Azole resistance surveillance in Aspergillus fumigatus: beneficial or biased?

Paul E. Verweij1,2, Pieter P. A. Lestrade1, Willem J. G. Melchers1,2 and Jacques F. Meis2,3

1Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands; 2Centre of Expertise in Mycology Radboudumc/CWZ, Nijmegen, The Netherlands; 3Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital (CWZ), Nijmegen, The Netherlands

Azole resistance is a growing concern with Aspergillus fumigatus, and may cause increased mortality in patients with azole-resistant invasive aspergillosis (IA). Microbial surveillance has been recognized as a fundamental component of resistance management. Surveillance information may be used to inform decisions regarding health services and research funding allocation, to guide local infection control in hospitals and communities, and to direct local and national drug policies and guidelines. Azole resistance frequencies have been based on screening of unselected A. fumigatus isolates, on the number of azole-resistant cases within a cohort of patients with a specific Aspergillus disease, or on analysis of patients within a specific risk group. The various surveillance approaches differ in their aims, as well as in their associated advantages and drawbacks. Nevertheless, a wide range of azole resistance frequencies has been reported, partly due to the denominator used. As most azole resistance is believed to develop in the environment and, as a consequence, azole-naive patients may present with azole-resistant aspergillosis, experts recommended a 10% resistance frequency threshold above which the standard treatment choice, i.e. voriconazole, should be reconsidered. We believe that local resistance rates based on Aspergillus disease and/or risk group should be leading for decisions regarding empirical antifungal therapy in specific units. In addition, patient factors should be considered, such as admission to the ICU. Collecting valid surveillance data may be challenging in azole resistance due to numerous factors that present potential biases. Surveillance research may benefit from further standardization, which may be facilitated through the recently instituted International Society for Human and Animal Mycology (ISHAM) Aspergillus Resistance Surveillance Working Group.

Introduction

Triazole resistance in Aspergillus fumigatus is an increasing concern, as it complicates the management of patients with Aspergillus diseases, including invasive aspergillosis (IA), with regard to detection of resistance, treatment and response evaluation. In the absence of management guidelines, a group of experts recently recommended reconsidering the use of empirical voriconazole monotherapy when a resistance threshold of 10% is exceeded. However, a wide variation in resistance frequencies has been reported in recent years, which may reflect true variation, but may also be due to differences in laboratory procedures or bias related to the surveillance system. Alanio et al. discuss this problem and advocate harmonization of mycological diagnosis and of reporting of azole resistance frequency.

Acquired azole resistance in A. fumigatus is a relatively recently recognized clinical problem and we are only now starting to understand the epidemiology, pathogenesis and clinical implications. As the number of drug classes for treatment of aspergillosis, i.e. azoles, polyenes and in specific settings echinocandins, is already very limited, the loss of the most important azole class directly causes challenges in patient management. Indeed, case series indicate high mortality rates in patients with documented azole-resistant IA, but a comparative trial with azole-susceptible cases has not been published. In 2009 a possible link between resistance of A. fumigatus to medical azoles and the use of azole fungicides in the environment was suggested, which, because of natural selection, increases the potential for migration of resistance traits. However, in vitro susceptibility testing of A. fumigatus is not common practice in clinical microbiology laboratories, and although resistance has now been reported in six continents (Asia, Africa, North America, South America, Europe and Australia), in many regions the azole resistance frequency in A. fumigatus remains unknown.

Microbial surveillance has been recognized as a fundamental component of resistance control, as surveillance information enables the assessment of the burden of resistance, determination of risk factors, and identification of trends in resistance phenotypes and genotypes. Such information may be used to inform decisions regarding health services and research funding allocation, to guide local infection control in hospitals and communities, and to direct local and national drug policies and guidelines.
guidelines. Several reports have underscored the need for surveillance in A. fumigatus, and the International Society for Human and Animal Mycology (ISHAM) recently instituted an Aspergillus Resistance Surveillance Working Group. However, the question is how azole resistance surveillance can best be performed, especially if resistance frequencies are used to guide empirical antifungal treatment decisions. Can we use unselected clinical A. fumigatus isolates? Or should isolates from selected respiratory specimens and from normally sterile sites be included? Or should we only use isolates from patients with documented Aspergillus disease, such as IA?

Validity of resistance surveillance systems

The validity of antimicrobial resistance surveillance data is influenced by several potential biases, previously summarized by Rempel and Laupland. The factors include bias related to the use of inadequate or inappropriate denominator data, case definitions and case ascertainment, sampling bias, failure to deal with multiple occurrences, and biases related to laboratory practice and procedures. For surveillance studies focusing on IA, the case definition relies on the European Organisation for Research and Treatment of Cancer (EORTC)/Mycosis Study Group (MSG) consensus definitions, which include subjective elements and cannot be used in all risk groups. For instance, the classification of IA can be problematic in non-neutropenic patients, such as those admitted to the ICU. Due to these problems, the case ascertainment in IA will be limited. Moreover, the performance of culture, which is the main diagnostic tool to identify resistance, is poor. The overall yield of culture positivity in high-risk patients is low; due to mould-active prophylaxis or the early use of biomarkers to initiate pre-emptive antifungal therapy. The number of A. fumigatus isolates available for resistance frequency assessment is therefore very low, especially when compared with bacterial resistance surveillance programmes. In addition, recent insights in the pathogenesis of azole-resistant IA indicate that azole-susceptible and azole-resistant coinfection may occur. Individual pulmonary lesions may be caused by genetically different A. fumigatus strains, some azole susceptible and others azole resistant. During azole therapy radiological evaluation may show improvement of pulmonary lesions while the azole-resistant strain disseminates. A positive Aspergillus culture may therefore contain mixed phenotypes, thus increasing the difficulty of identifying patients with azole-resistant IA. If culture is not performed or remains negative, azole resistance may also not be diagnosed. In addition to the wide variety of management strategies of different patient groups at risk of IA laboratory practices in mycology also vary widely. All these factors contribute to the challenge of collecting valid data that can be confidently used to guide empirical treatment strategies.

Basically three approaches to resistance surveillance can be followed: (i) resistance surveillance of unselected A. fumigatus isolates or from selected specimens; (ii) surveillance of A. fumigatus cultures relevant to an Aspergillus disease; and (iii) an audit of consecutive patients within a cohort of patients with a specific underlying disease. With the increasing availability of molecular techniques for resistance detection, these methods can also be incorporated in surveillance studies. The aims of the above-mentioned approaches differ and each has its advantages and disadvantages. An example of the three approaches to the surveillance of azole resistance in IA is shown in Table 1. Although the resistance frequency may vary between departments within the same hospital, between different hospitals and between geographical regions, the denominator used to calculate the resistance frequency has a major impact (Figure 1). When only patients with a specific underlying disease are considered, the resistance frequency will be low, as many of these patients will not develop IA. However, if patients with IA are included, the resistance frequency will be higher. The highest rates of resistance are reported when only patients with a positive culture are included, as the population of patients with IA and a positive culture is usually small.

From resistance frequency to treatment strategy

The wide variety in the reported resistance frequencies is confusing, especially if a cut-off resistance frequency is recommended at which a unit should consider moving away from azole monotherapy. An expert panel recommended reconsidering the use of azole monotherapy in regions with azole resistance rates exceeding 10%. Alternative empirical therapy included either liposomal amphotericin B or voriconazole and echinocandin combination therapy, but the efficacy of these alternative treatment options was the subject of much debate. There was concern as to whether liposomal amphotericin B would be as effective as voriconazole in azole-susceptible cases, as there are no comparative trials. Furthermore, the clinical efficacy of voriconazole and anidulafungin combination therapy in azole-resistant IA is unknown, and might be suboptimal in patients infected with isolates highly resistant to voriconazole.

The concept of resistance treatment thresholds is commonly used for bacterial infections such as urinary tract infections. The use of alternative antimicrobial drugs for empirical therapy based on such threshold recommendations is rare. Furthermore, treatment recommendations of bacterial infections are commonly based on population antimicrobial resistance prevalences. The European Conference on Infections in Leukaemia (ECIL) has published guidelines for empirical use of antibacterial therapy for febrile neutropenic patients taking into account the increasing rates of bacterial resistance. A cut-off prevalence of resistance that should prompt a unit to change empirical antibacterial therapy was not provided due to lack of literature data. Initial empirical antibacterial therapy was recommended to reflect the department/unit resistance epidemiology, the patients’ risk factors for resistant bacteria and the patients’ risk factors for a complicated course of infection.

With respect to azole resistance, the above-mentioned expert panel did not specify how the azole resistance rates should best be monitored. We believe that in an optimal scenario, resistance rates based on Aspergillus disease and/or risk group are preferred regarding decisions of first-line antifungal therapy in specific units. Although it may be laborious to collect these data and such collection may take several years to complete, the variety in resistance frequencies between departments and hospitals makes extrapolation from one department to another or between different hospitals more difficult. The recommended 10% threshold may help to decide locally whether moving away from azole
monotherapy is appropriate. Unfortunately resistance rates may vary over time, thus necessitating continuous or repeated audits.

In addition to resistance frequency, patient factors should also be considered. Patients that require admission to the ICU may benefit from upfront polyene-based therapy or azole/echinocandin combination therapy, in a setting with a significant proportion of azole resistance. The disease progression in these patients may be rapid and reported mortality rates in ICU patients with azole-resistant IA receiving azole monotherapy are very high. Several ICUs in the Netherlands have moved away from azole monotherapy due to local high resistance rates or are considering to do so.26

In conclusion, azole resistance surveillance would benefit from standardization, although unified laboratory practices and procedures will be very difficult to achieve at least to the extent advocated by Alanio et al.2 Although changes to treatment choices should ideally be based on resistance surveillance in specific risk groups at the department level, this seems an unrealistic goal given the poor performance of fungal culture and the time

<table>
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<tr>
<th>Table 1. Characteristics of three surveillance strategies for azole resistance</th>
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<td>Surveillance strategy</td>
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<tr>
<td>Screening of unselected clinical A. fumigatus isolates</td>
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<tr>
<td>Identify patients with IA based on a positive Aspergillus culture</td>
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<td>Determine the frequency of IA within a cohort of patients</td>
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![Figure 1. Impact of the denominator on the resistance frequency in azole resistance surveillance. Resistance rates are calculated for a theoretical population of 200 AML patients, with a 10% prevalence of IA and 33% culture positivity.](http://jac.oxfordjournals.org/)

Azole resistance frequency

200 AML patients 1% (2/200)

20 IA cases 10% (2/20)

6 culture positive 33% (2/6)
needed to collect a meaningful number of cases. National and international surveillance programmes using standardized protocols, such as through the ISHAM Aspergillus Resistance Surveillance Working Group, may be a first step to generate reliable surveillance data for different countries and patient groups that in some way support treatment discussions. Although several reports have recommended resistance surveillance in A. fumigatus, funding has proved more difficult than for similar programmes for antibacterial resistance. Given the substantial global burden of Aspergillus diseases, the worldwide dimensions of resistance and the high mortality rates in azole-resistant IA, allocation of funds for azole resistance surveillance programmes is urgently warranted in order to design control strategies and improve patient outcome.

**Transparency declarations**

**References**