OPINION ON AZOLE ANTIMYCOTIC RESISTANCE

ADOPTED BY

THE SCIENTIFIC STEERING COMMITTEE

AT ITS MEETING OF 27 –28 JUNE 2002

OPINION

I. BACKGROUND AND MANDATE

Clinically important fungal infections have become more prevalent in the past two decades, during which time there has been extensive agricultural use of azole fungicides. There has also been an increase in the resistance of agriculturally important fungi to azole fungicides during this period. The Commission therefore requested the Scientific Steering Committee to examine these issues and determine which options they could identify to manage them. More precisely, the Commission submitted the following questions:

- Is it true that resistance of pathogenic fungi against antimycotic drugs poses an increasing problem and concern in human medicine?

- If so, is it likely that the development of resistance is related to the use of azole fungicides in crop protection or might other uses of these substances, e.g. as biocides), be a more plausible reason for the observation? Which options can the Scientific Committee identify to manage this resistance?

II. OPINION

Having examined the available scientific evidence, the Scientific Steering Committee found that there had been an important problem caused by resistance to treatment of pathogenic fungi to antimycotic drugs. The prevalence of this resistance to treatment, however, is not increasing and in some areas is decreasing.

The cause of the resistance to treatment is not a simple issue. Based on "in vitro" data azole antifungals do not necessarily achieve fungicidal levels at doses normally given in clinical practice and successful treatment additionally relies to an important extent on the patient's ability to mount an immune response. As a result of improvements in medical treatment an increasing number of immune compromised patients have survived otherwise fatal diseases, and have required treatment for severe fungal infections. These have not been easily treated with azole fungicides.

The relationship between in vitro and in vivo antimicrobial activity is not as direct for antimycotics as it is for bactericidal agents. It is difficult to determine, therefore, whether the increased prevalence of resistance to treatment with antimycotics has been due predominantly to increasing resistance of pathogenic fungi or to failure of the patient's immune competence. An equivalent increase in the prevalence of resistance to treatment has, however, not been seen in otherwise healthy patients (not immune compromised) who also suffer severe fungal infections. An important component to resistance to treatment must therefore be treatment failure as opposed to changes in primary or secondary fungal resistance.

The introduction of Highly Active Antiretroviral Therapy (HAART) has been accompanied by a reduction in resistance to azole antifungal treatment. Hence, although the resistance of pathogenic and nosocomial fungi against treatment with antimycotic drugs has been increasing until recently, it is now stabilised and in some areas of Europe it has fallen.

For all of these reasons, the SSC does not consider that resistance of pathogenic fungi against antimycotic drugs poses an increasing problem and concern in human medicine today. This situation could, however, change and needs to be monitored in case the ability of HAART therapy to control HIV viral replication in AIDS patients become less successful.

Having examined the scientific evidence regarding the use of azole antimycotics in agriculture, the SSC do not consider that the increased resistance to treatment of fungal infections with azole antimycotics is related to the use of azole fungicides in agriculture. Although resistance to azole antimycotics has been observed amongst plant specific pathogens this problem has been limited in extent and largely contained as a result of the introduction of appropriate control measures.

There are other issues related to the use of antimycotics which may need attention. Some of them are addressed in the attached report.

REPORT ON AZOLE ANTIMYCOTIC RESISTANCE

I   INTRODUCTION

Clinically important fungal infections have become more prevalent during the past two decades because therapeutic advances have allowed the survival of an increasing number of immune compromised patients. However, resistance to treatment with antifungal drugs has also become a greater clinical problem.

Antifungals of a class similar to those used in clinical practice are also widely used in agriculture where resistance to antifungals has also been a problem. IN agriculture methods of application and treatment control have been devised which limit the consequences of this emerging resistance and so allow for the continued use of fungicides.

It has been proposed, however,that there maybe a direct relation between the development of resistance to azole fungicides used in agricultural practice and the development of resistance to antifungals observed in clinical practice. The Scientific Steering Committee was invited to consider these issues and determine which options they could identify to manage this resistance.

II   MANDATE

Commission Services requested the Scientific Steering Committee for an opinion on the following questions:

- Is it true that resistance of pathogenic fungi against antimycotic drugs poses an increasing problem and concern in human medicine?

- If so, is it likely that the development of resistance is related to the use of azole fungicides in crop protection or might other uses of these substances, e.g. as biocides), be a more plausible reason for the observation? Which options can the Scientific Committee identify to manage this resistance?

III   REPORT

III.1   PREAMBLE

An exposure of a (micro-) organism, be it fungus or bacteria, to an antibiotic substance involves a risk of resistance developing through the process of selection. The rate and extent of the emergence of resistant organisms (agents or strains), whether this resistance is reversible or not and whether it is an issue relevant to human medicine, depends upon a variety of factors and conditions, including the mechanisms of action of the substance (frequently unknown), the target site(s) in the organism and their number, the risk of transfer of the resistance between individual organisms and species, and the mechanism of that resistance.

Resistance is not transferred between pathogenic fungi in humans and the mechanisms for the development of resistance differ from those seen in bacteria. There is no evidence that the genes that confer resistance on fungi can be transferred experimentally, a key difference with bacteria that has important implications when considering the public health implications of drug resistance amongst fungal pathogens.

III.2. AZOLE RESISTANCE I N CLINICAL PRACTICE:

Most information on anti-fungal resistance relates to clinical observations (eg, on immuno-suppressed patients, on populations of women with vaginal infections and on hospital patients with very specific disorders). These observations have been confirmed by in vitro laboratory tests, combined, in some cases, with studies to establish the genetic identity of the organisms. The extent of antimycotic resistance resulting from the systemic use of azoles in medicine is affected by the fact that azoles are very quickly metabolised by the human to non active substances. Most of the data on laboratory confirmed antifungal resistance is based on studies in specific patient populations notably those with AIDS or other serious underlying disease states.

There are three important types of antifungal used in clinical practice (which have also a different mode of action) and important problems of resistance are linked to therapeutic use of two of these, the third is only involved to a limited extent.

a) **Flucytosine** has been used for over 20 years and yeasts are known to become resistant largely because the drug action is dependant on a number of stages involving penetration of cells (permease) and conversion to fluorouracil (deaminase), steps which involve specific enzymes which are subject to changes resulting from gene mutation. This has not emerged as a major clinical problem because the drug is used in combination with another antifungal usually amphotericin B and its usage is limited to systemic *Candida* infection and cryptococcal meningitis.

b) **Azoles**. Azole resistance emerged with the use of oral imidazole or triazole drugs for the long term treatment or suppression of fungal infection in patients with different forms of immunodeficiency, notably chronic mucocutaneous candidosis or oropharyngeal candidosis in patients with AIDS. The risk of developing resistance varies between different drugs and itraconazole, for instance, appears to be less associated with resistance than fluconazole or ketoconazole. Resistance is mainly seen in *Candida* species although there are a few cases involving *Cryptococcus neoformans*.

There are two forms of resistance, primary (or intrinsic) and secondary resistance. In primary resistance fungi can only be inhibited by high levels of an antifungal drug. For instance most strains of *Aspergillus fumigatus*, a common human pathogen, are intrinsically resistant to fluconazole and do not appear to respond to its clinical use. Similarly *Candida krusei* and *C.glabrata* strains are often intrinsically resistant to fluconazole. By contrast it appears that fluconazole resistance in *Candida albicans*, can emerge de novo during prolonged treatment. This is an example of secondary resistance (Perea *et al*, 2001).

There are at least three different mechanisms by which drug resistancemight arise. These involve increased efflux of drug, altered target demethylase sites and availability of alternative pathways for the synthesis of cell membrane sterols. *Candida krusei* shows intrinsic resistance to fluconazole largely due to the last mentioned mechanism. *Candida albicans* the commonest pathogen in humans can become resistant to azole

antifungals when these are given over a long period to patients with reduced immunity. The increased prevalence of resistant species appears to have followed use of these drugs in predisposed groups (Salonen *et al,* 2001). Resistant Candida strains may coexist in the same site as susceptible organisms (Lopez-Ribot *et al,* 1999). There is also evidence that in a clinical setting the adoption of differing strategies to treat patients over long periods or to prevent infections through antifungal prophylaxis can lead to selection of resistant organisms, either of the same or different species, but these do not pose the same general risk as bacteria in a similar context because there is no transfer of resistance genes between fungi.. The emergence of *C.dubliniensis* as a pathogenic organism as with other *Candida* species in some AIDS patients is thought to have followed selection because of its higher MIC values to azoles (Marr *et al,* 1998). Resistance in the setting of AIDS is mainly described with *Candida species* – although there are some cases of resistant *Cryptococcus neoformans* (Xu *et al,* 2001)

The development of resistance is also closely related to the use of antifungal drugs in immunosuppressed patients. In chronic vaginal candidosis, for instance, where the patients are immunologically normal yet continued or recurrent use of azoles is a common strategy there has not been an increased frequency of antifungal resistance amongst Candida species isolated. A recent study did not establish an association between exposure to OTC antifungals and drug resistant Candida species in the vaginal flora, although there were some resistant strains found (Mathema *et al,* 2001). There have been other studies which have also failed to establish a link between antifungal therapy and drug resistant *Candida* species in the vagina. Studies of dermatophytosis, where long term azole therapy is common, have also not shown a change in the development of resistance in fungi isolated from patients who are usually immunologically normal. The rise in the incidence of resistant fungi has been dominated by resistance occurring in AIDS patients and those with other similar immunodeficiency states. In AIDS patients the use of continuous drug therapy (as described previously), a strategy adopted in some units for suppression of oropharyngeal candidosis, or the use of long term suppressive therapy, e.g. for cryptococcal meningitis, have both been associated with azole resistance amongst Candida strains (Masia Canuto *et al,* 2000). A key feature is that this resistance occurs against a background of immunosuppression either due to disease or to therapeutic interventions. The reasons for this relationship between poor host immunity and resistance is not known although it is thought to occur because of the high number of colonising or infecting organisms seen with the immunosuppressed thus allowing a greater chance for the emergence of resistant strains. In addition some resistant yeasts may be less virulent. Resistance has been described in other severely ill patients but overall the pattern of this problem has been dominated by fungal infection secondary to HIV.

The widespread use of Highly Active Antiretroviral Therapy (HAART) in Europe for patients with AIDS has produced a number of changes in the pattern of this disease. This includes a significant fall in the numbers of

opportunist infections (Skolasky *et al*, 2001; Haddad, 2001) including fungal disease, the numbers of new cases of such secondary infections falling by as much as 60% or more in some cases. There is evidence that the incidence of orophayrngeal candidosis has also fallen substantially and also, from a limited number of studies, that the incidence of the isolation of azole resistant Candida species has also fallen (Martins *et al*, 1998; Ruhnke *et al*, 2000).

Changes in antifungal usage policies resulting from practical infection control measures (avoidance of long term use of antifungal suppressive therapy, standard dosage regimens etc.) can also produce a fall in the incidence of resistant *Candida albicans* strains (Lopez et al, 2001). The wider use of HAART and the institution of appropriate antifungal usage policies have both helped to modify the patterns of antifungal resistance experienced in European centres.

From clinical observations it also appears that moulds such as *Fusarium spp, Aspergillus spp* and other fungi living free in the environment are involved as agents of mycoses and many species show a primary resistance to anti-fungal drugs including azoles. This resistance indeed could be due to exposure to fungicides in agriculture, although the extent of primary resistance to certain azoles is unlike that seen with other fungi and suggests that prior exposure to antifungals may not be the cause However, research would be needed to substantiate this. The incidence of these infections has increased over the past thirty years. It is unlikely that this has been influenced by the use of prophylactic antifungal agents to which organisms such as *Aspergillus* are resistant. In the case of other mould fungi including *Fusarium* species the numbers of cases remains small even though they have increased in recent years.   The main explanation for the rise in aspergillosis and other mould infections in humans has been more likely the increased use of immunosuppressive regimens that ablate the patient's immune system, such as those used for conditioning prior to stem cell transplantation.

c) **Other antifungals**, apart from those discussed above , are associated with intrinsic resistance. For instance there is a higher prevalence of resistant strains of *Candida lusitaniae* to amphotericin B. However once again this is a rare organism and there is no possibility for transfer of resistance to other fungi.

The foregoing would suggest that there has been an increase in the frequency of isolation of resistant fungi, mainly *Candida* species to antifungals specifically to azoles. However the risk of resistance is correlated with the presence of immunodeficiency in the host population.

Importantly, there is evidence that the acquired resistance is to a large extent reversible because the introduction of a policy to reduce the use of azoles in populations at risk from antifungal resistance is accompanied by a reduction in colonization or infection by resistant *Candida* species (Lopez *et al,* 2001). In other words prudent infection control policies and the use of adjunctive therapies can re-instate the normal pattern of human fungal microflora. The risk of azole resistance is therefore real but associated with a) specific

organisms b) specific host conditions and c) is reversible by reducing immunosuppression or introducing policies for limiting exposure to the relevant antifungals.

In conclusion these observations suggest that there has been a rise in the incidence of drug resistant Candida infections but that this has now reached a stable level and in some units has actually fallen. This is associated with changes in the management of HIV infection and the implementation of appropriate control measures. This situation could change should, HAART therapy fail to control HIV viral replication in AIDS patients.

III.3. AZOLE RESISTANCE IN AGRICULTURE

Azoles are widely used in agriculture in the European Union and their use has gradually increased from the mid-seventies until the end of the century. It is estimated that currently slightly less than half of the total EU acreage under cereals and grapevine are treated annually with azole fungicides. This compares, for example to less than 5% of the total crop area treated annually in the USA. This difference in azole usage between the two major agricultural areas is important. Despite this difference in usage there is no difference in the prevalence of resistance to treatment.

There exists a large variability across fungi species and fungi strains with regard to their sensitivity to anti-fungal molecules and the development of resistance to fungicides amongst fungi is known. The existence of resistance amongst plant-specific fungi and field fungi which are not pathogenic to crops has been observed under field conditions. This problem has been considered so far to be limited in its extent and manageable and is not considered to have reached levels that cause widespread concern. Although levels of resistance to azoles have increased over a period of years; there has been no recent evidence of a rapid increase in resistance – due largely to containment as a result of good agricultural practices. There are also no indications that the incidence and extent of resistant fungi in the agricultural environment is currently increasing. The organisms that have demonstrated resistance to azole fungicides, and their prevalence seems to vary from year to year and the resistance observed so far seems to have been reversible in many cases.

One reason for this containment is that farmers have been encouraged to follow guidelines aimed at reducing the probability of developing resistance and/or to minimise its extent and/or to increase the chances that any developed resistance would still be reversible. Such practices include the rotational use of anti-fungal products with different modes of action, the preferential practice of a limited number of interventions with higher doses as opposed to more frequent applications with lower doses. These practices seem to have been effective in controlling the rate of development of resistance.

This does not, however, imply that the use of antifungal substances in agriculture should not be closely monitored because of the risk of accumulation of azoles in the soils (the ½ life time of azoles is approx>. 1 year) and because also the newer compounds may be at the origin of resistance development.

The above rather optimistic picture does not exclude the possibility that at the level of certain individual organisms, there may exist a problem of developed / acquired / natural reduced sensitivity to azole fungicides. The possible link, if any, with normal agricultural uses of azoles has, however, not been established.

An important limitation when addressing the issue of resistance linked to the agricultural use of azoles, is the lack of comprehensive data including:

- Quantities of azoles used; mode and frequencies of application; target crops; target organisms;
- Residue levels on food and feed; the relation (if any) between azole residue levels in/on foods and the development of resistance in certain human fungal pathogens;
- Effects on/or involvement of non-targeted organisms (banal fungi, saprophytic fungi, ...);
- Fungi and moulds present in the agricultural environment and affected by the use of azoles, that are also of potential interest in the medical environment (eg, with the potential of becoming opportunistic pathogens in immuno-supprssed patients);
- The effects of the use of azoles on the expansion of fungi that are naturally resistant and that may eventually constitute a new ecologically based risk (eg, residual levels of certain myco-toxins on foodstuffs);
- The prevalence of resistance in fungi that are a risk in the human clinical environment.

III.4. DO AZOLES USED IN CROP PROTECTION OR AS BIOCIDES IMPOSE A RISK OF RESISTANCE IN HUMAN POPULATION?

Primary (intrinsic) resistance

Intrinsic resistance against certain azoles used in agriculture is known for a number of micro-organisms / fungi such as Fusarium and it is not excluded that this may increase their presence in the environment where azoles are used because of a decreased competition with susceptible species. Although cases have been occasionally described in clinical practice of humans infected by such organisms (e;g., *Fusarium spp.*), their incidence is extremely low and without evidence of increasing. For these reasons they are not considered to be a matter of concern.

Secondary (acquired) resistance

The work described in previous sections shows that the main route for selection of resistant fungal populations depends on substitution of the normal flora with resistant strains. In order for this to happen the drug concentrations have to be sufficiently high to allow mutants with resistant genes to replace the original population which is suppressed by use of azole antimycotics. The small doses to which human populations are exposed via biocide mechanism are considered unlikely to affect the normal population of yeasts and the risk of drug resistance developing by this mechanisms is likely to be low. It is also apparent that the process of development of drug resistance is slow and not efficient and that the existence of large populations of yeasts as part of the normal or disease flora is a prerequisite for the development of resistance.

At present it is not possible to exclude the above mechanisms although it seems unlikely and not an immediate risk. It would be possible to carry out experimental animal work or in vitro laboratory experiments to establish whether resistance might arise through this mechanism and estimate the risk of this happening. There is a potential for azole cross resistance, from azoles used in agriculture to those used as medicines, but there is no conclusive evidence that this has occurred and once again this is amenable to scientific investigation.

III.5. CONCLUSIONS:

The resistance of pathogenic and nosocomial fungi against antimycotic drugs has been increasing until recently. The rate of that increase, however, appears to have halted and in some cases reversed as a result of innovative treatments and risk management strategies in clinical medicine. Similarly, the rate of increase in resistance of fungi in agriculture has also been successfully limited by the introduction of appropriate control measures. Experimental, clinical and field evidence indicating that the observed resistance in clinical practice might be related to the agricultural use of azole fungicides or biocides is lacking:

- Although there is evidence that certain agents (mainly *Candida albicans*) can mutate into other (more resistant) strains, there is no mechanism of transmission of that resistance to other species. Clinical observations have shown that if the selection pressure is reduced, the problem of resistance declines.

- There have been cases reported of humans affected by agents known as intrinsic azole resistant plant pathogens (e.g., *Fusarium spp*), but the number of cases is few, is not increasing and is too small to allow any conclusion. Other environmental organisms such as Aspergilli have been known as human pathogens for many years and there is no evidence to suggest that there has been a significant increase in azole resistance amongst these..

- Azole resistance in human medicine as described in the report of the Working Group, has been observed in both the EU and in the USA. However, the use of azoles in agriculture in the USA is extremely small as compared to the EU where up to 49% of the agricultural area is treated annually with azoles. This substantially reduces the likelihood that the development of resistance is related to the use of azole fungicides in crop protection.

- Although, the molecular, genetic and cellular mechanisms of this emerging resistance are poorly known, it seems, at the moment, that the resistances are linked to individual compounds and products and not to azoles as a group of compounds.

For all the above reasons, the Working Group does not consider that resistance against azole antimycotics is an increasing problem nor that there is evidence that the resistance to azole fungicides observed in clinical medicine is due to agricultural practice. However, two remarks need to be made:

- The increasing azole resistance in human medicine observed some years ago appears to have been halted and reduced because of risk management practices such as innovative treatments of HIV (e.g., the HAART therapy – see above). The effects of such innovations may be temporary and it is obvious that both the further evolution of azole resistance and the risk management efforts in medicine need to be maintained and monitored continuously.

- The fact that there is no apparent link between agricultural practices and azole resistance in human medicine does not imply that resistance risk management practices in agriculture could be reduced. Reducing them could create a different risk scenario with increasing selection pressure resulting from, for example, higher doses or the accumulation of azoles in the soil.

III.6. Options to manage the resistance

The options to manage this resistance are fundamentally similar to those already proposed by the SSC (Report of May 1998) for the management of bacterial antibiotic resistance most of which are already put into practice. They include

a) Prudent and restricted use of fungicides in terms of rotation of products, doses and periods of application;

b) Use of alternative fungicides in those cases where resistance to treatment is observed;

d) limiting the uses in agriculture by proper adherence to integrated pest management and resistance management strategies Using different azoles in agriculture from those used in human medicine.

There is further a need for :

a) Surveillance and collection of precise information which might permit scientific evaluation of the causes and options for management;

b) Research at epidemiological and laboratory level.

A difference has to be made between the primary or secondary resistances and between yeasts and moulds as a modulation of the risk assessment. Usually the secondary resistance, more frequently observed in yeasts, is not an increasing problem: this type of resistance is always in relation with a long-term azole therapy as it has been mentioned in the report. Moreover in most of the cases the fungus involved in secondary resistance is *Candida albicans* which is a well-known endosaprophytic yeast, living in the digestive tract of the patient, i.e., an environment more or less sheltered from the azoles sprayed on the fields.

Yet, there is an increase in infections due to intrinsically azole-resistant non-albicans *Candida spp* (= selection of primary resistant yeasts) as shown in numerous multicenter studie, but also probably due to the wide utilisation of azoles in vivo as prophylaxis and from there also independent of the use of azole in agriculture.

More alarming is the effect of invasive mould infections due to *Aspergillus spp, Fusarium sp, Scedosporium sp* or Mucorales which continue to rise. Here one has to deal with real exosaprophytic filamentous fungi living as saprophytes in the external environment and consequently in contact with the azoles used in agriculture and most of the species are considered as inherently resistant to one or more azoles. It has not been established for sure that these are always a real primary resistance.

Whereas there is no evidence that the acquisition of such resistance is due to agricultural practice, there is also no proof for its absence. In consequence, it is recommended that at least for those species to compare isolates from before and during the azole era.

Another field of research might include an assessment of the extent to which cosmetic (eg, shampoos) and medicinal uses (dermatological applications) are contributing to the development of azole resistance in fungi.

IV ACKNOWLEDGEMENTS

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