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Original Research Article

Current Trends of Antifungal Susceptibility Pattern of Dermatophytosis by E-Test in a Tertiary Care Hospital, Puducherry

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Abstract:

Introduction: The varied clinical presentation of dermatophytosis, which results in delay in diagnosis, poor compliance in follow up of cases consequently spread of infection in the community and antifungal resistance, had rekindled interest in rapid diagnostic method in identification of species and antifungal susceptibility testing. Antifungal susceptibility testing is receiving an increased attention with the advent of newer antifungal drugs.

Aims and Objectives: To determine the antifungal susceptibility of the isolates using the E-test method.

Materials and Methods: A total of 110 samples were collected from clinically suspected/ diagnosed cases of dermatophytosis patients visiting dermatology OPD. out of which, 82 were culture positive. All dermatophytes species were subjected to antifungal susceptibility testing by E-test method according to the manufacturer's instructions using E-test strips for Fluconazole, Ketoconazole and Itraconazole.

Results: The most active agent against all dermatophytes species was itraconazole with an MIC range of 0.094-12 µg/ml., MIC50 values of 0.094-0.5 µg/ml and MIC90 values of 2-8 µg/ml.

Conclusion: The E-test represented a simple and efficacious method for antifungal susceptibility testing of dermatophytes. The emergence of drug-resistant dermatophytes stresses the need of antifungal susceptibility testing.

Key words: Dermatophytes, Antifungal susceptibility testing, E-test, Fluconazole, Ketoconazole, Itraconazole.

Introduction

The varied clinical presentation of dermatophytosis, which results in delay in diagnosis, poor compliance in follow up of cases consequently spread of infection in the community and antifungal resistance, had rekindled interest in rapid diagnostic method in identification of species and antifungal susceptibility testing [1]. Antifungal susceptibility testing is receiving increased attention with the advent of newer antifungal drugs. Antifungal resistance is crucial if a treatment fails, and it is necessary to determine the sensitivity of the causative organism. In such cases antifungal drugs are ideally given on the basis of in vitro sensitivity of the isolates. The development of clinical correlations will be facilitated and the clinical utility of antifungal susceptibility testing will be increased by incorporating antifungal susceptibility testing methodologies into the clinical trials of new antifungal drugs.

Materials and Methods:

Anti- Fungal Susceptibility Testing:

A total of 110 samples were collected from clinically suspected/ diagnosed cases of dermatophytosis patients visiting dermatology OPD. This included 62 skin scrapings, 40 nail clippings and 8 hair samples. Out of which 82 were culture positive. All dermatophytes isolates were subjected to antifungal susceptibility testing by E-test method according to the manufacturer's instructions using E-test strips for Fluconazole, Ketoconazole and Itraconazole.

Inclusion Criteria: For the study, all culture positive dermatophytes species with minimum of 10 isolates were included.

Exclusion Criteria: The study excluded all dermatophytes species that had less than 10 isolates and those with negative culture results.

Inoculum preparation for dermatophyte: For each isolate, a suspension of mycelia from a 7-day culture was prepared in saline to a concentration of 10^6cells/ml. The suspensions were streaked into Muller Hinton Agar (MHA) plates with the aid of moistened swabs.

E-test: The plates were allowed to dry for 15 min before the E-test strips were applied. A strip of E-test was then carefully placed on the center of each plate and incubated at 28ºC for reading at 96 hours.

Test reading:

The MIC values were the drug concentrations at which the border of the elliptical inhibition zone intersected the scale on the antifungal strip. The MIC50 values were the MIC values which inhibited 50% of all isolates while MIC90 inhibited 90% of all isolates.

Quality control strains: Trichophyton *rubrum* (ATCC 40051) [2]

Results:

Among all the dermatophyte isolates T. *mentagrophytes* was the predominant isolate 49 (59.75%) followed by T. *rubrum* 17 (20.73%), M. *gypseum* 10 (12.19%) and E. *floccosum* 6 (7.31%). The most active antifungal agent against all dermatophytes species was itraconazole with an MIC range of 0.094-12 µg/ml., MIC50 values of 0.094-0.5 µg/ml and MIC90 values of 2-8 µg/ml., followed by ketoconazole with MIC range of 0.064-24 µg/ml., MIC50 values of 0.25-2 µg/ml. and MIC90 values of 2-8 µg/ml. The least active agent was fluconazole with MIC50 of 64-≥256 µg/ml and MIC90 of ≥256 µg/ml (Table-1). MIC values for dermatophytes species with less than 10 isolates were not calculated.

Table 1: The MICS of the antifungals against dermatophytes clinical isolates

Dermato-phytes species	Total number of isolates	Antifungal agents (µg/ ml)								
		Fluconazole			Ketoconazole			Itraconazole		
		MIC range	MIC 50	MIC 90	MIC range	MIC 50	MIC 90	MIC range	MIC 50	MIC90
T. *mentagrophytes*	49	128 - ≥256	128	>256	0.064 – 12	2	8	0.094 – 12	0.5	8
T. *rubrum*	17	128-≥256	128	>256	0.064 – 24	0.38	6	0.094 – 8	0.25	2
M. *gypseum*	10	64 – 256	64	256	0.25 – 2	0.25	2	0.094 – 2	0.094	2

Summary

The most active agent against all dermatophytes species was itraconazole with an MIC range of 0.094-12 µg/ml., MIC50 values of 0.094-0.5 µg/ml and MIC90 values of 2-8 µg/ml.

Discussion

The determination of fungus in vitro antifungal susceptibility has been reported to be important for the ability to eradicate pathogenic dermatophytes **[3]**. In this study, itraconazole had the highest antifungal activity for all dermatophytes species with the lowest MIC ranges among the tested azole drugs, including MIC50 (0.094–0.5 µg/ml) and MIC90 (2–8µg/ml). Similar results have been verified by other researchers [4,5]. These data can help to explain the promising results obtained for the treatment of dermatophytosis with this antifungal agent [6]. The highest MIC values in this study were for Fluconazole (MIC50: 64-256 µg/ml and MIC90: ≥256 µg/ml). The similar results were mentioned by Fernandez-Torres et al [4] and Barros et al [7].

To summarize, our data showed that itraconazole was the most active azole against all dermatophytes isolates, followed by ketoconazole and fluconazole. In addition, the increase in MIC values for azole medications identified for several of our isolates, after therapy raises the likelihood of an increase in antifungal resistance. [8-11] Further studies on larger samples of dermatophytes are recommended to correlate the MIC values with the clinical outcome for each isolate-drug combination to allow clinician adapting different therapeutic options with a high probability of successful results. In addition, these studies could be beneficial for investigation of development of in vitro resistance in dermatophytes species, and for management of cases clinically unresponsive to treatment.

Conclusions

The E-test represented a simple and efficacious method for antifungal susceptibility testing of dermatophytes. Regarding its performance, the E-test was not labor demanding, was easy to interpret, and with the potential of being used as an alternative method of antifungal susceptibility testing of dermatophytes. The emergence of drug-resistant dermatophytes stresses the need of antifungal susceptibility testing, antifungal stewardship and development of strong antifungal policy to help clinicians for instituting appropriate antifungals empirically and to change, if needed, after antifungal sensitivity testing results become available.

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