DOI: https://doi.org/10.53350/pjmhs22167634

ORIGINAL ARTICLE

Epidemiology and Susceptibility Profile of Aspergillus Species: An Experience from Tertiary Care Hospital

GULSHAN MUNIR[1], SADIA FARHAD[2], SHAISTA ALAM[3], ASMA AZIM[4], SAMAN HUSSAIN[5], SIKANDAR ALI KHAN[6], KHADIJA RAZIQ[7], SUDHAIR ABBAS BANGASH[8], INAM-U-LLAH[9], HINA MIR[10]

[1]*Department of Pathology, Hayatabad Medical Complex, Peshawar.*
[2]*Department of Pathology, Bacha Khan Medical College, Mardan, KPK*
[3]*Department of Pathology, Pak International Medical College, Peshawar*
[4]*Department of Pathology, Khyber Medical University, Peshawar.*
[5]*Department of Pathology, Northwest School of Medicine, Peshawar.*
[6]*Department of Biochemistry, Khyber Girls Medical College, Peshawar, Pakistan*
[7]*Department of Microbiology and Biotechnology, Abasyn University, Peshawar.*
[8]*Faculty of Life Science, Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar.*
[9]*Department of Food Science, the University of Haripur, KPK, Pakistan*
[10]*Department of Biochemistry, Shaheed Benazir Bhutto women University Peshawar*
Corresponding author: Sadia Farhad, Email: momina.khanbkmc@gmail.com, & Sikandar Ali Khan, Email: siki4sikandar@gmail.com

ABSTRACT

Objective: The primary emphasis of our research is on people with hematologic malignancies, and we want to learn more about the features of clinical and environmental Aspergillus isolates by doing so.

Study Design: Prospective study

Place and Duration: This study was carried out at Department of Pathology, Mardan Medical Complex from October 2021 to March 2022

Methods: There were 160 patients of both genders included in this study. Included patients were aged between 18-80 years. Patients with hematologic malignancy were included. Invasive aspergiluus isolates from all patients in which 80 were clinical and 80 were environmental. . With the help of SPSS 22.0, clinical data were analyzed and Aspergillus species-level cryptic identification, antifungal susceptibilities, and cyp51 gene sequencing were all carried out.

Results: Among 160 included patients, majority of the cases 95 (59.4%) were males and 65 (40.6%) patients were females. We found that 75 (46.9%) cases had age >50 years. Most common diagnostic criteria were probable IA found in 140 (87.5%) cases. Co-morbidities were pulmonary disease, neurological disease, autoimmune disease, cardiac disease and burns. Cryptic Aspergillus species composed 37.5% of environmental and clinical isolates. Section Nigri had a significant value (70.5%) of cryptic species, mostly among A. awamori and A. tubingensis the former was prevalent in ambient samples and the latter in clinical isolates (P 0.003). Twelve (7.5%) of 80 A. fumigatus isolates were azole resistant. At 90 days, A.fumigatus was 100% responsible for all deaths by resistant to azoles.

Conclusion: Comparing clinical and environmental isolates, this study reveals a large proportion of cryptic Aspergillus species, highlights the clinical consequences of azole resistance.

Keywords: Hematologic Malignancy, Drug Resistance Mechanisms, Aspergillus, Environmental Microbiology, Azoles

INTRODUCTION

High morbidity, death, and healthcare expenditures make invasive aspergillosis (IA) is the harmful invasive fungal diseases seen in the clinic [1]. Every year, Aspergillus species are known to infect about 200,000 people with IA [2]. However, additional species of Aspergillus, including Aspergillus fumigatus, Aspergillus fumigatus, Aspergillus niger, A. niger terreus, A. niger versicolor, and A. niger nidulans, may also create illnesses [3]. Chronic pulmonary infection that occurs (CPA) and acute IA both affect immunocompromised people and those with preexisting lung disorders [4]. Early identification of IA is challenging and its misinterpretation is common since Aspergillus infection seldom show distinctive signs and the particular pathogenics usually required excess time to discover [5].

Antifungal resistance is a major problem today [6], but azole antifungals like voriconazole, isavuconazole, fluconazole, itraconazole, etc. are essential for treating IA, either as first-line or secondary regimens. Aspergillosis is becoming more common, and Aspergillus spp. are developing an increasing level of resistance to azoles; nevertheless, the degree of resistance shown by A. flavus to azoles varies widely among geographic locations and nations. Single nucleotide in the gene encoding the CYP51-Aspergillus (CYP51A) protein have been linked to amino acid alterations that contribute to the evolution of azole-resistant A. flavus [8,9]. These modifications probably decrease the fungus's affinity for antifungal medicines. In order to treat invasive Aspergillus illnesses early in the clinical process and improve patient outcomes, it is necessary to understand and assess the most common strains, epidemiological characteristics, and drug sensitivity profile in the area.

Mostly in A. fumigatus, which seems to acquire resistance to azoles either via patient treatment or environmental exposure to azole fungicides [10]. Several cyp51A gene alterations have been linked to azole resistance, while the number of resistant isolates lacking cyp51A-mutations is rising in certain locations (Harrison E, Hughes SJ, Buied A, Bowyer P, Henning DW). Antimicrobial agents and chemotherapy: 49th Interscience Conference, California, September 2009, M-1720: The increasing incidence of azole resistance pathways in Aspergillus fumigatus. However, drug resistance may be innate, and the recent revisions to the classification of Aspergillus have had far-reaching effects on our knowledge of drug susceptibility patterns. [11,12]

One major source of resistance is the widespread use of fungicides in farming[13]. Because of the geographical variation in azole resistance, worldwide epidemiological studies are required. Furthermore, in order to have a holistic comprehension of antifungal resistance, it is necessary to compare the features of clinical and environmental isolates of azole-resistant Aspergillus. More epidemiological data, in addition to clinical data, including the result of therapy for IA caused by halide strains [14,15], is required to define an appropriate epidemiological cut-off (ECV) and future endpoint for A. species. Here, we set out to determine the evolutionary relationships between clinical and ambient Aspergillus isolates, the susceptibility profiles of these strains, and the distribution of cryptic species. In addition to examining the link or differences among isolates of environmental and clinical, we also assessed the treatment trajectory and treatment outcomes of IA by halide Aspergillus species.

MATERIAL AND METHODS

This prospective study was conducted at Department of Pathology, Mardan Medical Complex from October 2021 to March 2022 and

comprised of 160 cases. After obtaining informed written consent detailed demographics of all cases were recorded.

Aspergillus isolates tested from sterile locations or the respiratory system that had clinical relevance were included in the IA classification according to the new conditions of invasive fungal disease (IFD). Clinical isolates were found to include both pathogens and colonizers. We kept track of patient age, gender, race/ethnicity, race/ethnicity, race/ethnicity, race/ethnicity, race/ethnicity, race/ethnicity, race/ethnicity, race/ I the hospital's entrance gate, (ii) the main ward lobby, (iii) spotless hallway of a treatment ward, and (iv) a participant's place inside the clean-room, were all tested for air quality. High-rise structures, two parks, as well as the riverside are all within a kilometer of a hospital. Heating, ventilation, and air conditioning are all part of the hospital's setup. Because of its high-efficiency particle air filtration system, positive pressure, and 10,000-class clean-room status, the chemotherapy unit is among the cleanest in the hospital. Every other month, at the same time, air was collected from each place. After both 24 and 48 hours, we examined the dilution method plates underneath a microscope to verify the identity of the MECs. We used Chi-square test to compare categorical data. Correlation was studied using the Spearman rank correlation coefficient. The significance level for the two-tailed test was set at 0.05. SPSS 22.0 was used to analyze all data.

RESULTS

Among 160 included patients, majority of the cases 95 (59.4%) were males and 65 (40.6%) patients were females. Most common diagnostic criteria were probable IA found in 140 (87.5%) cases. Co-morbidities were pulmonary disease, neurological disease, autoimmune disease, cardiac disease and burns.(Table 1)

Table-1: Data of enrolled cases

Variables	Frequency	Percentage
Gender		
Male	95	59.4
Female	65	40.6
Diagnostic Criteria		
Probable IA	140	87.5
Others	20	12.5
Other Diseases		
pulmonary disease	44	27.5
neurological disease	30	18.8
autoimmune disease	22	13.8
cardiac disease	16	10
burns	7	4.4

We found that majority 75 (46.9%) cases had age >50 years, 50 (31.3%) had age 41-50 years and 35 (21.9%) had age 18-40 years.(figure 1)

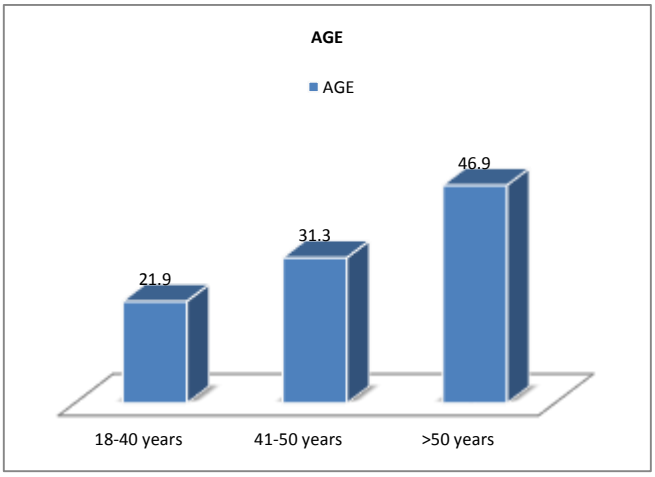

Figure-1: Included patients with age groups

Cryptic Aspergillus species composed 37.5% of clinical and environmental isolates. Most of the cryptic species in Section Nigri were caused by A. medium of transport and A. awamori, with the former being found in samples collected while the latter in strains isolated (P 0.003). Only 12 (7.5%) of 80 A. fumigatus isolates showed resistance to azoles (table 2)

Table-2: identification of cryptic species of Aspergillus

Variables	Clinical (80)	Environmental (80)
cryptic Aspergillus spp.		
Yes	30 (37.5%)	30 (37.5%)
No	50 (62.5%)	50 (62.5%)
Fumigati		
A. fumigatus	30	30
A. lentulus	2	0
A. udagawae	2	3
Nigri		
A. niger	26	20
A. tubingensis	35	29
Terrei		
A. terreus	2	4
Nidulantes		
A. nidulans	4	3
A. sydowii	3	1

While different antibiotics have been shown to be successful in reducing the fungal load, their clinical efficacy has been sadly hampered by the rise of fungal resistance, leading to a significant public health issue throughout the globe.

One hundred of the original 160 Aspergillus isolates were suitable for antifungal susceptibility testing because they had been stored appropriately and did not include spores that had already deteriorated. Particularly of interest are the geometric mean (GM), range, and MIC/MEC value. Out of total, 73 A. fumigatus isolates, 29 had MICs over 2 g/ml, 13 of 24 A. flavus isolates had MICs above 2 g/ml, and 13 of 22 A. niger isolates had MICs above 2 g/ml, however this was due to intrinsic resistance. Aspergillus spp. had high MICs for FC (>64 g/ml), whereas A. niger had a much lower MIC (10.62 g/ml). Each, however, showed MICs for MIF and CAS of 0.008 to 0.02 and 0.03 g/ml, respectively. Azole MICs of 5 ml were observed for the vast majority of the tested isolates of Pps (n = 95.3), ITR (85.7%), VRC (91.2%), and ISA (85%). Every single A. fumigatus sample tested positive for sensitivity to Pro (0.09 g/ml), with a similar percentage responding to ITR, ISA, and VRC. High azole sensitivity was observed in vitro for Aspergillus flavus, irrespective of the fact that 8.33percent of the population was robust to ISA and VRC. All azole drugs showed sensitivity patterns in the other Aspergillus (A. niger, A. — a rare, and A. nidulans).

DISCUSSION

Over 30 different species of Aspergillus have been linked to IA so far [16], with the most prevalent being A. flavus, A niger, A. — a rare, and A. nidulans. Infection locations, age, climate, geographic circumstances, agricultural practices, and other variables all contribute to the considerable variation in IA prevalence and the fungus spectrum that is associated with it [17].

Aspergillus fumigatus is the most often isolated Aspergillus species in China [18], followed by Aspergillus flavus and Aspergillus niger. Aspergillus species other than A. fumigatus have also been increasingly responsible for IA in recent years in China. According to a study by Wang et al. [19], individuals with hepatitis B disease hepatic failures had a higher risk of developing invasive lung aspergillosis (IPA) due to A. flavus than A. fumigatus. More instances of IA caused by A. niger and A. tb were discovered in clinical and ambient samples taken by Li et al. [20]

In this study, we analyzed the differences between pathogens, all clinical, and environmental isolates of Aspergillus, and determined their distribution and susceptibility. The prevalence of cryptic Aspergillus was comparable amongst the three groups, and the prevalence of azole among A. fumigatus was likewise

comparable between environmental and clinical isolates. Proven or suspected IA occurred in 87.5% of cases, although there was no statistically significant relationship in between the occurrence of IA and the month ambient fungal intensity as determined by air. This means that both the host and the environment have a role in the development of IA.

The prevalence of bacteria that are resistant to the antifungal drug azole has risen sharply during the last several decades [21]. Aspergillus azole-resistance is increasing and is becoming a worldwide health concern. As new resistance mechanisms emerge, the efficacy of azoles in treating aspergillosis is put at risk, which in turn affects clinical outcomes. According to results from the worldwide antifungal monitoring programme, 5.79 percent of A. fumigatus had increased MICs for at least one azole. The antifungal resistance complicates the treatment of candidiasis [22], and the reported prevalence of azole resistance varies greatly across countries/regions.

Furthermore, Blatzer & al. [23] shown that when exposed to amphotericin B, A. terreus isolates with varying degrees of resistance had different Hsp70 responses. Blocking HSP70 using specific inhibitors, mainly pifithrin-, improved medication efficacy. The cytosolic concentration of Hsp70 rose following the start of amphotericin B therapy, especially in resistant isolates.

4.2% of Aspergillus isolates in the Fumigati section were cryptic species. High MICs for AMB were observed for A. lentulus, which is consistent with earlier research. All azoles, AMB, and rimantadine had effective minimum inhibitory concentrations (MICs) against A. udagawae or A. turcosus (0.5 g/ml). The majority of the undetected species here belonged to the Nigri subgroup, namely the A. tubingensis or A. participants species. It was shown that A. tubingensis was much more prevalent in ambient isolates, whereas A. awamori was more prevalent in clinical isolates. Eighty-two percent of the clinical isolates from the section Nigri were first labelled as Aspergillus niger by ITS sequenced but were later shown to be A. awamori by products in stock sequencing. This confirms other reports [24,25] that A. awamori is a widespread cryptic species. A. awamori had a 1 g/ml voriconazole MIC90, but A. mode of transport required 4 g/ml. It is essential to construct a MIC set point that may represent the epidemiological studies and therapeutic comment based further on data collection, despite the fact that A. awamori is one of the primary morphological traits in clinically harmful isolates and is one of the most common of these isolates' morphological characteristics.

We focus on the most important findings from this research. The proportion of cryptic Aspergillus is the same for all clinical and ambient Fusarium isolates, at about one-third. Second, whereas A. awamori was significantly abundant in clinical isolates, Aspergillus tubingensis was more prevalent in ambient samples. Finally, our investigation revealed that 5.3percent of A. spreadss samples were ARAF-positive. In both human and environmental samples, azole resistance was shown to occur with a comparable frequency. There was an increased all-cause death rate at ninety days for patients having IA who had ARAF, making this a fourth-ranking risk. Findings from this research may have applications in a variety of areas, such as the collection of epidemiologic data and the development of brand-new therapies.

CONCLUSION

Comparing clinical and environmental isolates, this study reveals a large proportion of cryptic Aspergillus species, highlights the clinical consequences of azole resistance.

REFERENCES

1 Kim A, Nicolau DP, Kuti JL. Hospital costs and outcomes among intravenous antifungal therapies for patients with invasive aspergillosis in the United States. Mycoses. (2011) 54:e301–12. doi: 10.1111/j.1439-0507.2010.01903.x

2 Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. Sci Transl Med. (2012) 4:165rv13.

3 van de Veerdonk FL, Gresnigt MS, Romani L, Netea MG, Latge JP. Aspergillus fumigatus morphology and dynamic host interactions. Nat Rev Microbiol. (2017) 15:661–74. doi: 10.1038/nrmicro.2017.90

4 Sugui JA, Kwon-Chung KJ, Juvvadi PR, Latge JP, Steinbach WJ. Aspergillus fumigatus and related species. Cold Spring Harb Perspect Med. (2014) 5:a019786. doi: 10.1101/cshperspect.a019786

5 Bongomin F, Gago S, Oladele RO, Denning DW. Global and multi-national prevalence of fungal diseases-estimate precision. J Fungi. (2017) 3:

6 Soubani AO, Chandrasekar PH. The clinical spectrum of pulmonary aspergillosis. Chest. (2002) 121:1988–99. doi: 10.1378/chest.121.6.1988

7 Zhang R, Wang S, Lu H, Wang Z, Xu X. Misdiagnosis of invasive pulmonary aspergillosis: a clinical analysis of 26 immunocompetent patients. Int J Clin Exp Med. (2014) 7:5075–82.

8 Chowdhary A, Kathuria S, Randhawa HS, Gaur SN, Klaassen CH, Meis JF. Isolation of multiple-triazole-resistant Aspergillus fumigatus strains carrying the TR/L98H mutations in the cyp51A gene in India. J Antimicrob Chemother. (2012) 67:362–6.

9 Rybak JM, Ge W, Wiederhold NP, Parker JE, Kelly SL, Rogers PD, et al. Mutations in hmg1, challenging the paradigm of clinical triazole resistance in Aspergillus fumigatus. mBio. (2019) 10:e00437–19.

10 Howard SJ, Cerar D, Anderson MJ, et al. Frequency and evolution of azole resistance in Aspergillus fumigatus associated with treatment failure, Emerg Infect Dis, 2009, vol. 15 (pg. 1068-1076)

11 Snelders E, Huis in 't Veld RAG, Rijs AJJM, et al. Possible environmental origin of resistance of Aspergillus fumigatus to medical triazoles, Appl Envir Microbiol, 2009, vol. 75 (pg. 4053-4057)

12 Verweij PE, Snelders E, Kema GHJ, Mellado E, Melchers WJG. Azole-resistance in Aspergillus fumigatus: a side effect of environmental fungicide use?, Lancet Infect Dis, 2009, vol. 9 (pg. 789-795)

13 Verweij PE, Snelders E, Kema GH, Mellado E, Melchers WJ. 2009. Azole resistance in Aspergillus fumigatus: a side-effect of environmental fungicide use? Lancet Infect Dis 9:789 –795.

14 Lee HJ, Cho SY, Lee DG, Park C, Chun HS, Park YJ. 2018. TR34/L98H mutation in cyp51A gene in Aspergillus fumigatus clinical isolates during posaconazole prophylaxis: first case in Korea. Mycopathologia 183: 731–736.

15 Heo ST, Tatara AM, Jiménez-Ortigosa C, Jiang Y, Lewis RE, Tarrand J, Tverdek F, Albert ND, Verweij PE, Meis JF, Mikos AG, Perlin DS, Kontoyiannis DP. 2017. Changes in in vitro susceptibility patterns of aspergillus to triazoles and correlation with aspergillosis outcome in a tertiary care cancer center, 1999-2015. Clin Infect Dis 65:216 –225.

16 Lockhart SR, Frade JP, Etienne KA, Pfaller MA, Diekema DJ, Balajee SA. Azole resistance in Aspergillus fumigatus isolates from the ARTEMIS global surveillance study is primarily due to the TR/L98H mutation in the cyp51A gene. Antimicrob Agents Chemother. (2011) 55:4465–8.

17 Chen M, Xu Y, Hong N, Yang Y, Lei W, Du L, et al. Epidemiology of fungal infections in China. Front Med. (2018) 12:58–75.

18 Yang X, Chen W, Liang T, Tan J, Liu W, Sun Y, et al. A 20-year antifungal susceptibility surveillance (From 1999 to 2019) for Aspergillus spp. and proposed epidemiological cutoff values for Aspergillus fumigatus and Aspergillus flavus: a study in a tertiary hospital in China. Front Microbiol. (2021) 12:680884.

19 Wang W, Zhao CY, Zhou JY, Wang YD, Shen C, Zhou DF, et al. Invasive pulmonary aspergillosis in patients with HBV-related liver failure. Eur J Clin Microbiol Infect Dis. (2011) 30:661–7.

20 Li Y, Wan Z, Liu W, Li R. Identification and susceptibility of Aspergillus section nigri in china: prevalence of species and paradoxical growth in response to echinocandins. J Clin Microbiol. (2015) 53:702–5

21 Jensen RH, Hagen F, Astvad KM, Tyron A, Meis JF, Arendrup MC. Azole-resistant Aspergillus fumigatus in Denmark: a laboratory-based study on resistance mechanisms and genotypes. Clin Microbiol Infect. (2016) 22:570 e1–9.

22 Lestrade PPA, Meis JF, Melchers WJG, Verweij PE. Triazole resistance in Aspergillus fumigatus: recent insights and challenges for patient management. Clin Microbiol and Infect. (2019) 25:799–806.

23 M. Blatzer, G. Blum, E. Jukic, W. Posch, P. Gruber, M. Nagl, et al.Blocking Hsp70 enhances the efficiency of amphotericin B treatment against resistant Aspergillus terreus strains.Antimicrob Agents Chemother, 59 (2015), pp. 3778-3788

24 Howard SJ, Harrison E, Bowyer P, Varga J, Denning DW. 2011. Cryptic species and azole resistance in the Aspergillus niger complex. Antimicrob Agents Chemother 55:4802–4809.

25 Won EJ, Shin JH, Kim SH, Choi MJ, Byun SA, Kim MN, Lee WG, Lee K, Uh Y, Shin MG, Suh SP. 2018. Antifungal susceptibilities to amphotericin B, triazoles and echinocandins of 77 clinical isolates of cryptic Aspergillus species in multicenter surveillance in Korea. Med Mycol 56:501–505.