Introduction

Aspergillus species are ubiquitous saprophytes causing various fungal infections. Most commonly encountered species are Aspergillus flavus, A. fumigatus and A. niger. They primarily cause pulmonary infection with involvement of other body sites like paranasal sinuses and cutaneous tissue. Aspergillosis is a systemic infection, one of the most common causes of invasive mold infection in immunocompromised patients, after candidiasis, with an estimated every year 300,000 cases worldwide.

Azoles are the mainstay of treatment; however, excessive use of these antifungals clinically as well as in agriculture has influenced the susceptible species of saprophytic flora leading to the genetic changes, which is further contributing to the emergence of resistance among Aspergillus species thereby challenging management.

As such azole resistance rate of A. fumigatus is globally varying from 2-31%. Hence to determine the resistance, antifungal susceptibility testing (AFST) among clinical and community environment isolates of Aspergillus species against amphotericin B, itraconazole, voriconazole and caspofungin was performed.

Material and Methods

Clinical as well as soil samples from community environment were processed as per Standard Mycological Techniques. Growth of Aspergillus species was identified by phenotypic methods and AFST was performed by micro broth dilution as per CLSI M38-A2 protocol.

Results

Among clinical samples, 30 (75%) were A. flavus, 9 (22.5%) A. fumigatus and one (2.5%) was A. candidus.

All 30 (75%) isolates of A. flavus were sensitive to itraconazole while one strain of A. flavus was found resistant to voriconazole with MIC value of ≥0.2 µg/ml.

Another strain was found to be resistant to amphotericin B with MIC value of 8 µg/ml and also had high MEC value of 0.12 µg/ml to caspofungin.

All 9 (100%) isolates of A. fumigatus were found to be sensitive to itraconazole and voriconazole while one (11.11%) isolate showed elevated MIC value of 4 µg/ml for amphotericin B and 2 (22.22%) isolates had elevated MEC value of 0.12 µg/ml to caspofungin.

MIC value of A. candidus isolate (2.5%) for itraconazole, voriconazole, amphotericin B was 0.25 µg/ml, 0.06 µg/ml and 1 µg/ml, respectively. MEC value of A. candidus for caspofungin was 0.12 µg/ml.

Among 22 Aspergillus isolates of community environment; 15 (68.18%) isolates were of A. niger, 4 (18.18%) isolates were of A. flavus and 3 (13.63%) isolates were of A. fumigatus.

All species of Aspergillus from community environment were found to be sensitive for itraconazole and voriconazole while 2 (13.33%) isolates of A. niger, 3 (75%) isolates of A. flavus and one (33.33%) of A. fumigatus had elevated MEC value of 0.12 µg/ml for caspofungin.

One (25%) strain of A. flavus was also found to be resistant to amphotericin B with MIC value of 4 µg/ml.

Discussion

In India, Aspergillus flavus is more prevalent in the environment and the most frequent species causing aspergillosis. Where as in developed countries, Aspergillus fumigatus is more common species found in the environment. Similarly, in the present study also, Aspergillus flavus is found to be more common in the environment as well as in clinical cases causing aspergillosis. Triazoles such as itraconazole, voriconazole and posaconazole are usually the antifungals of choice used as the effective drugs in the treatment of different clinical forms of aspergillosis and also as a prophylactic therapy in severe cases. Azole drugs act as competitive cyp51 inhibitors. So azole resistance involves mutation in the cyp51 gene. In present study, also one strain of A. flavus was found to be resistant to voriconazole with MIC value of ≥0.2 µg/ml.

Polyenes compound target ergosterol in cell membrane such as amphotericin B deoxycholate and lipid formulations of amphotericin B. Echinocandins target 1,3-beta-D-glucan synthesis in cell wall such as caspofungin, anidulafungin and micafungin while caspofungin is used as a salvage therapy for invasive aspergillosis. In the present study, various isolates both from clinical and environment showed elevated MIC and MEC values to amphotericin B and caspofungin respectively which is posing a threat of emerging resistance in the near future even for salvage drugs. So rational usage of drugs following the proper guidelines and routine antifungal susceptibility of the isolates should be adopted in order to combat the emerging resistance.

Conclusions

These results suggest that resistance is emerging for salvage drugs such as amphotericin B and caspofungin while first line drugs such as azoles are comparatively sensitive for Aspergillus isolates. Considering the importance of emerging resistance, surveillance studies should be performed routinely and Antifungal Stewardship Programme should be followed so that considerable improvement in the outcome of the patients can be achieved at an early stage.

References