 2 methods on the basis of inter-individual variabilities in serum drug levels.\(^6\) Our findings confirm that both Etest and the Vitek 2 system are accurate methods to determine MICs of temocillin for \(E.\) coli, including ESBL- and AmpC-producing strains by using Fuchs et al.’s\(^4\) breakpoint values. New calibration and/or re-design of the AST-N045 and AST-N046 Vitek cards is warranted for use with the BSAC breakpoints. In our study, Neo-Sensitabs discriminated better between susceptible and resistant strains than paper discs.

**Funding**

This study was partially supported by a grant from Eumedica s.a., Brussels, Belgium.

**Transparency declarations**

We are not members of any Eumedica advisory board and we do not have any conflicts of interest that may affect the conclusions of the present article.

**References**


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### Table 1. Rate of error in temocillin susceptibility categorization of clinical \(E.\) coli strains (\(n=153\)) by test method in comparison with agar dilution method

<table>
<thead>
<tr>
<th></th>
<th>Minor error (%)</th>
<th>Major error (%)</th>
<th>Very major error (%)</th>
<th>Total discrepancies (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Fuchs(^a) BSAC systemic(^a)</td>
<td>Fuchs(^a) BSAC systemic(^a)</td>
<td>Fuchs(^a) BSAC systemic(^a)</td>
<td>Fuchs(^a) BSAC systemic(^a)</td>
</tr>
<tr>
<td>Neo-Sensitabs</td>
<td>5 (3)</td>
<td>0</td>
<td>2 (1)</td>
<td>7 (5)</td>
</tr>
<tr>
<td>Paper discs</td>
<td>23 (15)</td>
<td>8 (5)</td>
<td>0</td>
<td>31 (20)</td>
</tr>
<tr>
<td>Vitek 2</td>
<td>0</td>
<td>3 (2)</td>
<td>6 (4)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Etest</td>
<td>0</td>
<td>4 (3)</td>
<td>3 (2)</td>
<td>4 (3)</td>
</tr>
</tbody>
</table>

\(^a\)MIC breakpoints.


Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dkp023
Advance Access publication 15 February 2009

In vitro susceptibility of non-Aspergillus allergenic fungal species to azoles

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Keywords: itraconazole, posaconazole, voriconazole, asthmatics, allergens

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Sir,

Over the last decade, the importance of fungal organisms as allergens has increased. Allergic bronchopulmonary aspergillosis (ABPA) is an inflammatory disease of the airways caused by hypersensitivity to the Aspergillus fumigatus antigen and occurs in ∼1% of asthmatics and 15% of adult cystic fibrosis patients. The widespread use of itraconazole for the treatment of ABPA has been reported to improve lung function and aid in reducing the concentration of glucocorticosteroids required.1 Other manifestations of fungal allergy of the respiratory tract include allergic fungal sinusitis and severe asthma with fungal sensitization (SAFS).2 Recently, a randomized placebo-controlled study of itraconazole treatment of SAFS has shown major improvements in asthmatic control and quality of life in patients sensitized to one of several fungi including Penicillium, Cladosporium and others.2 These data and the introduction of the new azole drugs voriconazole and posaconazole led us to examine the azole susceptibility of other potentially allergenic moulds.3 In this study, the in vitro activity of posaconazole and voriconazole is compared with that of itraconazole.

Eighteen mould isolates were evaluated: two Alternaria alternata, two Botrytis cinerea, one Cladosporium cladosporioides, two Cladosporium herbarum, two Epicoccum purpurascens, one Helminthosporium halodes, two Helminthosporium maydis, two Penicillium chrysogenum, two Trichophyton interdigitale and two Trichophyton rubrum. Eleven isolates were obtained from the culture collection at Centraalbureau voor Schimmelcultures (CBS), the Netherlands, and both C. herbarum and one E. purpurascens were environmental isolates (identified by CBS). All Trichophyton spp. were clinical isolates from the culture collection at The Regional Mycology Laboratory, Manchester, UK.

MICs were determined according to CLSI (formerly NCCLS) M38-A guidelines,4 with modified temperature and length of incubation (due to growth requirements of organisms being tested). Final drug concentrations ranged from 0.015 to 8 mg/L.

Isolates were grown on Sabouraud agar at either room temperature or 30°C, and inocula were prepared in PBS containing 0.05% Tween 80. A spectrophotometric method for inoculum preparation was used for all isolates, except Penicillium (counted by a haemocytometer). Plates were incubated at room temperature or 30°C for durations of 2–8 days, depending on the organism tested, in a moist chamber. Plates were checked daily and read when sufficient growth was apparent in the positive control, giving full well coverage (each species was read at the same incubation time). MICs were read visually to determine a no growth endpoint, and MFCs were also determined (99% kill).

The differences between drugs were analysed by a one-way analysis of variance with a Bonferroni correction for multiple comparisons (SPSS).

In general, the species tested had a susceptibility order of: posaconazole > itraconazole > voriconazole, with geometric mean MICs of 0.08, 0.16 and 1.21 mg/L, respectively (Table 1). All isolates had MICs of posaconazole that were lower or equal to those of itraconazole or voriconazole, except one Penicillium isolate, which had a lower MIC of itraconazole of 0.125 compared with an MIC of posaconazole of 0.25 mg/L. There was no significant difference (P>0.05) between MICs of posaconazole and itraconazole; however, significant differences (P<0.05) were seen between MICs of voriconazole and each of the other drugs.

Incubating P. chrysogenum at 30°C instead of at room temperature mostly increased MICs of itraconazole and voriconazole, but no change was seen with posaconazole, suggesting that MIC values can be influenced by changes in incubation time and temperature at least for some drugs.

Using the CLSI M38-A guidelines for inoculum preparation, the colony counts for one-third of the isolates were not within the CLSI range, although each drug was tested simultaneously, enabling a valid comparison to be made.

Many studies have compared antifungal activity against filamentous fungi, but they cannot be directly compared with our results due to method differences. Furthermore, a small number of isolates were tested in this study. Cuenca-Estrella et al.5 demonstrated that when using CLSI methods to perform susceptibility testing on Penicillium species, posaconazole was the most active, then itraconazole and voriconazole, consistent with our findings, although the MIC90 values were much higher than

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