



Recent trends in the epidemiology, diagnosis, treatment, and mechanisms of resistance in clinical *Aspergillus* species: A general review with a special focus on the Middle Eastern and North African region



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ABSTRACT

Globally, more than billion people suffer from fungal infections each year. The early diagnosis of aspergillosis is mandatory for successful treatment outcome. As careful testing takes time, epidemiological surveillance is crucial to guide individual patient therapy and to promote a high standard of health care. In this paper, we first present current trends in the epidemiology and antifungal susceptibility patterns of *Aspergillus* spp. in Middle Eastern and North African (MENA) countries in order to support infectious disease specialists and health workforces in this geographic area to treat adequately patients with aspergillosis. Then we discuss the existing literature data regarding the available diagnostic tools and antifungal resistance mechanisms of *Aspergillus* spp. Although a limited number of studies were reviewed here, the currently available data show that *Aspergillus* infections are not negligible in the MENA region, and that the emergence of antifungal resistance is a growing health issue, especially among immunocompromised patients.

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Introduction

The increase in fungal diseases challenges healthcare providers globally, with more than a billion people still falling ill with fungal infections and over 1.6 million related deaths each year [1]. In fact, *Aspergillus* is one of the most common causes of deadly fungal infections. Despite major improvements in diagnosis and treatment of aspergillosis, severe hospital-related fungal diseases are still difficult to prevent and to treat, and mortality remains high, particularly among immunocompromised patients with invasive infections [2]. Infections are typically caused by *Aspergillus fumigatus* (approximately 90%), followed by *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus terreus* and *Aspergillus versicolor* [3]. However, depending on the countries, non-*fumigatus* pathogens are now increasingly reported as common etiologic agents [4]. Precise species identification is crucial because of variations in the antifungal susceptibility profiles and of differences in the clinical presentations produced by the different species. Unfortunately, there is a lack of advanced diagnostic tools allowing an accurate identification at the species level and an antifungal susceptibility testing of fungal isolates in the majority of Middle Eastern and North African (MENA) countries. Along with these serious issues, antimicrobial resistance has a major negative health and economic impacts on MENA communities [5]. In this context, the main aim of this review is to present the current trends in the epidemiology and antifungal susceptibility profiles of *Aspergillus* spp. in MENA region in order to promote the adequate treatment of aspergillosis in this geographic area. In a second time, we discuss the existing literature data regarding the available diagnostic tools and antifungal resistance mechanisms of *Aspergillus* spp.

Epidemiology of aspergillosis in the MENA

Aspergillus spp. are widespread in the environment and are commonly isolated from both the outdoor and indoor environments including tertiary care centers. Globally, the prevalence of aspergillosis is increasing as a result of developing advanced medical practices with a rise in the proportion of immunocompromised populations due to cancer treatment, organ transplantation, and prolonged immunosuppressive therapy. A ten-fold increase in the frequency of invasive aspergillosis (IA) was seen over the last two decades [6].

Therefore, we searched PubMed, Science Direct, Scopus and Google Scholar databases for studies published between 2000 and 2018. We used a combination of the words “*Aspergillus* spp.”, “Aspergillosis”, “Burden of fungal infections”, “Burden of aspergillosis”, “Epidemiology of aspergillosis”, “Invasive aspergillosis”, “Antifungal resistance”, “MENA region”, “Amphotericin B resistance”, and “Azole resistance”. Indexed original articles in English and French of any design and sampling strategy; and of any enrollment timing (retrospective, prospective or cross-sectional) were eligible for inclusion. Other types of reports were excluded. To be included, eligible studies must have reported original information regarding the epidemiology of aspergillosis in MENA region. After importation of search results, two authors (A. Zakaria and M. Osman) independently first screened citations for their relevance using title and abstract, and all eligible investigations were retained for full text assessment (Fig. 1). Data extraction was performed by the same aforementioned authors.

Despite the growing issue of aspergillosis, *Aspergillus* spp. are not well documented in the majority of MENA countries. Only sporadic data are available concerning the epidemiology, distribution of species, and antifungal susceptibility patterns of *Aspergillus* isolates in MENA region. Moreover, high percentage of the available papers was data collection and did not provide enough epidemiological information in order to assess the morbidity and mortality related to aspergillosis in the MENA (Table 1). Likewise, the information is limited because of the common lack of advanced diagnostic tools. Moreover, no epidemiological investigations were reported in Libya, Palestine, Cyprus, UAE, Yemen, and Oman.

- *Invasive aspergillosis*

The lower estimated burden of IA in the MENA was observed in Qatar with 0.6 cases per 100,000 [7], followed by Jordan (1.3/100,000) [8], Iraq (2.6/100,000) [8], Turkey (4.9/100,000) [9], Algeria (7.1/100,000) [10], Saudi Arabia (7.6/100,000) [11], and Egypt (10.7/100,000) [12]. A clinical review conducted in Lebanon and Saudi Arabia during the period 2011–2012 showed a higher incidence in Lebanon (1.21 per 1000 discharges) than in Saudi Arabia (0.4 per 1000 discharges), with a predominance of *A. fumigatus*, followed by *A. niger* and *A. flavus* [13]. However, an Iranian study focused on immunocompromised patients suffering from IA in Mashhad found that *A. flavus* was the predominant species (75%) in bronchoalveolar lavage fluid samples, followed by *Aspergillus tubingensis* (15%) and *A. fumigatus* (10%) [4]. A Tunisian study on patients followed-up for acute leukemia in the hematology unit of the Farhat Hached hospital of Sousse also described *A. flavus* as the predominant species (37.5%) in sputum samples, followed by *A. niger* (35.4%), *A. tubingensis* (18.8%), *A. fumigatus* (4.2%), *Aspergillus westerdijkiae* (2.1%), and *Aspergillus ochraceus* (2.1%) [14]. In addition, 67% of the *A. flavus* isolates showed a reduced susceptibility to amphotericin B while *A. tubingensis* and *A. niger* were susceptible. On the other hand, 22% of *A. tubingensis* and *A. flavus* were itraconazole-resistant and caspofungin-resistant, respectively [14]. On the contrary, in Bahrain, *A. fumigatus* was found to be responsible for 53% of IA, followed by *A. niger* and *A. flavus*. This study also found that voriconazole is the best choice for the treatment of aspergillosis since the mortality rate was significantly lower in patients receiving this triazole (36%) than in patients receiving other types of antifungal therapy (75%) [15]. Interestingly, only one study in the MENA analyzed at the molecular level the resistance mechanism of *Aspergillus* spp. to triazoles [16]. The sequencing of the *cyp51A* gene revealed that 86.8% of the triazole-resistant *A. fumigatus* isolates exhibited the TR34/L98H mutation.

- *Aspergillus otomycosis*

A recent study showed that out of 149 cases of otomycosis diagnosed at Mustapha Bacha hospital in Alger, 9.3% were caused by *Aspergillus* spp. [17]. Another Algerian study on otomycosis performed at Oran University hospital reported the predominance of *A. niger* (50%), followed by *A. flavus* (41.7%) and *A. terreus* (8.3%) [18]. In the same context, an Egyptian study conducted in 2011 at Tanta University Hospital found that *A. niger* was responsible for 91% of otomycosis cases, followed by *A. flavus* (9%) [19]. In Syria, a recent study conducted in Almouassat University Hospital and Syrian Arab Red Crescent clinic from February 2015 to December 2016 showed

Table 1
Epidemiology of aspergillosis in MENA countries.

Country	Period	Type of study	Types of samples	Number of <i>Aspergillus</i> isolates	Detection method	Incidence of invasive aspergillosis	Identified species	Resistance rates	Reference
Morocco	1992–2011	Monocentric	Nails	53	Conventional microbiological diagnosis ^a	NA	ND	ND	[23]
Algeria	2015	National collected data	Respiratory	2865	ND	7.1/100,000	ND	ND	[10]
	2013	Monocentric	Ear discharges	14	Conventional microbiological diagnosis ^a	NA	ND	ND	[17]
	2016	Monocentric	Ear discharges	12	Conventional microbiological diagnosis ^a	NA	<i>A. niger</i> (50%) <i>A. flavus</i> (41.7%) <i>A. terreus</i> (8.3%)	ND	[18]
	2016	Monocentric	Respiratory	22	Galactomannan anti- <i>Aspergillus</i> antibody	NA	ND	ND	[62]
Tunisia	2014	Monocentric	Respiratory	48	Conventional microbiological diagnosis ^a MALDI-TOF-MS Sequencing of rDNA and β-tubulin genes	NA	<i>A. flavus</i> (37.5%) <i>A. niger</i> (35.4%) <i>A. fumigatus</i> (4.2%) <i>A. westerdijkiae</i> (2.1%) <i>A. ochraceus</i> (2.1%)	<i>A. flavus</i> 67% AmB 22% CAS 0% VCZ 0% POS <i>A. niger</i> 0% AmB 0% VCZ 0% POS <i>A. tubingensis</i> 0% AmB 22% ITC 0% VCZ 0% POS	[14]
Egypt	2012–2015	Multicentric	Skin scrapings; pus; hair fragments; cuttings from finger or toenails	19	Conventional microbiological diagnosis	NA	<i>A. niger</i> (36.6%) <i>A. flavus</i> (27.3%) <i>A. terreus</i> (13.6%) <i>A. fumigatus</i> (5.6%) <i>Aspergillus</i> spp. (16.9%)	ND	[24]
	2017	National collected data	Respiratory	9001	ND	10.7/100,000	ND	ND	[12]
	2011	Monocentric	Ear discharges	110	Conventional microbiological diagnosis ^a	NA	<i>A. niger</i> (91%) <i>A. flavus</i> (9%)	ND	[19]
Turkey	1920–2017	National collected data	ND	3911	Data collection	4.9/100,000	ND	ND	[9]
	2014–2015	Monocentric	Corneal scraping	4	Conventional microbiological diagnosis ^a	NA	<i>A. flavus</i> (100%)	0% AmB 0% ITR 0% VCZ 25% POS 0% ANF 0% CAS 100% FLC 100% 5FC	[26]
	1999–2012	Monocentric	ND	746	Conventional microbiological diagnosis ^a	NA	<i>A. fumigatus</i> (100%)	0% AmB 10.2% ITC 10.2% VCZ 9.6% POS	[16]

Table 1 (Continued)

Country	Period	Type of study	Types of samples	Number of <i>Aspergillus</i> isolates	Detection method	Incidence of invasive aspergillosis	Identified species	Resistance rates	Reference
Syria	2015–2016	Multicentric	Ear discharges	45	Conventional microbiological diagnosis ^a	NA	<i>A. niger</i> (71.1%) <i>A. versicolor</i> (22.2%) <i>A. fumigatus</i> (4.5%) <i>A. flavus</i> (2.2%)	ND	[20]
Jordan	2011–2013	National collected data	ND	ND	Data collection	1.3/100,000	ND	ND	[8]
Lebanon & Saudi Arabia	2011–2012	Multicentric	Respiratory	10	Conventional microbiological diagnosis ^a Galactomannan PCR Radiology	NA	<i>A. fumigatus</i> (60%) <i>A. niger</i> (10%) <i>A. flavus</i> (10%) <i>Aspergillus</i> spp. (20%)	ND	[13,63]
Saudi Ara-bia	2013	National collected data	Respiratory	2146	Data collection	7.6/100,000	ND	ND	[11]
	1991–1997	Monocentric	Paranasal sinus; surgical specimens	23	Conventional microbiological diagnosis ^a Radiology	NA	<i>A. flavus</i> (65%) <i>A. fumigatus</i> (9%) <i>Aspergillus</i> spp. (26%)	ND	[25]
Kuwait	1993–2011	Monocentric	Collection of <i>A. flavus</i> strains (mycology reference laboratory)	99	Conventional microbiological diagnosis ^a Sequencing of β-tubulin and calmodulin genes	NA	<i>A. flavus</i> (100%)	10% AmB 0% VCZ 0% POS 0% ANF 0% CAS 0% MIF	[21]
Bahrain	2009–2013	Monocentric	Respiratory	60	Conventional microbiological diagnosis ^a Radiology	NA	<i>A. fumigatus</i> (53%) <i>A. niger</i> (28%) <i>A. flavus</i> (12%) <i>Aspergillus</i> spp. (7%)	ND	[15]
Qatar	2009–2014	National collected data	Respiratory	11	Data collection	0.6/100,000	ND	ND	[7]
Iraq	ND	National collected data	ND	ND	Data collection	2.6/100,000	ND	ND	[8]
Iran	2012	Multicentric	Hospitals bioaerosols	ND	Conventional microbiological diagnosis ^a	NA	<i>A. fumigatus</i> (66.4%) <i>A. niger</i> (33.6%)	ND	[27]
	2017	Monocentric	Ear discharges	43	Sequencing of β-tubulin gene	NA	<i>A. tubingensis</i> (74.5%) <i>A. niger</i> (25.5%)	100% FLC	[22]
	2014–2015	Multicentric	Respiratory	20	Conventional microbiological diagnosis ^a Sequencing of β-tubulin gene	NA	<i>A. flavus</i> (75%) <i>A. tubingensis</i> (15%) <i>A. fumigatus</i> (10%)	ND	[4]

*NA: not applicable; ND: not determined; *A. flavus*: *Aspergillus flavus*; *A. fumigatus*: *Aspergillus fumigatus*; *A. niger*: *Aspergillus niger*; *A. tubingensis*: *Aspergillus tubingensis*; *A. versicolor*: *Aspergillus versicolor*; *A. terreus*: *Aspergillus terreus*; *A. ochraceus*: *Aspergillus ochraceus*; *A. westerdijkiae*: *Aspergillus westerdijkiae*; FLC: Fluconazole; 5FC: 5-Flucytosine; ANF: Anidulafungin; AmB: Amphotericin B; CAS Caspofungin; ITC: Itraconazole; MIF: Micafungin; POS: Posaconazole; VCZ: Voriconazole; MALDI-TOF-MS: Matrix-Assisted Laser Desorption Ionization Time-Of-Flight - Mass spectrometry.

^a Conventional microbiological diagnosis includes microscopical examination and/or culture.

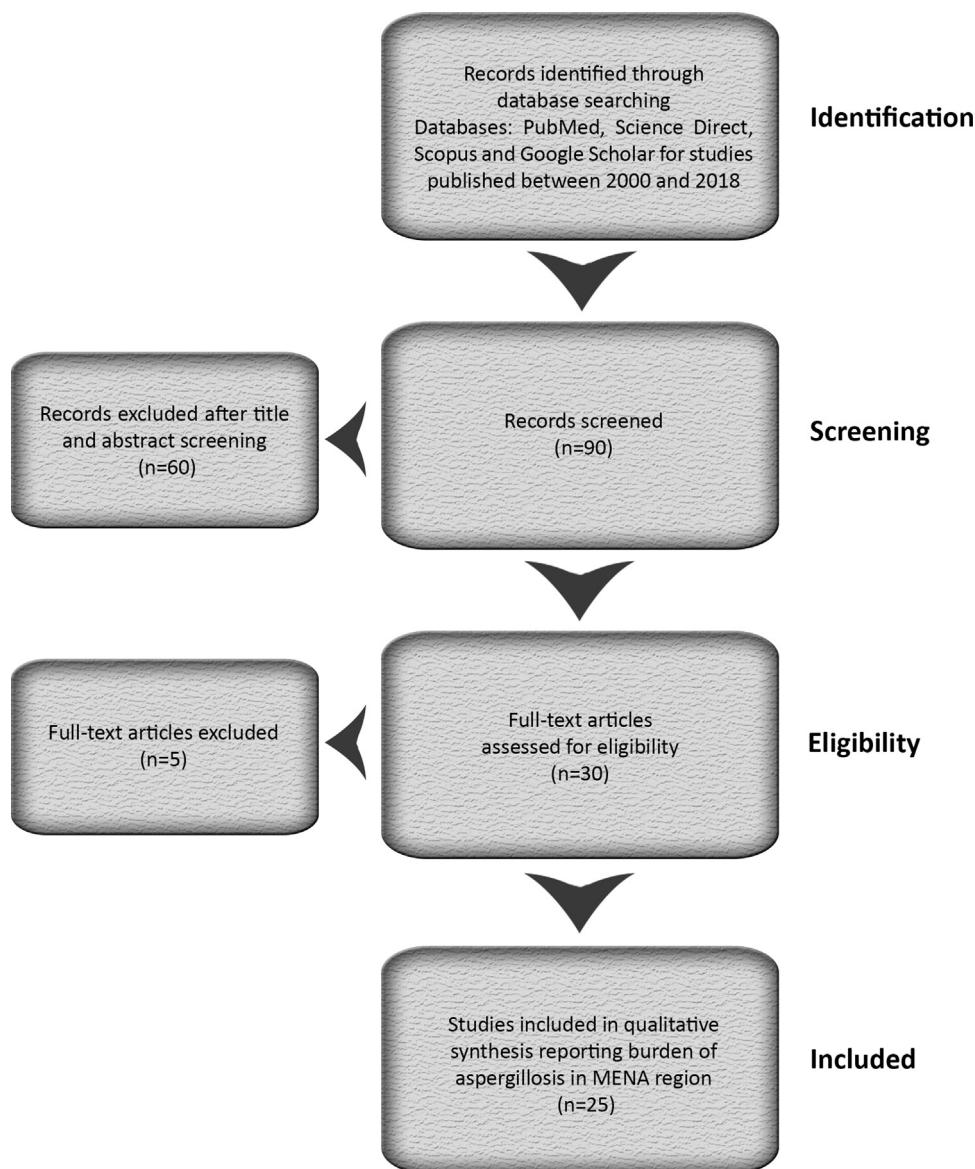


Fig. 1. Flow diagram describing paper selection and inclusion/exclusion process for the review according to PRISMA guidelines.

that 23.4% of ear infections were due to fungal pathogens, with a predominance of *A. niger* (46%), followed by *A. versicolor*, *Penicillium* spp., *A. fumigatus* and *A. flavus* [20]. In Kuwait, all identified *Aspergillus* clinical isolates recovered from respiratory secretions and ear swabs (as well as environmental isolates) in 2013 belonged to *A. flavus* [21]. Nevertheless, another monocentric study showed a predominance of *A. tubingensis* (74.4%) followed by *A. niger* (25.6%) in patients with otomycosis at a referral center in Tehran, Iran [22]. Regarding susceptibility to antifungals, all *A. tubingensis* and *A. niger* isolates recovered in this last study were resistant to fluconazole [22].

- *Aspergillus skin and soft-tissue infections*

Regarding other localized *Aspergillus* infections, a retrospective study in Morocco evaluated the frequency of moulds involved in onychomycosis at Ibn Sina hospital between 1992 to 2011, and found a predominance of *Aspergillus* spp. (35.3%) [23]. Similarly, a recent study made in Assiut (Egypt) between 2012 and 2015 found that 19.5% of non-dermatophytic fungi recovered from patients with superficial skin infections were *Aspergillus* species. Of them,

A. niger was the most common affecting 36.4% of the patients, followed by *A. flavus* (27.2%), and *A. terreus* (13.8%) [24]. Furthermore, an old Saudi Arabian study reported that *A. flavus* was the most frequently isolated species among immunocompetent patients with paranasal sinusitis at King Faisal Specialist Hospital and Research Center [25]. Moreover, in a recent Turkish study conducted in 2014–2015 at Cukuvora University Hospital in Adana, *A. flavus* was found to be responsible for 6.3% of fungal keratitis [26]. Concerning susceptibility to antifungals, all *A. flavus* isolates were susceptible to amphotericin B, itraconazole and voriconazole, while 25% and 100% of the isolates were resistant to posaconazole and fluconazole, respectively [26]. Interestingly, the resistance rates were higher among *A. fumigatus* isolates recovered from various clinical samples in Turkey, with 10.2% of them resistant to both voriconazole and itraconazole [16].

- *Aspergillus in environment*

A multicentric study conducted in five educational Iranian hospitals in 2012 found that *A. fumigatus* and *A. niger* were the most common in bioaerosol, with a prevalence of 14.6% and 7.4% respec-

tively [27]. Furthermore, a recent study focused on the genetic characterization and antifungal susceptibility profiles of clinical and environmental isolates of *A. flavus* in Kuwait reported low anti-fungal resistance rates, with only 10% of the isolates resistant to amphotericin B and none resistant to voriconazole and posaconazole [21].

Laboratory tools for the diagnosis of aspergillosis

The prognosis of IA is essentially associated with the delay of diagnosis, along with comorbid conditions in patients, insufficient awareness among multiple medical specialties, and performance of routine microbiological tests [2]. Most clinical laboratories, especially in developing world, use conventional tools based on morphological characters such as direct microscopic examination, histopathological analysis, and standard microbiological cultures for the detection of aspergillosis. Recently, the matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS) has greatly improved the diagnosis of aspergillosis [28]. Several serological methods also exist, including complement fixation, immunodiffusion, different enzyme-linked immunosorbent assays (ELISA), and Western blotting [29]. Most ELISA assays target the galactomannan antigen, a polysaccharide composed of galactofuranosyl side chains on a mannan backbone released by the aspergilli during their active growth. On the other hand, nucleic acid amplification testing is a simple, rapid, sensitive and easy-to-apply technique that could be used every day in routine clinical laboratories and capable to identify an infection at an early stage [30]. Most PCR-based techniques use primers targeting the rDNA internal transcribed spacer (ITS) region of *Aspergillus* spp., which allow the identification at the species complex level. Other loci such as the β -tubulin (*benA*) or calmodulin (*caM*) genes, are secondary identification markers that are usually required for the identification of individual species within the complex [31]. Moreover, multiple other molecular detection techniques have been described such as fluorescently labeled probes, electrospray ionization mass spectrometry, *in situ* hybridization probes and multiplexed arrays [32]. However, to our knowledge, no molecular assays have been approved by the Food and Drug Administration (FDA) for diagnosis of aspergillosis.

In addition to the above-mentioned methods, medical imaging has a crucial role in detecting pulmonary infections and evaluating response to therapy. The findings delivered by this method, in association with results of other diagnostic tools, can predict the infection and thus helps to access to an appropriate diagnosis and treatment mainly in the case of invasive pulmonary aspergillosis.

Regarding antifungal susceptibility testing of *Aspergillus* species, many assays are available. The broth microdilution is the gold standard and the reference technique (CLSI M38 – EUCAST). This method allows to evaluate the activity of drugs and thus to manage treatment of the patients, especially immunocompromised ones. Other assays commercially available or proposed for susceptibility testing include disk diffusion assays, Etest MIC strip kits, fluorescence-activated cell sorting, the Sensititre YeastOne colorimetric assay, MTT dye assays, and direct measurement of oxygen consumption. Moreover, molecular tools targeting mutated sequences in the *cyp51A* gene encoding the lanosterol 14- α -demethylase provide a faster and reliable assessment of the acquired resistance to azole drugs compared to conventional tools [33].

Treatment of aspergillosis and mechanisms of resistance

Numerous antifungal agents exist for the treatment of fungal infections. Of them, amphotericin B, azoles and echinocandins

(Fig. 2) are the main active compounds against aspergillosis. Even if voriconazole is the preferred agent for the treatment of IA in most patients, combination therapy is recommended in the treatment of mild to severe cases [34]. The treatment should be continued for a minimum of 6–12 weeks, depending on the degree and duration of immunosuppression, site of disease, and evidence of disease improvement.

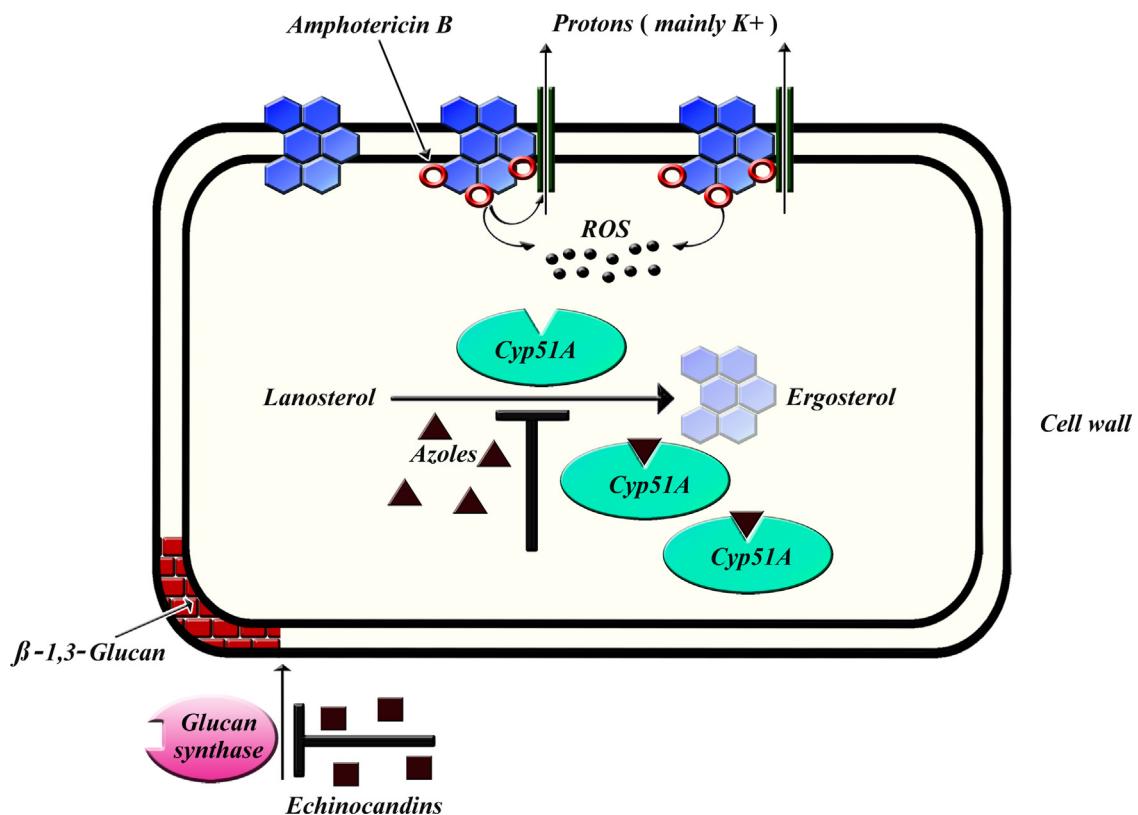
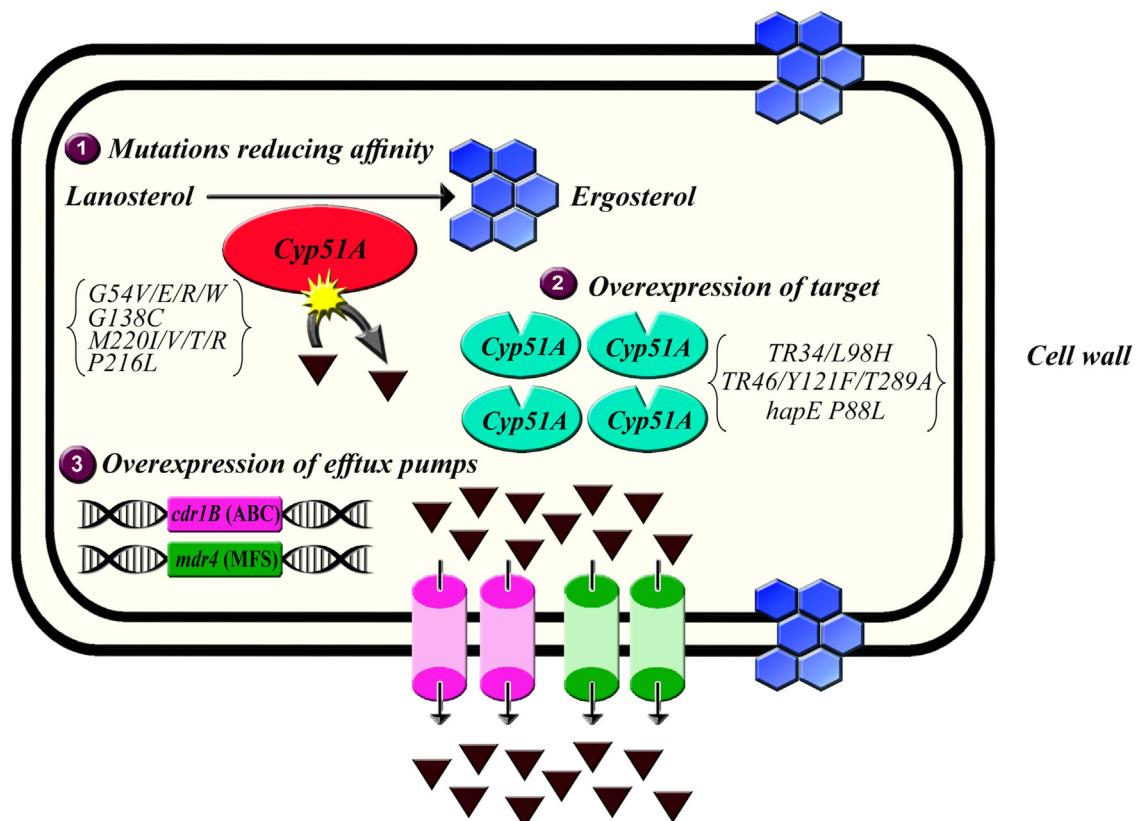
Although antifungals have been proven to be effective in terms of ability to reduce the fungal burden, their clinical effectiveness is regrettably affected by the emergence of fungal resistance, resulting in a serious public health crisis worldwide. The molecular mechanisms underlying antifungal resistance in *Aspergillus* spp. are reviewed here, and the triazoles resistance mechanisms are summarized in Fig. 3.

Amphotericin B

Ergosterol, an analogue of cholesterol in animal cells, is an essential steroid component of fungal membranes. It is responsible for membrane fluidity and gives the signal for cell division. Hence, this component constitutes an important target for many antifungal drugs. Amphotericin B, which is a polyenic antifungal, binds to ergosterol. The intercalation into the fungal membrane of a ring of 8–10 polyene molecules linked to an equal number of ergosterol molecules is responsible for the formation of aqueous pores, inducing cell death by leaking of protons, potassium and other monovalent cations, and essential cytoplasmic materials. Amphotericin B, therefore, is fungicidal and inhibits fungal growth through physicochemical interaction but not enzymatic inhibition. Furthermore, amphotericin B can generate reactive oxygen species and thus promote oxidative damage of cell membranes [35]. Since the fungicidal effect of amphotericin B can be attributed to its oxidative action, the resistance could be induced by the capacity to produce high levels of neutralizing enzymes like catalases and superoxide dismutases [2]. In vivo and in vitro studies indicate that the high majority of *A. terreus* strains are naturally resistant to amphotericin B. An experimental study using a murine model and comparing the responses to amphotericin B among amphotericin B-resistant and amphotericin B-susceptible *A. terreus* showed that ergosterol content plays a minor role in amphotericin B resistance, which is mainly associated with a decrease in intracellular drug concentration by decreased absorption and increased discharge, and a better protection against oxidative damage [36]. However, a recent study described that the high-level of resistance to amphotericin B is correlated with the modification of ergosterol [37]. Another study highlighted the importance of Hsp90 in amphotericin B resistance in clinical isolates of *Aspergillus* spp. recovered from bronchoalveolar lavage fluid samples [38]. Moreover, Blatzer et al. [39] provided evidence that susceptible and resistant *A. terreus* isolates exhibit distinct Hsp70 responses when treated with amphotericin B. The cytosolic level of Hsp70 was increased after the beginning of amphotericin B treatment, particularly in resistant isolates, and blocking of HSP70 with specific inhibitors, especially pifithrin- μ , increased the drug efficiency [39]. Fortunately, despite the extensive use of amphotericin B in the treatment of life-threatening fungal infections for more than half a century, resistance to this drug still remains particularly uncommon [40].

Azoles

Azoles are fungistatic agents under low concentrations and fungicidal agents under high concentrations. This class constitutes the largest group of antifungals used for more than two decades. Azoles are commonly used in different fields such as clinical, food production, plant protection, and material preservation. The mode of action of these drugs relies on the inhibition of the fungal

**Fig. 2.** Mechanisms of action of antifungal agents active against *Aspergillus* spp.*ROS: reactive oxygen species (e.g.: H₂O₂).**Fig. 3.** Mechanisms of triazoles resistance in *Aspergillus* spp.

*cdr1B: ABC efflux transporter, mdr4: major facilitator superfamily transporter.

lanosterol 14- α -demethylase, a member of the cytochrome P450 family, CYP51 encoded by *cyp51* gene, which is a key enzyme in the ergosterol biosynthesis pathway. In fungi, CYP51 catalyzes the demethylation of lanosterol to create an important precursor that is finally converted into ergosterol. The binding of the nitrogen atom of the five-membered triazole ring to the iron atom of the heme group present in the active site of CYP51A protein inhibits the demethylation step, thus resulting in the blockage of ergosterol synthesis. Unfortunately, the fungistatic activity at a low dose and the excessive and long-term use of azoles increased the percentage of resistant fungal strains. Despite the existence of several resistance mechanisms, point mutations in the *cyp51* gene and/or tandem insertions at its promoter have been associated with the main azole-resistant phenotypes [41]. *Aspergillus* spp. possess two isoenzymes CYP51A and CYP51B but none of them individually is essential for cell growth, notwithstanding this, the inactivation of both genes is lethal [42].

Point mutations

Over the years, the emergence of nonsynonymous hot-spot mutations, particularly in the *cyp51A* gene, was shown to be the main resistance mechanism for this antifungal class. The wild-type *A. fumigatus* CYP51A possesses two ligand entry channels (channel 1 and 2), which are immersed in the membranes of the endoplasmic reticulum. Mutations in *cyp51A* gene induce the replacement of small hydrophobic residues by larger ones, which leads to the closure of channel 2 entrance, disruption of the docking of large drug molecules, and alteration of azoles affinity for the enzyme causing drug tolerance [42,43]. Even though the existence of two ligand access channels, the mutation in one of them seems to be sufficient for the appearance of *Aspergillus* resistant isolates. Of note, the majority of mutations are in close proximity to the opening of channel 2. Some mutations like single amino acid substitutions at glycine 54 (G54V, G54E, G54R, G54W) and 138 (G138C) cause a cross-resistance to itraconazole and posaconazole. Other mutations such as G448S induce voriconazole resistance and reduce itraconazole and posaconazole activity. The substitution at methionine 220 (M220I, M220V, M220T, M220K) is related to various patterns of reduced susceptibility to azoles [2]. Other point mutations, such as A284T, F219C, F219I, G434C, G432S, H285Y, P216L, and Y431C, have also been sporadically found [44]. The point mutations are generally reported among patients receiving long-term azole therapy (usually more than 4 months), particularly in case of chronic aspergillosis [2]. The possibility that deletions can occur during chronic *Aspergillus* infections was clearly demonstrated using next-generation sequencing tools. These mutations lead to the emergence of resistant isolates, thus revealing the dynamic alterations that occur in the *Aspergillus* genome within the host during infection and treatment [45].

On the other hand, several other polymorphisms including A9T, D255E, F427K, F46Y, F219I, G54E, M172V, N248T, and P216L, are also reported alone or in combination, causing an increase in minimum inhibitory concentration (MIC) values. However, these mutations are not necessarily associated with the emergence of azole-resistant *Aspergillus* strains [2].

Tandem repeats in cyp51A

In addition to point mutations in the coding sequence of *cyp51A*, changes in the promoter region of this gene have been described in some resistant isolates, consisting of the insertion of tandem repeats or transposable elements as TR34, TR46 and TR53. These alterations generally lead to an overexpression of *cyp51A* responsible for reduced azole susceptibility. Indeed, the increase in mRNA levels correlates with an increased cellular CYP51A level resulting in decreased susceptibility to azoles [46]. For instance, the combination of the insertion of a 34-bp tandem repeat in *cyp51A* promoter

with L98H substitution in CYP51A has been detected several times associated with an up to 8-fold increase in the expression of *cyp51A* [47]. TR34/L98H which is the most frequent resistance mechanism in environmental isolates, confers pan-azole resistance with resistance to itraconazole and reduced susceptibility to voriconazole and/or posaconazole [2,48]. Likewise, the TR46/Y121F/T289A mutation is an emerging resistance mechanism with high-level of resistance to voriconazole, and elevated MICs to all other azoles [49]. In addition, a 53-bp tandem repeat sequence was also detected in *cyp51A* promoter region without any amino acid substitution in CYP51A. This rare mutation conferred a resistance to itraconazole and voriconazole and lower susceptibility to posaconazole [50]. The low frequency of TR53 mutation predicts limitations on fungal survival in the environment associated with this mutation [50].

Non-cyp51 gene mutations

Although many of the resistance phenotypes described in the literature are related to *cyp51*, there are also other mechanisms of resistance independent of *cyp51*. No mutations within the *cyp51* locus are found in up to 50% of azole-resistant clinical *Aspergillus* strains [51]. Hence, other pathways and factors have been suggested to confer secondary resistance. Among them, the unfolded protein response (UPR), iron-responsive transcriptional networks, and chaperone proteins such as HSP90 were previously reported to be potentially involved in sterol biosynthesis and drug interactions, and their alteration would provide additional opportunities for *Aspergillus* resistance [52]. One of the best-known CYP51-independent mutations is P88L in heme activator protein E, which is an important subunit of the CCAAT-binding transcription factor complex (CBC). This substitution leads to a significant loss of binding activity, subsequently causing upregulation of *cyp51A* gene, and conferring azole resistance [53]. Furthermore, Gsaller et al. [54] revealed that CBC acts complementary to the sterol-regulatory element binding protein (SrbA) as a negative regulator of ergosterol biosynthesis. Hence, decrease in CBC activity leads to an increased expression of several enzymes of the ergosterol biosynthesis pathway including HMG-CoA-synthase, HMG-CoA-reductase and sterol C14-demethylase, via the transcriptional activation of the encoding genes, and thus confers resistance to fluconazole and voriconazole [54]. On the other hand, *srbA* plays a critical role in maintaining ergosterol biosynthesis and cell polarity, and arbitrating resistance to azole drugs [55]. Significantly, the *srbA* null mutant was reported as highly susceptible to azoles [55]. Recent molecular investigations of the HAP complex have reported it to interact with the *cyp51A* promoter at the repeat elements found in azole-resistant strains, thereby opposing the action of the sterol response factor SrbA. Nevertheless, the number of clinical isolates exhibiting the P88L substitution is very low, but maybe underestimated, in comparison with other azole resistance mechanisms. In the same context, deletions and nonsynonymous mutations in *afyap1* (F487L) and *aldA* (G357S) genes, encoding oxidative stress-responsive transcriptional regulator and putative aldehyde dehydrogenase, respectively, have been reported among azole-resistant *Aspergillus* isolates during chronic infection [45].

Efflux pump-mediated azole resistance

One of the important mechanisms of azole resistance is the over-expression of efflux pumps responsible for a reduced intracellular accumulation of drugs by expelling the drugs out of the cell.

The two main types of efflux pumps are ATP-Binding Cassette (ABC) transporters and members of the Major Facilitator Superfamily (MFS) [56]. The detailed mechanism of ABC transporters leading to azole resistance has not yet been completely clarified. The ABC efflux transporter Cdr1B (protein with high homology to *Candida albicans* efflux pump Cdr1p) is the best-studied transporter in cor-

relation with drug resistance. The overexpression of this protein, originally regulated by the zinc cluster transcription factor AttrR, induces resistance to fluconazole in *A. fumigatus* [51].

Echinocandins

Echinocandins are parenteral antifungals with a narrow-spectrum restricted to *Candida* and *Aspergillus*. Because of their good antifungal activity and favorable safety profile, echinocandins have emerged as major drugs against invasive fungal infections [57]. However, the big disadvantage is that echinocandins have only fungistatic activity against *Aspergillus* spp. Recent investigations described the importance of the synergistic effect of echinocandins in combination with a polyene or azole in experimental aspergillosis [58]. Synergy between antifungals can allow lowering the doses, thus decreasing drug toxicity. Three echinocandins are available, approved by both the FDA and the European Medicines Agency (EMA) for clinical use: anidulafungin, caspofungin, and micafungin. Although echinocandins have a similar spectrum of activity, caspofungin remains the sole compound with an indication for the treatment of patients with IA. However, recent in vitro studies reported that anidulafungin and micafungin could be more potent than caspofungin [21]. But till now, there are less clinical data for use of anidulafungin and micafungin against severe aspergillosis. These two compounds are mainly used for antifungal prophylaxis in stem cell transplantation.

The mode of action of echinocandins relies on the inhibition of the glucan synthase, an enzyme encoded by the *FKS1* and *FKS2* genes and required for the biosynthesis of β-1,3-glucan, leading to defects in the integrity of the cell wall. Hence, modifications in the glucan synthase may be responsible for echinocandin resistance. Resistance to echinocandins can occur via point mutations within highly conserved regions of the *FKS1* and *FKS2* genes [59]. A previous study showed that the presence of the S678Y substitution in Affks1p leads to echinocandin resistance among *A. fumigatus* isolates [60]. Similarly, another study found that the S678P substitution in Fks1p was also able to confer echinocandin resistance [61].

Concluding remarks

In conclusion, to our knowledge, this is the first review regarding the epidemiology of *Aspergillus* spp. in the MENA region. Despite the limited number of studies dealing with the epidemiology and susceptibility to antifungals of *Aspergillus* spp. in this region, the currently available data shows that *Aspergillus* infections are not negligible, and that the emergence of antifungal resistance is a growing health issue, especially among immunocompromised patients. This highlights the need for reliable, sensitive, and above all rapid diagnostic tools followed by a susceptibility test in order to initiate the appropriate antifungal therapy as soon as possible and to stem the morbidity and mortality related to aspergillosis. Further epidemiological studies on aspergillosis are recommended to confirm the significance of these findings, particularly among immunocompromised populations.

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