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

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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 MIC_{90} values were much higher than

Table 1. MIC and MFC results for mould isolates using a modified CLSI M38-A microtitre method[4]

Organism/actual final inoculum (cfu/mL)	MIC/MFC (mg/L)		
	itraconazole	posaconazole	voriconazole
A. alternata			
2.25×10^4	0.25/0.25 ($n=1$)	0.125/0.125 ($n=1$)	8/8 ($n=1$)
2.0×10^4	0.25/0.25 ($n=1$)	0.125/0.125 ($n=1$)	2/2 ($n=1$)
B. cinerea			
1.25×10^3	≤0.015/≤0.015 ($n=1$)	≤0.015/≤0.015 ($n=1$)	0.125/0.125 ($n=1$)
4.25×10^4	0.03/0.03 ($n=1$)	0.03/0.03 ($n=1$)	1/1 ($n=1$)
C. cladosporioides			
7.5×10^3	0.25/0.25 ($n=1$)	0.125/0.125 ($n=1$)	4/4 ($n=1$)
C. herbarum			
2.5×10^3	0.125/0.125 ($n=1$)	0.06/0.06 ($n=1$)	8/8 ($n=1$)
5.5×10^3	0.06/0.06 ($n=1$)	0.03/0.03 ($n=1$)	2/2 ($n=1$)
E. purpurascens			
1.0×10^4	2/2 ($n=1$)	0.125/0.125 ($n=1$)	2/2 ($n=1$)
5.0×10^3	>8/>8 ($n=1$)	0.5/0.5 ($n=1$)	4/4 ($n=1$)
H. halodes			
7.5×10^3	0.06/0.06 ($n=1$)	0.03/0.03 ($n=1$)	8/8 ($n=1$)
H. maydis			
2.25×10^4	0.06/0.06 ($n=1$)	0.03/0.03 ($n=1$)	0.5/0.5 ($n=1$)
5.0×10^3	0.03/0.03 ($n=1$)	0.03/0.03 ($n=1$)	0.25/0.25 ($n=1$)
P. chrysogenum (room temperature)			
4.25×10^4	0.125/0.125 ($n=1$)	0.25/0.25 ($n=1$)	1/1 ($n=1$)
3.5×10^4	0.25/0.25 ($n=1$)	0.25/0.25 ($n=1$)	2/2 ($n=1$)
T. interdigitale			
8.75×10^4	0.125/0.125 ($n=1$)	0.06/0.06 ($n=1$)	0.25/0.25 ($n=1$)
1.25×10^5	0.125/0.125 ($n=1$)	0.125/0.125 ($n=1$)	0.5/0.5 ($n=1$)
T. rubrum			
1.25×10^3	0.125/0.125 ($n=1$)	0.06/0.06 ($n=1$)	0.125/0.125 ($n=1$)
3.75×10^5	0.5/1 ($n=1$)	0.25/0.5 ($n=1$)	1/>8 ($n=1$)
Geometric mean	0.16/0.17	0.08/0.08	1.21/1.41
Mean	1.13/1.16	0.12/0.14	2.49/3.32
Median	0.125/0.125	0.09/0.09	1.5/2
Range	≤0.015 to >8/≤0.015 to >8	≤0.015–0.50/≤0.015–0.50	0.125–8/0.125 to >8

those obtained in our study. High MIC_{90} values of itraconazole, posaconazole and voriconazole for *Alternaria* (all >8 mg/L) were also shown by Cuenca-Estrella *et al.*,[5] but we only found this with voriconazole (2 and 8 mg/L). Susceptibility of *Cladosporium* species to itraconazole was investigated by Pujol *et al.*,[6] and MICs obtained ranged from ≤0.035 to >20 mg/L ($n=6$), but we found that the three isolates tested were susceptible to itraconazole with MICs ranging from 0.06 to 0.25 mg/L.

All three azole drugs exhibited good activity against *Trichophyton* species (all MICs ≤0.5, except one *T. rubrum* isolate with an MIC of 1 mg/L). When using CLSI methods, previous studies have shown similar and also much higher MIC values of posaconazole and itraconazole compared with our findings.

MFCs were also determined in this study as it has been previously shown that they may correlate better with clinical outcomes than MICs.[7] We have demonstrated that all isolates were also killed by the drug concentration that inhibited them, except one isolate of *T. rubrum*.

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Decreasing prevalence of levofloxacin-resistant *Streptococcus pneumoniae* in Hong Kong, 2001 to 2007

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Sir,

Streptococcus pneumoniae is the most common identifiable cause of community-acquired pneumonia. With the rise in resistance to penicillin and macrolides, other classes of antibiotics, notably the newer fluoroquinolones, are increasingly being used for the empirical treatment of pneumonia. As observed for other antibiotics, fluoroquinolone resistance has emerged in *S. pneumoniae* among sporadic isolates as well as in the major epidemic clones.[1] Our previous work documented the rapid emergence of pneumococcal resistance to the fluoroquinolones in Hong Kong since the late 1990s.[2,3]

This study assessed the recent epidemiology of levofloxacin-resistant *S. pneumoniae* (LRSP) using the database of a clinical microbiology laboratory in Hong Kong. This laboratory provides service to a network of five hospitals, including one acute care university teaching hospital (HKW1) with 1400 beds and all the clinical disciplines and four chronic care hospitals (HKW2, HKW3, HKW4 and HKW5) with 110–524 beds. HKW4 only admits children. The hospital network is estimated to provide clinical service to a population of approximately 1 million. The data on *S. pneumoniae* from 2001 to 2007 were extracted, and duplicate isolates were removed by the initial isolate per patient method, irrespective of susceptibility and specimen source. Only the first isolate from each patient was included during the whole surveillance period. During this period, all isolates were routinely tested for their susceptibility to penicillin (oxacillin), erythromycin, co-trimoxazole, chloramphenicol and levofloxacin by the disc diffusion method. Penicillin MICs were determined by Etest (AB Biodisk, Solna, Sweden). All results were interpreted according to the CLSI.[4] Strains were identified as *S. pneumoniae* by Gram stain, colony morphology, optochin susceptibility and bile solubility.[3,5]

There were a total of 2290 *S. pneumoniae* isolates during the 7 year period, and the selection criteria identified 1680 isolates for analysis. These included 203 isolates from outpatients and 1477 from inpatients. All isolates were obtained from clinical samples submitted for investigation. The specimen sources were respiratory ($n=1466$), blood ($n=139$), wound ($n=61$) and other body fluid ($n=14$). Overall, 11% of these isolates had reduced susceptibility to levofloxacin; 15 (0.9%) were intermediate (equivalent MIC breakpoint, 4 mg/L) and 169 (10.1%) were resistant (equivalent MIC breakpoint, $\geq$8 mg/L). Most LRSP exhibited co-resistance to penicillin (MIC $>$ 0.06 mg/L, 97.6%), co-trimoxazole (93.5%), chloramphenicol (92.3%) and erythromycin (97%). The 169 LRSP isolates were obtained from sputum ($n=148$), blood ($n=13$), tracheal aspirate ($n=4$), bronchoalveolar lavage ($n=3$) and pleural fluid ($n=1$). All patients with LRSP were adults (aged $>$17 years). The great majority of patients with LRSP were elderly; 87.6% were $>$64 years and 72.1% were $>$74 years.

During the study period, there were fluctuations in the annual proportion of LRSP. The proportions of LRSP by year were 11.7% (39/334) for 2001, 12.9% (40/311) for 2002, 8.7% (19/218) for 2003, 12.2% (24/196) for 2004, 10.3% (18/174) for 2005, 7.8% (18/232) for 2006 and 5.1% (11/215) for 2007