Review

Is the emergence of fungal resistance to medical triazoles related to their use in the agroecosystems? A mini review

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Abstract

Triazole fungicides are used broadly for the control of infectious diseases of both humans and plants. The surge in resistance to triazoles among pathogenic populations is an emergent issue both in agriculture and medicine. The non-rational use of fungicides with site-specific modes of action, such as the triazoles, may increase the risk of antifungal resistance development. In the medical field, the surge of resistant fungal isolates has been related to the intensive and recurrent therapeutic use of a limited number of triazoles for the treatment and prophylaxis of many mycoses. Similarities in the mode of action of triazole fungicides used in these two fields may lead to cross-resistance, thus expanding the spectrum of resistance to multiple fungicides and contributing to the perpetuation of resistant strains in the environment. The emergence of fungicide-resistant isolates of human pathogens has been related to the exposure to fungicides used in agroecosystems. Examples include species of cosmopolitan occurrence, such as Fusarium and Aspergillus, which cause diseases in both plants and humans. This review summarizes the information about the most important triazole fungicides that are largely used in human clinical therapy and agriculture. We aim to discuss the issues related to fungicide resistance and the recommended strategies for preventing the emergence of triazole-resistant fungal populations capable of spreading across environments.

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Introduction

Fungicides are a key component in human therapy and the control of plant diseases caused by fungi that threaten human health and crop production. Among the several types of fungicides, theazole group (triazole and imidazole derivatives) was first introduced in the 1970s. Since then, azoles, especially the triazoles, have been widely used for the control of fungal diseases of several plants and human mycoses. As opposed to other systemic fungicides, the specific site of action of triazoles is an inherent advantage that has led to improved control efficacy of the target fungus. However, experience has shown that these compounds are prone to resistance in the pathogenic population, especially without the following of recommended practices that are aimed at prolonging the effectiveness of these fungicides.

In this context, the efficacy of triazole fungicides can be affected due to cross-resistance or when an isolate develops resistance to all fungicides in a chemical group. Some authors have also suggested that cross- and multidrug-resistance may be driving forces in the development of resistance in fungi that are at the interfaces of agroecosystem, domestic, and hospital environments. For instance, emerging fungi in clinical environments include saprophytic or plant pathogenic fungi that have previously exposed to triazole fungicides and end up spreading into the environment and infecting humans.

In this mini review, we summarize key aspects of the triazoles for therapeutic use and discuss the possible link between triazole-resistant clinical isolates and the widespread use of triazole fungicides for the control of fungal diseases, which would have a major impact in agriculture.

Basic aspects and therapeutic use of triazoles

The azole fungicides are of synthetic origin and are characterized by the presence of an aromatic five-membered heterocycle. These include triazoles (two carbon atoms and three nitrogen atoms), imidazoles (three carbon atoms and two nitrogen atoms), and thiazoles (three carbon atoms, one nitrogen atom and one sulfur atom). The characteristics of the azole rings, which are distinguished by the number of nitrogen and sulfur atoms, change the physical and chemical properties, toxicity, and therapeutic efficacies of these compounds. Therefore, the addition of different substituents to the pristine 1,2,4-triazole molecule influences its fungicide or fungistatic effect.

Triazoles affect the biosynthesis of ergosterol, a fundamental component of the fungal cell plasma membrane. The main target of antifungal azole drugs is lanosterol 14α-demethylase (Erg11 protein), a cytochrome P450 enzyme that is involved in the conversion of lanosterol to 4,4-dimethylcholesta-8(9),14,24-trien-3β-ol. The azole agents link to this enzyme using the aromatic five-membered heterocycle and thereby inhibit the cytochrome P450 catalytic activity. The absence of ergosterol and the increase of intermediate compounds alter fungal membrane integrity as well as cell morphology, which inhibits fungal growth.

Triazoles are among the most common systemic fungicides used in the control of plant diseases. Triazoles are absorbed and translocated in the plant, where they act preventively (before infection) or curatively (in the presence of symptoms) by affecting germ tube and appressoria formation or haustoria development and/or mycelial growth. By widening the window of protection beyond protectant fungicides, which act only preventatively and are not translocated, the advantages of triazoles represent a breakthrough in increasing the productivity of various crops affected by fungal diseases. Around a third of all fungicides used for the protection of crop yields include triazoles, among which more than 99% are inhibitors of demethylation (DMJ). However, triazole fungicides are also known to present long-term stability, allowing them to remain active in certain ecological niches, such as soil and water, for several months.

The number of antifungals available in the medical field for the treatment of systemic infections is relatively limited compared to those used for controlling diseases in plants, which is mainly due to problems related to erratic efficacy, drug toxicity, and intrinsic resistance. These compounds are usually effective in both topical and prophylactic treatments of invasive fungal infections. However, new triazoles that are less toxic to humans and with more specific targets have been investigated. The first generation of triazoles for human therapy included itraconazole and fluconazole. The second generation is represented by voriconazole and posaconazole, which proved to be less toxic, safer, and with a broader spectrum of activity, including activity against fungi that were resistant to the previous generation. Presently, isavuconazole, ravuconazole, and albaconazole are being investigated in phase III clinical trials as extended-spectrum triazoles with fungicidal activity against a wide number of clinically important fungi.

Development and monitoring of triazole resistance

The development of resistance to triazoles as a result of selective pressure by the continued use of regular or sub-regular dosages of fungicide is typically quantitative and expressed by a gradual change in the frequency of resistant isolates. The main mechanisms involved have been reviewed and relate to the overexpression of the CYP51 gene due to mutations (insertions or duplications) in the promoter region and an increase in molecular efflux by ABC transporters caused by the overexpression of genes coding for membrane transport. Recently, a study that examined A. fumigatus isolates from a range of clinical environments suggested point mutations of CYP51 and TFAB1/98L genomic regions in isolates obtained from patients with long term use of triazole-based therapy for the treatment of chronic aspergillosis.

A key element in the sustainable use of fungicides is to monitor the sensitivity of the pathogen population to a certain compound. There are a number of direct and indirect methods recommended for specific fungi that are aimed at estimating the EC50 (effective concentration at which 50% of fungal growth is inhibited) and MIC (minimum inhibitory concentration) values.

In the medical field, the surveillance and prevention of resistance to antifungal agents have been subject to many
restrictive actions in recent years. More specifically, the FDA (Food and Drug Administration) and the EMA (European Medicines Agency) regulate and approve the use of antimicrobials in North America and Europe, respectively. Simultaneously, the Clinical and Laboratory Standards Institute (CLSI), together with the Subcommittee on Antifungal Susceptibility Testing (AFST) of the European Committee for Antimicrobial Susceptibility Testing (EUCAST), publish in vitro test protocols periodically for monitoring fungi sensitivity to antifungal agents of clinical and veterinary use. These actions allow for the standardization of parameters for the evaluation of in vitro resistance in the laboratory. However, these actions and protocols do not involve the monitoring of resistance of plant pathogenic fungi, thus challenging the use of antifungal agents in clinical therapy.

In agriculture, the Fungicide Resistance Action Committee (FRAC), a technical group maintained by the industry, provides guidelines for the management of fungicide resistance, such as the need to estimate a baseline resistance level in isolates sampled from the population prior to the commercial use of a fungicide. During commercial use, reports of failures in disease control and detection of resistant isolates (those with sensitivity levels lower than the baseline) are indicators of the risk of developing fungicide resistance. Periodically, information is provided by the FRAC about the risk of plant pathogens that ranges from low to high. Currently, many studies are known that report steadily increasing resistance to triazoles in plant pathogenic fungi.

**Triazole resistance in clinical isolates and agricultural use**

In the medical field, the first report of DMI’s resistance in *A. fumigatus* isolates dates back more than three decades ago. However, the resistance to itraconazole by *Aspergillus* spp. from the clinical environment was first reported in 1997 for three isolates obtained from California in the late 1980s. The prescription of triazoles as a preferential choice for the treatment of patients with respiratory diseases has been considered to contribute to the development of resistance to this group of fungicides. Multidrug-resistance (MDR) is considered to be the cause of the failure of a wide range of antifungal agents available on the market. As an emergent fungus in clinical environments, *A. fumigatus* holds a history of cross-resistance and multi-resistance to azoles. It is probable that millions of people are not effectively treated due to infections by fungi exhibiting antifungal resistance, among which 4.8 million cases are related only to the species of *Aspergillus*.

The triazole antifungals commonly used in the medical field for the treatment of fungal diseases and pathogens that have exhibited some level of resistance are listed in Table 1.

**Table 1 – Pathogenic fungi with intrinsic or developed resistance to triazoles for human therapeutic use.**

| Triazole     | Fungi                                                                 | References   |
|--------------|-----------------------------------------------------------------------|--------------|
| Itraconazole | *Aspergillus fumigatus*; *Fusarium solani*; *F. oxysporum*; *Zygomycetes*; *Candida* spp. | 83-87, 63    |
| Fluconazole  | *Candida* spp.; *Saccharomyces cerevisiae*; *Trichosporon* spp.; *Fusarium* solani; *F. oxysporum*; *Scedosporium* spp.; *Penicillium* spp.; *Bipolaris* australiensis; *B. hauaiensis*; *B. spicifera*; *Aspergillus* spp.; *Dermatophytes*; *Zygomycetes*; *dimorphic fungi*; *Cryptococcus* neoformans | 73, 84, 85, 88-93 |
| Voriconazole | *Aspergillus fumigatus*; *Zygomycetes* *Trichosporon* spp.; *Penicillium* spp. | 86, 87, 94, 95 |
| Posaconazole | *Aspergillus fumigatus*                                                  | 96           |
| Ravuconazole | *Fusarium* solani; *Exymporum*; *Zygomycetes*; *Pseudallescheria* spp.; *Scedosporium* spp.; *Acremonium* spp.; *Sporothrix* schenckii; *Scopulariopsis* spp.; *Paecilomyces* spp. | 20, 24       |
| Albavucanazole | *Fusarium* solani; *Zygomycetes*                                         | 34           |
| Isavuconazole | *Aspergillus fumigatus*                                                 | 97           |

It has been shown that exposure of environmental fungi to triazole fungicides may cause shifts from susceptible to resistant populations, especially in the absence of adaptive costs which may facilitate the spread of resistant populations into diverse environments. The surge of “emerging fungi” in the medical field or fungi that are otherwise harmless to humans, such as the *zygomycetes* and other hyaline filamentous fungi, has led some authors to hypothesize that other mechanisms may be leading to resistance, such as the large amount of fungicides used in agroecosystems. This hypothesis was initially suggested by studies conducted in the Netherlands and later corroborated by studies conducted in Spain, Belgium, Norway, Great Britain, Denmark, France, China, Italy, Austria, and India.

A few studies have jointly examined the sensitivity of isolates that cause diseases in both plants and humans to triazoles. These studies suggested that the selection of fungicides with a similar mode of action as those used in human drug therapy for triazole-resistant isolates could contribute to the development of multi-resistant populations. The development of cross-resistance to triazoles and the low number of triazoles recommended for human therapy relative to the high number of triazoles used in agriculture may affect triazole efficacy for human therapy. For instance, the fungus *Colletotrichum graminicola* that causes anthracnose of corn plants is an emerging pathogen in humans. Resistance to tebuconazole as well as to multiple otherazole antifungals has been reported in plant pathogenic populations used in clinical medicine. Similarly, cross-resistance to triazoles was observed in clinical isolates of *Candida albicans* and agricultural environmental yeasts.

Several other fungi have been found in association with human and animal diseases, including species of several genera such as *Bipolaris*, *Macrophomina*, *Aspergillus*, *Fusarium*, *Alternaria* and *Muco* (Table 2). The pathogenicity of clinical isolates of the *Fusarium solani* species complex was confirmed in plants of the Cucurbitaceae family, which exhibited similar aggressiveness to isolates originating from diseased plants. *Cryptococcus neoformans* is also found in different environmental niches, such as plants and animals.
Fluconazole is the most prevalent clinical antifungal used to treat cryptococcosis. However, the continued use of this antifungal is an increasing concern due to the frequency of isolates resistant to triazoles used in human therapeutic use. There is a need for attention to azole resistance and optimal therapy in regions with high incidence of cryptococcosis, such as the Asian-Pacific region (5.1–22.6%), Africa/Middle-East (7.0–33.3%), and Europe (4.2–7.1%). In addition to fluconazole resistance in these regions, the new point of mutation in the ERG11 gene of C. neoformans afforded resistance to voriconazole (VRC). In these cases, the spread of isolates exhibiting resistance to triazoles into the environment and those capable of causing human diseases may affect the efficacy of therapeutic control with fungicides of the same group, especially in the presence of cross-resistance.

The mutagenesis in TR34/L98H in azole-resistant Aspergillus may have originated due to the use of triazole fungicides in agroecosystems. Such mutation was detected in 89% of A. fumigatus-resistant isolates from air samples, flowers, and soils from hospital areas. Microsatellite sequencing of clinical and environmental isolates that lead to the TR34/L98H mutation revealed high genetic homology, which suggests a common ancestor.

### Future directions

Triazole antifungals largely used in plant protection are also important as antifungal treatments in the human medical field even though they possessing structural differences. However, sensitive populations that co-inhabit environments may be reduced by the selection of isolates resistant to fungicides. Fungi arising from agricultural ecosystems as opportunistic pathogens may carry cross-resistance to triazoles used in the medical field. The restricted number of antifungal agents for clinical use, which contrasts with the large number of agricultural fungicides with similar modes of action, may be a risk factor that limits the success of the therapeutic use of these drugs.

Currently, genome-wide studies, together with novel T-cell-based therapeutic approaches for the prophylaxis and treatment of opportunistic fungal infections, have promising avenues of research in the detection of potentially new antifungal targets. Thus, different strategies should be the main goals of the pharmaceutical industry.

### References

1. Horsfall JG. Fungi and fungicides. The story of a nonconformist. Annu Rev Phytopathol. 1975;13:1–14.
2. Hof H. Critical annotations to the use of azole antifungals for plant protection. Antimicrob Agents Chemother. 2001;45:2987–2990.
3. Russel PE. A century of fungicide evolution. J Agric Sci. 2005;143:11–25.
4. Dehne HW, Deising HB, Gisi U, Kuck KH, Russell PE, Lyr H. Modern. Fungicides and antifungal compounds. In: International Reinhardtbrun Symposium Friedrichroda. Vol. 15. 2007:45–51.
5. Salam KP, Tomas JG, Beard C, Loughman R, Mac Leod WJ, Salam MJ. Application of meta-analysis in plant pathology: a case study examining the impact of fungicides on wheat yield loss from the yellow spot-septoria nodorum blotch disease complex in Western Australia. Food Secur. 2013;5:319–325.
6. Snelders E, Huis In’t Veld RA, Rijks AJ, Kema GH, Melchers WJ, Verweij PE. Possible environmental origin of resistance of Aspergillus fumigatus to medical triazoles. Appl Environ Microbiol. 2009;75:4053–4057.
7. Brown GD, Denning DW, Levitz SM. Tackling human fungal infections. Science. 2012;336:647.

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**Table 2 – Main genera of fungi reported as the causative agents of diseases in plants and in humans.**

| Genus      | Species                                                                                   | References       |
|------------|-------------------------------------------------------------------------------------------|------------------|
| Fusarium   | F. dimerum; F. verticillioides; F. solani; F. oxysporum; F. graminearum; F. poae; F.    | 18, 98, 99       |
|            | sporotrichoides; F. culmorum                                                             |
| Alternaria | A. alternate                                                                              | 100, 101         |
| Aspergillus| A. flavus; A. par                                                                        | 18, 98, 102–104  |
|            | B. aticicus; A. terreus                                                                   | 105              |
| Curvularia | C. lunata                                                                                 | 106              |
| Cladosporium| C. cladosporioides                                                                       | 107              |
| Colletotrichum| C. gloeosporioides, C. cocodes                                                           | 68, 108          |
| Mucoor     | M. pinforms                                                                              | 109              |
| Absidia    | Absidia spp.                                                                              | 110              |
| Rhizopus   | R. arrizhus                                                                               | 72               |
| Macrophomina| M. phaseolina                                                                             | 109              |
| Bipolaris  | B. australiensis; B. hawaiiensis; B. spicifera                                           | 73, 112          |

**Conflicts of interest**

The authors declare no conflicts of interest.

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8. Cools HJ, Fraaije BA. Update on mechanisms of azole resistance in Mycophthora graminicola and implications for future control. Pest Manage Sci. 2013;69:150–155.
9. Ma Z, Michailides TJ. Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. Crop Prot. 2005;24:853–863.
10. Deising HB, Reimann S, Pascholati SF. Mechanisms and significance of fungicide resistance. Braz J Microbiol. 2008;39:286–295.
11. McGrath MT. Fungicide resistance in curcubit powdery mildew: experiences and challenges. Plant Dis. 2001;85:237–245.
12. Denning DW, Park S, Lass-Florl C, et al. High-frequency triazole resistance found in nonculturable Aspergillus fumigatus from lungs of patients with chronic fungal disease. Clin Infect Dis. 2011;52:1123–1129.
13. Snelders E, van der Lee HA, Kuipers J, Rijjs AJMM, Varga J. Emergence ofazole resistance in Aspergillus fumigatus and spread of a single resistance mechanism. PLOS Med. 2008;5:1629–1637.
14. Snelders E, Camps SM, Karawajczyk A, et al. Triazole fungicides can induce cross-resistance to medicinal triazoles in Aspergillus fumigatus. PLoS ONE. 2012;7:e31801.
15. Bowyer P, Denning DW. Environmental fungicides and triazole resistance in Aspergillus. Pest Manage Sci. 2013;70:173–178.
16. Lelièvre L, Groh M, Angebault C, Maherault AC, Didier E, Bougnoux ME. Azole resistant Aspergillus fumigatus: an emerging problem. Med Mal Infect. 2013;43:139–145.
17. Mehl HL, Epstein L. Fusarium solani species complex isolates conspecific with F. solani f. s. curcubitae race 2 from naturally infected human and plant tissue and environmental sources are equally virulent on plants, grow at 37 °C and are intervertebrate. Environ Microbiol. 2007;9:2189–2199.
18. De Lucía AJ. Harmful fungi in both Agriculture and Medicine. Rev Iberoam Micol. 2007;24:3–13.
19. Kaur S, Dhillon GS, Brar SK, Vallad EG, Chaud R, Chauhan BV. Emerging phytopathogen Macrophoma phaseolina: biology, economic importance and current diagnostic trends. Crit Rev Microbiol. 2012;38:136–151.
20. Catalán M, Montejo JC. Antifúngicos sistémicos. Farmacodinámica Y Farmacocinética. Rev Iberoam Micol. 2006;23:39–49.
21. Sheppard D, Lampires HW. Agentes antifúngicos. In: Katzung BG, ed. Farmacologia Básica e Clinica. Rio de Janeiro: Brazil: McGraw Hill Interamericana do Brasil, Guanabara Koogan; 2008:707–714.
22. Brent KJ. Fungicide Resistance in Crop Pathogens, How Can it be Managed. Brussel: Global Crop Protection Federation; 1995.
23. Becker R, Wirsel SGR. Fungal cytochrome P450 sterol 14α-demethylase (CYP51) and azole resistance in plant and human pathogens. Appl Microbiol Biotechnol. 2012;95:825–840.
24. Becker R, Hettwer U, Karlovsky P, Deising HB, Wirsel SGR. Adaptation of Fusarium graminearum to tebuconazole yielded descendants diverging for levels of fitness, fungicide resistance, virulence, and mycotoxin production. Phytopathology. 2010;100:444–453.
25. Serfling S, Ordon F. Virulence and toxin synthesis of an azole insensitive Fusarium culmorum strain in wheat cultivars with different levels of resistance to Fusarium head blight (FHB). Plant Pathol. 2014;63:1230–1240.
26. Buchenauer H. Mechanism of action of triazolyl fungicides and related compounds. In: Lyhr H, ed. Modern Selective Fungicides: Properties, Applications, Mechanisms of Action. Harlow, United Kingdom: Longman Scientific and Technical; 1987:205–231.
27. Pontzen R, Scheinflug H. Effects of triazole fungicides on sterol biosynthesis during spore germination of Botrytis cinerea, Venturia inaequalis and Puccinia graminis f. sp. tritici. Netherl. J Plant Pathol. 1989;95:151–160.
28. Chowdhary A, Kathuria S, Randhawa HS, Gaur SN, Klaassen CH. Isolation of multiple-triazole-resistant Aspergillus fumigatus strains carrying the TR/I98H mutations in the cyp51A gene in India. J Antimicrob Chemother. 2012;67:362–366.
29. Hamey PY, Harris CA. The variation of pesticide residues in fruits and vegetables and the associated assessment of risk. Regul Toxicol Pharmacol. 1999;30:34–41.
30. Bergold AM, Georgiadis S. Novidades em fármacos antifúngicos: uma revisão new antifungal drugs: a review. Visão Acad. 2004;5:159–172.
31. Hay R. Antifungal drugs. In: Katsambas A, Lotti T, eds. European Handbook of Dermatological Treatments. Berlin, Germany: Springer; 2003:700–710.
32. Carrillo-Munóz AJ, Giusiano G, Ezcurra PA, Quindóz G. Antifungal agents: mode of action yeast’s cell. Rev Esp Quimioter. 2006;19:130–139.
33. Girmenia C. New generation azole antifungals in clinical investigation. Expert Opin Invest Drugs. 2009;18:1279–1295.
34. Pasqualotto AC, Thiele KO, Goldani LZ. Novel triazole antifungal drugs: focus on isavuconazole, ravuconazole and albaconazole. Curr Opin Invest Drugs. 2010;11:165–174.
35. Thompson GR, Cadena J, Patterson TF. Overview of antifungal agents. Clin Chest Med. 2009;30:203–215.
36. Vandeputte V, Ferrari S, Coste AT. Antifungal resistance and new strategies to control fungal infections. Int J Microbiol. 2012;71:3687:1–26.
37. Coleman JJ, Mylonakis E. Efflux in fungi: la pièce de résistance. PLoS ONE. 2009;5:1–7.
38. Ammar GA, Tryono R, Do K, Karlovsky P, Deising HB, Wirsel SGR. Identification of ABC transporter genes of Fusarium graminearum with roles in azole tolerance and/or virulence. PLoS ONE. 2013;8:2–13.
39. Parnell S, van den Bosch F, Gilligan CA. Large-scale fungicide spray heterogeneity and the regional spread of resistant pathogen strains. Phytopathology. 2006;96:549–555.
40. Suzuki F, Yamaguchi J, Koba A, Nakajima T, Arai M. Changes in fungicide resistance frequency and population structure of Pycyrriza oryzae after discontinuation of MBI-D fungicides. Plant Dis. 2010;94:329–334.
41. Van der Heyden H, Dutilleul P, Brodeur L, Carisse O. Spatial distribution of single nucleotide polymorphisms related to fungicide resistance and implications for sampling. Phytopathology. 2014;104:604–613.
42. Edgington LV, Khaw KL, Barron GL. Fungitoxic spectrum of benzimidazole compounds. Phytopathology. 1971;61:42–44.
43. Liu X, Yin Y, Wu J, Jiang J, Ma Z. Identification and characterization of carbendazim-resistant isolates of Gibberella zeae. Plant Dis. 2010;94:1137–1142.
44. Spolti P, Jorge BC, Del Ponte EM. Sensitivity of Fusarium graminearum causing head blight of wheat in Brazil to tebuconazole and metconazole fungicides. Trop Plant Pathol. 2012;37:419–423.
45. Spolti P, Del Ponte EM, Dong Y, Cummings JA, Bergstrom GC. Triazole sensitivity in a contemporary population of Fusarium graminearum from New York wheat and competitiveness of a tebuconazole-resistant isolate. Plant Dis. 2014;98:607–613.
46. Aparicio JF, Mendes MV, Antón N, Recio E, Martín JF. Polyenemacrolide antibiotic biosynthesis. Curr Med Chem. 2004;1:645–1656.
47. Brent KJ, Hollomon DW. Fungicide resistance in crop pathogens: how can it be managed? FRAC Monograph n.1. 2007.
48. FRAC. FRAC cod list. FRAC information: list of resistance. Available in: http://www.frac.brasil.org.br (accessed 05.05.15).
49. Denning DW, Venkateswarlu K, Oakley KL, Anderson MJ, Manning PJ, Stevens DA. Itraconazole resistance in Aspergillus fumigatus. Antimicrob Agents. 1997;41:1364–1368.
50. Shao PL, Huang LM, Hseuh PR. Recent advances and challenges in the treatment of invasive fungal infections. Int J Antimicrob Agents. 2007;6:487–495.
51. Gore RB. The utility of antifungal agents asthma. Curr Opin Pulm Med. 2010;16:36–41.
52. Guishan K, Moey-Rowley WS. Multidrug resistance in fungi. Eukaryot Cell. 2007;6:1933–1942.
53. Fischbach MA, Walsh CT. Antibiotics for emerging pathogens. Science. 2009;325:1089–1093.
54. Pfaffer MA, Diekema DJ. Progress in antifungal susceptibility testing of Candida sp.: by use of clinical and laboratory standards institute broth microdilution methods, 2010 to 2012. J Clin Microbiol. 2012;50:2846–2856.
55. Howard SJ, Webster I, Moore CB, Gardiner RE, Park S, Perlin DS. Multi-azole resistance in Aspergillus fumigatus. Int J Antimicrob Agents. 2006;28:450–453.
56. Denning D, Fleuvry A, Cole D. Global burden of ABPA in adults with asthma and its complication chronic pulmonary aspergillosis in adults. Med Mycol. 2013;51:361–370.
57. Verweij E, Kema GH, Zwaan B, Melchers WJ. Triazole fungicides and the selection of resistance to medical triazoles in the opportunistic mould Aspergillus fumigatus. Pest Manage Sci. 2013;69:165–170.
58. Ruping MJGT, Vehreschil JJ, Cornely OA. Patients at high risk of invasive fungal infections: when and how to treat. Drugs. 2008;68:1941–1962.
59. Tobudic S, Kratzer C, Presterl E. Azole-resistant Candida sp.: emerging pathogens. Mycoses. 2012;55:24–32.
60. Rodriguez-Tudela JL, Alcazar-Fuoli L, Mellado E, Alastruy-Izquierdo A, Monzon A, Cuenca-Estrella M. Epidemiological cutoffs and cross-resistance toazole drugs in Aspergillus fumigatus. Antimicrob Agents Chemother. 2008;52:2468–2472.
61. Howard SJ, Pasqualotto AC, Denning DW. Azole resistance in allergic bronchopulmonary aspergillosis and Aspergillus bronchitis. Clin Microbiol Infect. 2010;16:683–688.
62. Mortensen KL, Mellado E, Lass-Flörl C, Rodriguez-Tudela JL, Johansen HK. Environ mental study of azole-resistant Aspergillus fumigatus and other aspergilli in Austria, Denmark, and Spain. Antimicrob Agents Chemother. 2010;54:4545–4549.
63. Burgel PR, Baixench MT, Amsellem M, Audureau E, Chapron J, Kanaan R. High prevalence of azole-resistant Aspergillus fumigatus isolates in adults with cystic fibrosis exposed to itraconazole. Antimicrob Agents Chemother. 2012;56:869–874.
64. Lockhart SR, Frade JP, Etienne KA, Pfaffer MA, Diekema DJ, Balajee SA. Azole resistance in Aspergillus fumigatus isolates from the ARTEMIS global surveillance study is primarily due to the TR/L39H mutation in the cyp51A gene. Antimicrob Agents Chemother. 2011;55:4465–4468.
65. Van der Linden JW, Arendrup MC, Verweij PE. SCARE-Network. Prospective International Surveillance of Azole Resistance in Aspergillus fumigatus (SCARE-Network). Chicago, USA: ICAC; 2011.
66. Meneau I, Sanglard D. Azole and fungicide resistance in clinical and environmental Aspergillus fumigatus isolates. Med Mycol. 2005;43:307–311.
67. Drummond DE, Reimão QJ, Dias ALT, Siqueira AM. Comportamento de mostras ambientais e clínicas de Cryptococcus neoformans frente a fungicidas de uso agronômico e ao fluconazol. Rev Soc Bras Med Trop. 2007;2:209–211.
68. O’Quinn RP, Hoffman JL, Boyd AS. Colletotrichum species as emerging opportunistic fungal pathogens: a report of 3 cases of phaeohyphomycosis and review. J Am Acad Dermatol. 2001;45:56–61.
69. Serfling A, Wohlrab J, Deising HB. Treatment of a clinically relevant plant pathogenic fungus with an agricultural azole causes cross-resistance to medical azoles and potentiates caspofungin efficacy. Antimicrob Agents Chemother. 2007;51:3014–3016.
70. Mehnna HM, Kuo T, Chaplin J, Taylor G, Morton RP. Fungal laryngitis in immunocompetent patients. J Laryngol Otol. 2004;118:379–381.
71. Srinivasan A, Wickes BL, Romanelli AM, Debenenko L, Rubinstein JE. Cutaneous Infection caused by Macrophomina phaseolina in a child with acute myeloid leukemia. J Clin Microbiol. 2009;47:1969–1972.
72. Cunha KC, Sutton DA, Fothgill AW, et al. Diversity of Bipolaris species in clinical samples in the United States and their antifungal susceptibility profiles. J Clin Microbiol. 2012;50:4061–4066.
73. Chowdhary A, Randhawa HS, Sundar G, et al. In vitro antifungal susceptibility profiles and genotypes of 308 clinical and environmental isolates of Cryptococcus neoformans var. grubii and Cryptococcus gattii serotype B from north-western India. J Med Microbiol. 2011;60:961–967.
74. Gullo FP, Rossi SA, Sardi Jde C, Teodoro VL, Mendes-Giannini MJ, Fucso-Almeida AM. Cryptococcosis: epidemiology, fungal resistance, and new alternatives for treatment. Eur J Clin Microbiol Infect Dis. 2013;32:1377–1391.
75. Paul S, Doering TL, Moey-Rowley WS. Cryptococcus neoformans Yap1 is required for normal fluconazole and oxidative stress resistance. Fungal Genet Biol. 2015;74:1–9.
76. Pfaffer MA, Diekema DJ, Gibbs DL, et al. Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: 10-year analysis of susceptibilities of noncandidal yeast species to fluconazole and voriconazole determined by CLSI standardized disk diffusion testing. J Clin Microbiol. 2009;47:117–123.
77. Sionov E, Lee H, Chang YC, Kwon-Chung KJ. Cryptococcus neoformans overcomes stress ofazole drugs by formation of disomy in specific multiple chromosomes. PLoS Pathog. 2010;6:1–13.
78. Williams DA, Lemke TL. Faye’s Principles of Medicinal Chemistry. 5th ed. New Jersey, USA: Philadelphia, Lippincott Williams and Wilkins; 2002.
79. Singh PK, Gaur SN, Hagen F, et al. Possible environmental origin of resistance of Aspergillus fumigatus to medical triazoles. Appl Environ Microbiol. 2009;75:4053–4057.
80. Barrena A, Alastruy-Izquierdo A, Martín MJ, Cuesta I, Vizcaino JA. Analysis of the protein domain and domain architecture content in fungi and its application in the search of new antifungal targets. PLoS Comput Biol. 2014;10:1–16.
81. Mancini N, Marrone L, Clementi N, Sautto GA, Clementi M, Burioni R. Adaptive T-cell therapy in the treatment of viral and opportunistic fungal infections. Future Microbiol. 2015;10:665–682.
82. Alastruy-Izquierdo A, Cuenca-Estrella M, Monzón, Emilia Mellado, A Rodriguez-Tudela JL. Antifungal susceptibility profile of clinical Fusarium spp. isolates identified by molecular methods. J Antimicrob Chemother. 2008;61:805–809.
84. Howard SJ, Cerar D, Anderson MJ, Albarrag A, Fisher MC. Frequency and evolution of azole resistance in Aspergillus fumigatus associated with treatment failure. Emerg Infect Dis. 2009;15:1068–1076.

85. Almeida LMM, Souza EAF, Bianchin DB, Svidzinski TIE. Resposta in vitro de fungos agentes de micoses cutâneas frente aos antifúngicos sistêmicos mais utilizados na dermatologia. An Bras Dermatol. 2009;84:249–255.

86. Beernaert LA, Pasmans F, Van Waeyenberghe L, et al. Avian Aspergillus fumigatus strains resistant to both itraconazole and voriconazole. Antimicrob Agents Chemother. 2009;53:2199–2201.

87. Dotta KFD, Freitas AR, Consolaro MEL, Svidzinski TIE. A challenge for clinical laboratories: detection of antifungal resistance in Candida species causing vulvovaginal candidiasis. Lab Med. 2011;42:87–93.

88. Sanguinetti M, Posteraro B, Fiori B, Ranno S, Torelli R, Fadda G. Mechanisms of azole resistance in clinical isolates of Candida glabrata collected during a hospital survey of antifungal resistance. Antimicrob Agents Chemother. 2015;49:668–679.

89. Quintero CHG. Resistencia de levaduras del género Candida al fluconazol Candida yeast’s resistance to fluconazol. Infect. 2010;14:172–180.

90. Cheong JW, McCormack J. Fluconazole resistance in cryptococcal disease: emerging or intrinsic. Med Mycol. 2013;51:261–269.

91. Adimi P, Hashemi SJ, Mahmoudi M, et al. In-vitro activity of 10 antifungal agents against 320 dermatophyte strains using microdilution method in Tehran. IJPR. 2013;12:537–545.

92. Wang JF, Xue Y, Zhu XB, Fan H. Efficacy and safety of echinocandins versus triazoles for the prophylaxis and treatment of fungal infections: a meta-analysis of RCTs. Eur J Clin Microbiol Infect Dis. 2015;34:651–659.

93. Hashemi SM, Badali H, Faramarzi MA, et al. Novel triazole alcohol antifungals derived from fluconazole: design, synthesis, and biological activity. Mol Divers. 2015;19:15–27.

94. Van der Linden JW, Jansen JR, Bresters D, et al. Azole-resistant central nervous system aspergillosis. Clin Infect Dis. 2009;48:1111–1113.

95. Chowdhary A, Kathuria S, Agarwal K, et al. Voriconazole-resistant Penicillium oxalum: an emerging pathogen in immunocompromised hosts. Open Forum Infect Dis. 2014;1:1–7.

96. Chouki F, Botterel F, Sitterlé E, et al. Prospective evaluation of azole resistance in Aspergillus fumigatus clinical isolates in France. Med Mycol. 2015;53:593–596.

97. Gregson L, Goodwin J, Johnson A, et al. In vitro susceptibility of Aspergillus fumigatus to isavuconazole: correlation with itraconazole, voriconazole, and posaconazole. Antimicrob Agents Chemother. 2013;57:5778–5780.

98. Naiker S, Odhav B. Mycotic keratitis: profile of Fusarium species and their mycotoxins. Mycoses. 2004;47:50–56.

99. Mehl HL, Epstein L. Sewage and community shower drains are environmental reservoirs of Fusarium solani species complex group 1, a human and plant pathogen. Environ Microbiol. 2008;10:219–227.

100. Brandt ME, Warnock DW. Epidemiology, clinical manifestations, and therapy of infections caused by dermatitiece fungi. J Chemother. 2003;2:36–47.

101. Chhabra V, Rastogi S, Barua M, Kumar S. Alternaria alternata infection associated osteomyelitis of maxilla: a rare disease entity. Indian J Dent Res. 2013;24:639–641.

102. Diener UL, Cole RJ, Sanders TH, Psyne GA, Lee LS, Kich MA. Epidemiology of aflatoxin formation by Aspergillus flavus. Annu Rev Phytopathol. 1987;25:249–270.

103. Abbas HK, Williams WP, Windham GL, Pringle CH, Xie W, Shier WT. Aflatoxin and fumonisin contamination of commercial corn (Zea mays) hybrids in Mississippi. J Agric Food Chem. 2002;50:5246–5254.

104. Lass-Flori C, Mayr A, Perkhöfer S, et al. Activities of antifungal agents against yeasts and filamentous fungi: assessment according to the methodology of the European Committee on Antimicrobial Susceptibility Testing Antimicrob Agents Chemother. 2008;52:3637–3641.

105. Navi SS, Bandyopadhyay R, Reddy RK, Thakur RP, Yang XB. Effects of wetness duration and grain development stages on sorghum grain mold infection. Plant Dis. 2005;89:872–878.

106. Tournas VH. Spoliation of the vegetable crops by bacteria and fungi and related health hazards. Crit Rev Microbiol. 2005;31:33–44.

107. Weikl F, Radt V, Munch JC, Pritsch K. Targeting allergenic fungi in agricultural environments aids the identification of major sources and potential risks for human health. Sci Total Environ. 2015;52:223–230.

108. Cao J, Guarro J, Molecular Gené J. Morphological identification of Colletotrichum species of clinical interest. J Clin Microbiol. 2004;42:2450–2454.

109. Kobayashi M, Hiruma M, Matsushita K, Kawai M, Ogawa H, Udagawa S. Cutaneous zygomycosis: a case report and review of Japanese reports. Mycoses. 2001;44:311–315.

110. Miceli MH, Lee SA. Emerging moulds: epidemiological trends and antifungal resistance. Mycoses. 2011;54:666–678.

111. Cornely OA. Aspergillus to Zygomycetes: causes, risk factors, prevention, and treatment of invasive fungal infections. Infection. 2006;6:296–313.

112. El Khizzi N, Bakheshwain S, Bipolaris Parvez S. A plant pathogen causing human infections: an emerging problem in Saudi Arabia. Res J Microbiol. 2010;5:212–217.