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Collateral consequences of agricultural fungicides on pathogenic yeasts: A One Health perspective to tackle azole resistance

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Abstract

Candida and *Cryptococcus* affect millions of people yearly, being responsible for a wide array of clinical presentations, including life-threatening diseases. Interestingly, most human pathogenic yeasts are not restricted to the clinical setting, as they are also ubiquitous in the environment. Recent studies raise concern regarding the potential impact of agricultural use of azoles on

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CONFLICTS OF INTEREST

All authors declare that they have no conflict of interest.

resistance to medical antifungals in yeasts, as previously outlined with *Aspergillus fumigatus*. Thus, we undertook a narrative review of the literature and provide lines of evidence suggesting that an alternative, environmental route of azole resistance, may develop in pathogenic yeasts, in addition to patient route. However, it warrants sound evidence to support that pathogenic yeasts cross border between plants, animals and humans and that environmental reservoirs may contribute to azole resistance in *Candida* or other yeasts for humans. As these possibilities could concern public health, we propose a road map for future studies under the One Health perspective.

INTRODUCTION

Most medically relevant fungal species are ubiquitous in the environment and have a worldwide distribution. They affect over a billion people and include major pathogens responsible for chronic or life-threatening diseases, such as invasive aspergillosis, candidiasis and cryptococcosis.[1, 2] However, the antifungal armamentarium is severely limited.[3] Five distinct classes are globally available (azoles, echinocandins, polyenes, allylamines and pyrimidine analogues), and a sixth, a triterpenoid antifungal, has recently been approved by the US Food and Drug Agency (FDA) for the treatment of vaginal candidiasis. Among these six antifungal classes, azoles are most widely used to treat both candidiasis and aspergillosis. Azole resistance has been associated with long-term azole therapy and clinical failure, as illustrated in patients with oropharyngeal candidiasis or pulmonary aspergillosis.[4] However, another route of azole resistance acquisition, through exposure to azole fungicides in the environment, has been confirmed as a driver of increasing azole resistance in *A fumigatus*.[5–8] In this context, new environmentally selected resistance mutations have been described, suggesting that this phenomenon is continuously evolving.[9, 10] Finally, several environmental hotspots for the development of azole resistance in *A fumigatus* have been identified.[11]

A vast array of medically relevant yeasts is also found in the environment where they may, like *A fumigatus*, be exposed to agrochemical compounds. This observation raises important questions: Could this environmental exposure select for yeast genotypes with cross-resistance to medical antifungals, as previously reported for *A fumigatus*? Do we have evidence to support that resistance is already evolving in the environment, animals or humans? Are environmental fungal populations currently subject to selective pressure for antifungal resistance or simply a reservoir for genotypes already selected by clinical exposure or naturally present as standing genetic variation? To answer these questions, we undertook a literature review to search for lines of evidence that environmentally derived azole resistance may also occur in pathogenic yeasts.

AGRICULTURAL FUNGICIDES, WHY ARE THEY USED AND WHAT ARE THE CONSEQUENCES OF THEIR USE?

Fungicides are chemical compounds that kill fungi or inhibit their growth. They are widely used in agriculture to preserve the yield and quality of crops by preventing and controlling fungal diseases and as growth regulators. Fungicides are also used in floriculture (bulb industry and flower fields) in which they are used as growth regulators and for disease

control. The number of agricultural fungicides and drug classes exceeds that of medical antifungals. However, not all have broad-spectrum activity, and for many major pathogens, fungicide options are increasingly limited both by the evolution of resistance to multiple fungicide classes and by regulatory changes, such as the recent withdrawal of chlorothalonil from the European Union market. Benzimidazoles, dithiocarbamates, strobilurins (Qol) and azoles are the most common classes used in the fields, of which azoles constitute the largest class, with more than 30 licenced compounds.[12, 13] Azoles have been particularly successful due to their low cost and systemic action, allowing both prevention and treatment of fungal diseases, long-lasting stability in the environment and broad antifungal spectrum.[11, 14] For many plant pathogens, azole resistance has evolved in a step-wise, quantitative manner, such that robust doses of new, more active azoles can still achieve good levels of plant disease control, in contrast to methyl benzimidazole carbamates and QoI resistance, which has resulted in control failure in many crop pathogens.

Fungicides have become an integral part of efficient food production where the global consumption has evolved from 207–269 kg/Ha in 2000–2014 to 263 kg/Ha in 2018, however, with notable geographical variations (source FAOSTAT.org). Fungicide use exceeded 5 kg/Ha in Brazil, Italy and the Netherlands; 10 kg/Ha in China, Guatemala, Japan and Taiwan; and reached 20 kg/Ha in Costa Rica and Ecuador during 2018. In Europe, fungicides represent roughly 40% of total pesticide sales. The consumption of azole fungicides has quadrupled in the United States over the last decade.[15] Environmental uses of fungicides outside of agriculture include timber treatment and other material preservation.

Fungicides have been demonstrated to select for resistance in fungal phytopathogens, which threatens food production.[12, 16] Fungicides with a long degradation time, accumulate in the environment[17, 18] and have been found in soil and surface water from agricultural areas and urban surroundings in various countries.[19–21] As most of these fungicides have a broad-spectrum activity, environmental exposure may trigger ecological imbalances and increase tolerance and resistance to these compounds in non-targeted fungi. Because agricultural and medical azoles share the same mode of action and have similar structures, the risk of cross-resistance with medical azoles was noted as early as 2001[14] and subsequently demonstrated for *A fumigatus*.[6, 8] Initially, five triazole fungicides, all introduced in the fields between 1990 and 1996, were identified as potential drivers of this resistance, before the isolation of the first azole-resistant environmental strain.[22] This suggested that environmental practices could alter the susceptibility of a human fungal pathogen, potentially leading to clinical failure in patients receiving medical azoles.[23]

Cross-resistance to medical and agricultural azoles has also been found in clinically relevant *Candida* and other yeasts from cultivated areas, raising the hypothesis that azole fungicides may also trigger cross-resistance in yeasts.[24] Indeed, in vitro studies have associated exposure to azoles fungicides with decreasing susceptibility to medical azoles in various *Candida* and/or *Cryptococcus* species.[25–31] As an example, in vitro exposure to prochloraz leads to stable fluconazole and voriconazole resistance in *C glabrata*.[25] Similarly, exposure to tebuconazole and tetraconazole selects for resistance to fluconazole in C *parapsilosis*, *C orthopsilosis* and *C metapsilosis*.[29] Besides antifungal resistance, some studies underlined other genotypic/phenotypic changes, such as genetic instability[31] or increased virulence.[32]

Some experiments identified active efflux and/or overexpression of the gene encoding the azole target lanosterol 14-alpha-demethylase as possible mechanisms explaining cross-resistance with medical azoles.[25–27, 29] Interestingly, these mechanisms and target gene mutations are also common in fungal filamentous phytopathogens following environmental exposure to fungicides.[16] These observations suggest that antifungal resistance may emerge in any fungus upon selection by antifungal pesticides, including human pathogenic yeasts.

THE ENVIRONMENTAL SPHERE: IS OUR ENVIRONMENT AN UNDERESTIMATED RESERVOIR FOR PATHOGENIC YEASTS WITH ACQUIRED ANTIFUNGAL RESISTANCE?

Opulente et al, studied yeast biodiversity in environmental samples across the United States and identified 54 species of budding yeasts, including the four most prevalent human pathogenic species *C albicans*, *C parapsilosis*, *C glabrata* and *C tropicalis*.[33] Except for *Cryptococcus*,[34] little is known about the potential role of the environment as a reservoir of opportunistic yeasts. However, *Candida* species have been repeatedly isolated from all types of managed soils, including agricultural, orchards and vineyards,[35] trees and other plants[33, 36, 37] and compost.[38] In addition, yeasts are found in surface water or sediment from aquatic environments and polluted wetlands and in hospital potable water.[39–41] Pathogenic yeasts are also found in dwellings, as illustrated with dishwashers and laundry machines,[42, 43] and on vegetables, fruits and cheese.[44, 45] Even *Candida auris*, a multidrug-resistant species which has recently emerged worldwide in healthcare settings, has also been found in the environment.[46, 47]

Whether these yeasts have been introduced through animal/human faeces, waste or are natural parts of the environment is a matter of debate, but whole-genome analyses of *C albicans* and *C glabrata* isolates suggest that environmental populations of these species can evolve independently.[48, 49] Taken together, these observations challenge the usual concept that these pathogenic yeasts are ‘obligate’ commensals and suggest that yeast ecology and lifestyle could be more complex than previously thought.[50]

Opportunistic yeasts present in topsoil, composting soils and plant microbiota may be exposed to agrochemicals which may in turn affect their biology and resistance to antimicrobials. To the best of our knowledge, the first description of acquired antifungal resistance in environmental yeasts dates from 2008, with the recovery of fluconazole-resistant *C tropicalis* from freshwater environments in Brazil.[51] Subsequently, Zuza-Alves et al reported a rate of fluconazole resistance as high as 43% in environmental *C tropicalis* from a Brazilian sand beach, with 24% of the isolates being multi-azole resistant.[52] Similar observations were reported from soil samples in Taiwan,[53] and among *C albicans* isolates from environmental sources in Brazil, Japan and South Africa.[54–56] Additionally, various levels of resistance to the medical antifungals fluconazole and flucytosine have been described in environmental *Cryptococcus* isolates.[57, 58]

However, studies that have focused on the topic of environmentally acquired resistance in yeasts are scarce and associated with some limitations: i) the use of non-standardised

methods for antifungal susceptibility testing; ii) the frequent occurrence of trailing growth during in vitro susceptibility assays, especially among *C tropicalis*, which may hamper MIC reading and potentially lead to overestimation of azole resistance; iii) the lack of molecular investigations to understand the genetic basis of resistant phenotypes; iv) the lack of correlation between the observed resistance/reduced susceptibility and fungicide exposure. Nevertheless, these observations are, however, intriguing and highlight the need for in-depth studies to confirm these findings and identify possible antifungal resistance hotspots.

THE ANIMAL SPHERE: ACQUIRED RESISTANCE IN YEASTS FROM ANIMALS AND THEIR ROLE AS POTENTIAL RESERVOIR AND SPREADERS OF RESISTANT ISOLATES

Candida species are common members of cutaneous and mucosal microbiota of animals, acting both as commensals and pathogens. The most frequently colonised anatomical sites include the gastrointestinal, urogenital and respiratory tracts, eye conjunctiva, ear canal and skin.[59] *Candida albicans*, *C tropicalis*, *C parapsilosis*, *C famata*, *C krusei*, *C guilliermondii* and *C glabrata* are the most commonly described commensal species.[60–69] *Candida* may act as primary pathogens, causing oral, esophageal or crop candidiasis in birds and mastitis in cows. Under favourable conditions such as young age, inadequate husbandry, disruption of epithelial barriers, use of antibacterial drugs, impaired immune system, etc, they may also become opportunistic pathogens.[59]

As opposed to human clinical isolates, antifungal susceptibility testing of *Candida* from veterinary sources is seldom performed. Nevertheless, there is an increasing number of reports of azole-resistant *Candida* from various animal species, including wild and domestic mammals, birds, reptiles and crustaceans.[60–69] These findings suggest the presence of an intense selective pressure in animal-associated microbial niches, which is particularly intriguing because systemic antifungal drugs are not commonly used in veterinary practices, and resistant isolates have been recovered from wild animals.[62]

Two different scenarios, not mutually exclusive, explain how environmental fungicides may affect animal microbiota: (i) direct acquisition of resistant *Candida* from the environment by ingestion or through direct contact of body surfaces with the environment; (ii) repeated exposure to fungicides accumulated in water, soils or in the food chain (*ie*, vegetables, invertebrates and vertebrates) might exert selective pressures on the animal's commensal microbiota, resulting in antifungal resistance. The latter scenario resembles what has already been described with antibiotic-resistant bacteria.[70] Animals colonised or infected with antifungal-resistant *Candida* may subsequently shed resistant isolates in the environment and contribute to their replication and dissemination. This is particularly relevant for those with migratory habits such as birds, which may carry around resistant yeasts contributing to their geographical spread, as documented with yellow-legged gulls and *C glabrata*.[65, 71]

The few attempts that have been made to elucidate the mechanisms underlying azole resistance in *Candida* from animals have identified active efflux as a mechanism,[63, 69, 72]

but the genomic changes responsible for efflux pump overexpression in animal isolates need to be determined. Future work is required to investigate whether amino acid changes in the lanosterol demethylase gene, a resistance mechanism commonly identified in human clinical isolates, also plays a role in resistance in *Candida* isolates from animals.

THE HUMAN SPHERE: ARE THERE DATA THAT SUPPORT ENVIRONMENTAL EXPOSURE OF PATHOGENIC YEASTS TO FUNGICIDES IMPACTING THE HEALTHCARE SETTING?

Antifungal resistance is increasing in yeasts. This poses a new challenge as several million people worldwide are affected each year by *Candida* and *Cryptococcus*.[2, 4, 12] In addition to the emergence of *C auris*, the most important challenges include acquired echinocandin resistance in *C glabrata* and azole resistance in *C tropicalis* and *C parapsilosis*. This situation and the paucity of available antifungal drug classes have led the CDC to list antifungal-resistant *Candida* as a serious threat of global public health importance.[73]

Azole resistance in *C tropicalis* has become particularly problematic in the Asia-Pacific region since 2010. One of the early warnings came from the nationwide TSARY surveillance programme in Taiwan.[74] Later, a single-centre prospective study during 2011–2017, showed fluconazole non-susceptible isolates in 16,9% of patients with candidemia in Taiwan.[75] At the same time, an increasing prevalence of fluconazole non-susceptible *C tropicalis*, from 11,2% to 42,7%, was observed in 10 hospitals in China between 2009 and 2014.[76] High rates of fluconazole resistance (>10%) have also been reported at different centres in Singapore,[77] Thailand and Vietnam.[78] The geographical variation of fluconazole resistance among *C tropicalis* isolates has been noticed by the 20-year SENTRY Antifungal surveillance programme (135 medical centres, 39 countries), which reported a greater than three times higher prevalence in Asia (9.2%), when compared to Europe, Latin America or North America.[79] In Canada, fluconazole resistance in *C tropicalis* was higher than *C glabrata* (12% vs 9%) based on 4,715 isolates collected during 2014–2018.[80]

In parallel, during 2016–2017, the SENTRY programme identified a mean rate of 8,8% fluconazole resistance in *C parapsilosis* with the highest prevalence in Europe (15,1%).[81] This is over 35 times higher than the mean prevalence of fluconazole resistance in *C albicans* from the same region (0,4%). There are however, huge differences between centres, with several exhibiting low resistance rates in candidemia isolates.[82] Outside Europe, emerging fluconazole resistance in *C parapsilosis* has been reported in South Korea during a multicentre surveillance programme involving eight hospitals,[83] and from different medical centres across Kuwait[84] and South Africa, with rates as high as 50%.[85] Eventually, a progressive decrease in fluconazole susceptibility has also been reported in *Cryptococcus*.[86–88]

However, whether this apparent increase in antifungal resistance in the clinical setting is only related to clinical antifungal use or is partly related to environmental use of fungicides, as demonstrated for *A fumigatus* remains to be determined? This environmental route has already been proposed for *C tropicalis*[53, 75] and to explain the recent emergence of *C*

auris.[89] However, documenting an environmental route of resistance for yeasts is difficult for two reasons. First, as the molecular mechanisms underlying fungicide resistance in environmental yeasts have not been thoroughly investigated, by contrast to *A fumigatus*, no specific genetic signature associated with environmental resistance has been identified. Second, in the healthcare setting, the recovery of azole-resistant strains from azole-naïve patients, which may suggest an environmental acquisition, can be alternatively explained by inter-human horizontal transmission of resistant isolates, as reported for *C parapsilosis*.[90–93] As opposed to *A fumigatus* and *Cryptococcus*, the potential role of the environment as a reservoir of resistant *Candida* can be counter intuitive, as infection with *Candida* is not air-borne, but rather involves transmission by direct contact. Interestingly, genetically related genotypes of *C tropicalis* have been found in both human hosts and environmental samples (soil and fruits) in Taiwan and China, suggesting that this species can circulate between different niches.[44, 53, 75] Besides, population genomics shows no distinction between environmental *Pichia kudriavzevii* and pathogenic *Candida krusei* which is used to make some fermented foods.[94] Even alarming that *C auris* which was first identified in 2009, has become a global concern because of multidrug resistance and increase rapidly.[95] *Candida auris* colonises human skin successfully, may contaminate and persist in the environment, and cause outbreaks in the healthcare settings or long-term care facilities.[47] Thus, it is possible that a strain with environmentally driven antifungal resistance may colonise humans through contact or, indirectly, through food and water in the community. More studies are therefore required to investigate the trafficking of pathogenic yeasts.

CALL FOR ACTION: WHAT SHOULD WE DO NOW?

Overall, the available literature indicates that *Candida* colonising environmental substrates and animals may exhibit acquired cross-resistance to medical azoles, which suggests the existence of selective pressures in certain ecological niches. Some in vitro studies support fungicide exposure, especially to agricultural azoles, as a contributor to this phenomenon. However, azoles are probably not the only agrochemical compounds capable of selecting resistance to medical azoles, as in vitro exposure to strobilurins, natamycin, benomyl and non-fungicide compounds, have been shown to affect the in vitro susceptibility to azole antifungals or polyenes, possibly due to non-target-site resistance mechanisms such as efflux.[28, 30, 31, 96]

So far, animals seem to be a hotspot for the emergence of azole resistance among *Candida* isolates, but antifungal susceptibility data from environmental and animal isolates remain scarce. Evidence of agricultural fungicides as a driver of antifungal resistance in the clinical setting is lacking. Even though the Asian data are intriguing and led some authors to suggest a possible role of environmental fungicides,[75] no similar trends have been observed in European countries, despite the massive use of agricultural fungicides. Thus, further studies are necessary to draw firm conclusions on whether ecological niches are yielding resistant strains of *Candida*. This is important as the results could guide governmental decision making to optimise the use of agrochemicals.

The concept of a vicious cycle contributing to the development of antifungal resistance among pathogenic yeasts is proposed, encompassing the three ecological spheres (Figure 1).

It emphasises the importance of the One Health approach to prevent the emergence of azole-resistant *Candida*. While this concept still remains theoretical, the initial steps to alleviate emergence of antimicrobial resistance should include antifungal susceptibility surveillance programmes, which would help to understand the epidemiology and mechanisms of antifungal resistance in human, animal and environmental isolates.[97] Altogether, these data would allow the identification of the most important drivers of resistance and the ecological niches under the greatest selective pressures (hotspots of resistance development).

To gain further insights into this potential concern, an international initiative is needed to draft a consensus protocol for the isolation of pathogenic yeasts from environmental and animal sources. Soil and water samples from fungicide-exposed and unexposed areas should be analysed. For animals, both farm animals and wild animals should be assessed. The inclusion of wild and domestic animals with diets based on plants (herbivores, frugivores, granivores), including livestock, is critical due to potential dietary exposure to more agrochemical-treated plant material. This approach should elucidate how fungicide exposure can affect the antifungal susceptibility of pathogenic yeasts. In the clinical setting, epidemiological surveillance is necessary including colonising isolates of *Candida*, especially from superficial sites, which are more likely to have come from environmental reservoirs. Once identified to the species level, antifungal susceptibility should be determined according to international standards (EUCAST or CLSI) for the main classes of medical antifungals to determine which antifungal drugs are the most affected. Ideally, the data should be used for longitudinal surveillance, and the results correlated with fungicide concentrations in environmental samples. A plan should be put in place to share data and collate the results. Importantly, resistant isolates should be further analysed to elucidate the molecular mechanisms underlying antifungal resistance and identify possible genetic markers associated with fungicide exposure in the environment. Coupling these on-site sampling strategies with in vitro evolution experiments could be relevant to accelerate the identification of fungicides able to confer cross-resistance with medical antifungals in yeasts. Eventually, molecular typing strategies, especially whole-genome sequencing, should be used to trace the spread of resistant strains and to study their evolution in different ecological spheres.

CONCLUSIONS

We present the hypothesis that agricultural fungicides and, possibly other agrochemicals, could act as drivers selecting resistance to medical antifungals, especially azoles, among *Candida* and other pathogenic yeasts. Indeed, azole-resistant *Candida* yeasts have been recovered from environmental sources and azole-naïve animals, suggesting the presence of selective pressures in these niches, possibly associated with the use of agrochemicals. However, it is still unknown whether pathogenic *Candida* species regularly migrate between environmental niches, animals and humans and if the environment and animals act as reservoirs of resistant *Candida* isolates for humans. Further studies are needed to determine whether environmental fungicides use may contribute to antifungal resistance in pathogenic yeasts in the clinical setting. Multidisciplinary efforts under the One Health perspective are also required to accelerate the development of innovative and more sustainable therapeutic strategies allowing to fight fungal diseases with limited collateral damages.

SEARCH STRATEGIES AND SELECTION CRITERIA

Relevant publications were selected using PubMed database among English language journals using the terms: ‘environment’, ‘fungicides’, ‘agrochemical compounds’, ‘pesticides’, ‘antifungals’, ‘environment’, ‘soil’, ‘water’, ‘antifungal resistance’, ‘cross-resistance’, ‘environmental resistance’, ‘animals’. Only articles related to the most common opportunistic yeasts causing human infections (*Candida* and *Cryptococcus*) were selected. Preference was given to studies published within the past decade, although older but classical references were included when necessary.

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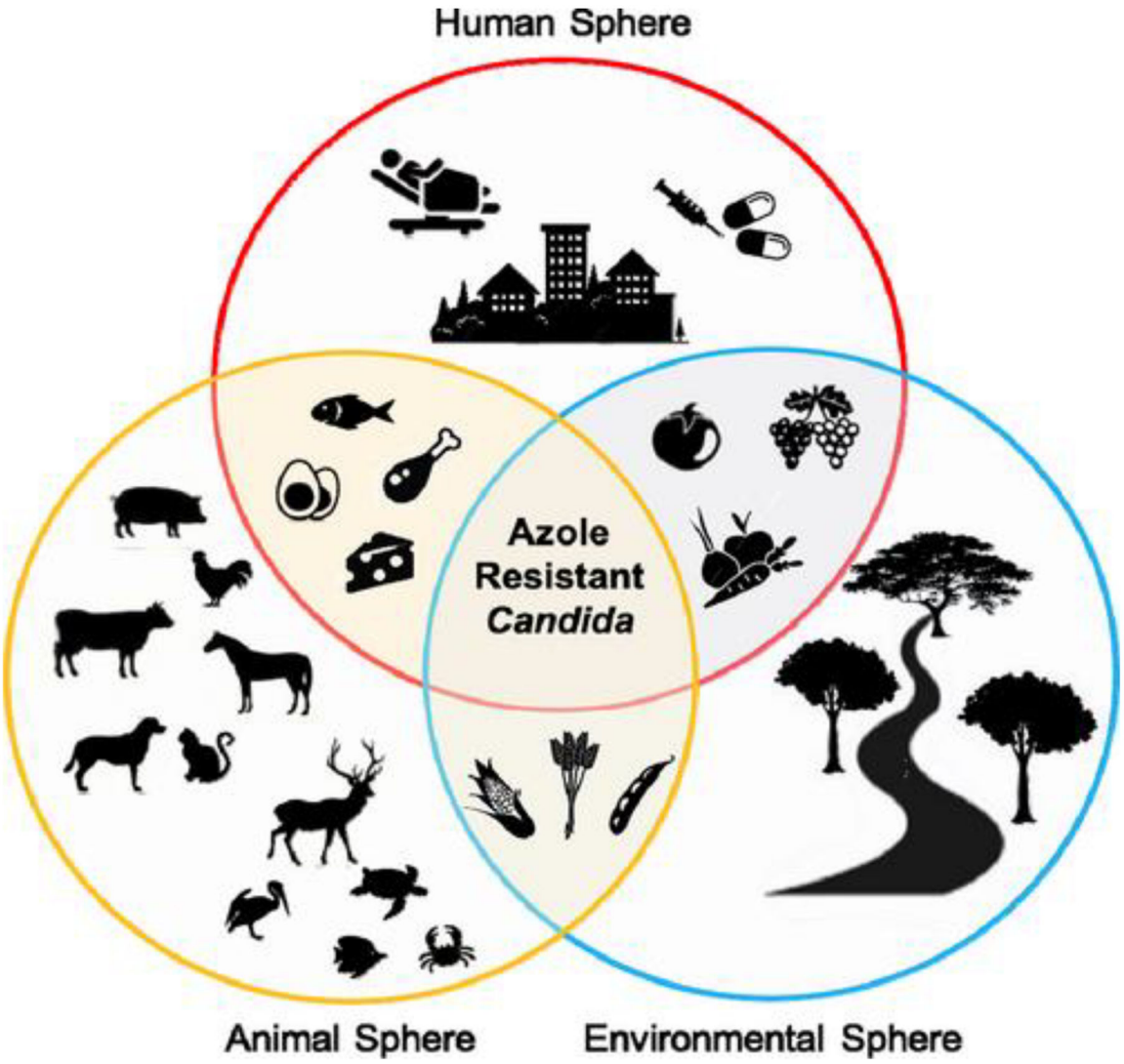

FIGURE 1.

Azole resistance cycle under the One Health perspective, applied to medically relevant yeasts. Environmental sphere: Fungicides are used in agricultural practices and are deposited in crops, soils and water causing a selective pressure in environmental *Candida* strains, which subsequently may colonise or infect humans and other animals. Animal sphere: Ingestion of residual fungicides within food items and water, and use of clinical antifungal drugs in wild and domestic animals, exerting selective pressures on commensal *Candida* or animals may be colonised or infected with resistant strains from the environment or from humans. These resistant isolates will be shed in the environment through animal faeces and farm waste. Human sphere: Clinical antifungal usage in hospital or community settings and residual fungicides within food items of animal and vegetable origins and water exert selective pressures on commensal *Candida* or humans may be colonised or infected with resistant strains from the environment or from animals. These resistant isolates will be shed in the environment through hospital or domestic sewage