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"Filling the Void in the Web Space"- Microbiological Perspective of Toe Web Space Infections in a Tertiary Care Hospital

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Abstract:

Toe web space infections are the most common superficial dermatoses of the feet. They are often unnoticed and progress to cause discomfort. There is lack of enough evidence on the microbiological profile and antifungal susceptibility pattern.

Settings and Design: *A prospective study was conducted between August 2015 to February 2017 in the Department of Microbiology at a tertiary care hospital.*

Methods and Material*: 200 samples were collected from interdigital spaces of Dermatology outpatients and inpatients. Bacterial and fungal pathogens were isolated and identified using conventional laboratory techniques. Antifungal susceptibility testing was done to determine the Minimum inhibitory concentration (MIC) by E-test. A small representative sample of Fusarium isolates were subjected to speciation by PCR and their MIC was determined by broth microdilution method for comparison.*

Statistical analysis used: p value was determined by Chi square test using SPSS software.

Results: *Our study demonstrated a male preponderance of 59 %, mostly between 41 to 50 years. Both the feet were affected in 43/48 (89.5 %) of the diabetic patients, the fourth web space being commonly involved. 198/200 (99%) of the samples were positive for either bacterial or fungal growth. There were a total of 156 fungal and 204 bacterial isolates. Out of 156 fungal isolates, Candida spp 62 (39.7 %) and Fusarium spp 50 (32 %) were the most common isolated yeast and mould respectively and 38 (24.3 %) dermatophyte species were isolated. Results of E-test and broth microdilution were comparable. Voriconazole and amphotericin B had lower MIC. Out of 204 bacterial isolates, Staphylococcus aureus 56 (27.4 %) and Pseudomonas aeruginosa 44 (21.5 %) were the most common isolates.*

Conclusions: *A good microbiological diagnosis would assist in the better alleviation of the symptoms and provide accurate treatment of web space infections.*

Key-words: ***Antifungal susceptibility, Candida, Fusarium, Toe web space.***

Introduction

Intertrigo/web space infection in *latin* means, "to rub in between". It is a complex nidus of sweat, irritation and superimposed infection which leads to discomfort and disease in the individual.[1],[2]

Toe web space infection is a common clinical entity, presents as either simplex (inflammation) or complex type

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(maceration, malodour and secondary infection). It is most commonly a fungal infection, often superimposed by a bacterial infection.[3],[4],[5] and may mimic dermatitis, psoriasis and acanthosis nigricans.[6] Due to its varied etiology, a clinico-microbiological correlation is essential for better diagnosis and treatment outcomes.

Subjects and Methods

A prospective study was conducted between August 2015 to February 2017 for a period of one and a half years at the Department of Microbiology in a tertiary care hospital. A total of 200 samples were collected mostly from outpatient and also from inpatients of the Department of Dermatology. Detailed history and examination of the lesion was done. The toe web space was cleaned with 70% alcohol and allowed to dry. Any purulent discharge was collected with a sterile swab and scrapping was taken from the intertriginous area with the blunt end of a sterile no. 22G blade and collected on a clean glass slide. The samples collected in swab were subjected to Gram stain and streaked on 5% sheep blood agar and MacConkey agar and incubated at 37°C. The plates were examined at 24 hrs and 48 hrs and the bacterial colonies were identified using conventional biochemical tests and antibiogram was performed by Kirby Bauer disc diffusion method according to CLSI 2014 guidelines. The scrapings collected on the slide were divided into three parts, one part was subjected to direct microscopy using 10 % KOH mount. The other two parts were inoculated into two sets of Sabouraud dextrose agar (SDA) slants with and without cycloheximide. One set of tubes were incubated at room temperature and the other at 37°C and were examined twice a week upto six weeks. The yeasts were identified using Gram stain, Germ tube test and Chrome agar. Moulds were identified by obverse colony morphology, reverse pigmentation and Lacto Phenol Cotton Blue (LPCB) mount. The fungal isolates were subjected to antifungal susceptibility testing and MIC was determined by using Ezy MIC strip (HiMedia, Mumbai, India) for voriconazole (0.002-32 mcg/ml), ketoconazole (0.002-32mcg/ml), fluconazole (0.016-256 mcg/ml), itraconazole (0.002-32mcg/ml) and amphotericin-B (0.002-32 mcg/ml). Four to five distinct colonies of yeasts or a loopful of mycelial growth was emulsified in 5ml of sterile saline and turbidity was adjusted to 0.5 McFarland standard. A sterile cotton swab was soaked in the prepared suspension and was streaked on to a SDA plate in the form of a lawn culture. The plates were allowed to dry for 15 minutes and an Ezy MIC strip was placed on the inoculated plate.(HiMedia product catalogue) The plates were incubated at room temperature for moulds and at 37°C for yeasts and were examined after two to four days for growth. MIC reading was taken at the point where the elliptical zone of inhibition intersects the strip. Further, a small number of the *Fusarium* isolates (15 numbers) were subjected to speciation by PCR and antifungal susceptibility for voriconazole, itraconazole, posaconazole, amphotericin B and caspofungin was studied by broth microdilution. The following primer pairs were used for DNA amplification of partial regions of two separate genes namely the panfungal marker Internal Transcribed Spacer (ITS) ITS1 5'-TCCGTAGGTGAACCTGCGG-3' and ITS 4 5'-TCCTCCGCTTATTGATATGC-3'and the marker of choice for *Fusarium* speciation Translation Elongation Factor (TEF-1α) EF1 5'-ATGGGTAAGGARGACAAGAC-3' and EF2 5'-GGARGTACCAGTSATCATG-3'.The samples which were positive in direct microscopy and failed to show growth were suggested to be sent for histopathological examination. The results were compiled, statistical analysis was done and p value was calculated by Chi square test using SPSS software.

Results

A total of 200 patients were included in the study. There were 118/200 (59 %) male and 82/200 (41 %) female patients in the age group of 18 to 80 years. The most common age group affected was 41 to 50 years i.e, 58/200 (29 %). 48/200 (24 %) of the patients were diabetic and 43/48 (89.5 %) among them had involvement of the both the feet with a significant *p value of 0.001* and the rest had either right or left foot involved with fourth web space invariably being involved, however, there was concomitant involvement of third web space in 160/200 (80 %) patients and second web space in 12/200 (6 %) patients. 170/200 (85 %) of the samples were positive for both fungal and bacterial growth, 2/200 (1 %) did not yield any growth as depicted in table -I.

Table I: Association of the bacteriological and fungal isolates

RESULTS	NO OF SAMPLES (N=200)	PERCENTAGE (%)
Positive bacterial growth No fungal growth	14	7
Negative bacterial growth Positive fungal growth	12	6
Positive bacterial and fungal growth	170	85
No growth	2	1

There were a total of 204 bacterial isolates and 156 fungal isolates. Out of 204 bacterial isolates, *Staphylococcus aureus* (27.4%) was the most common organism isolated followed by *Pseudomonas aeruginosa* (21.5 %) as summarised in table-II.

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Table II: Bacterial and fungal isolates

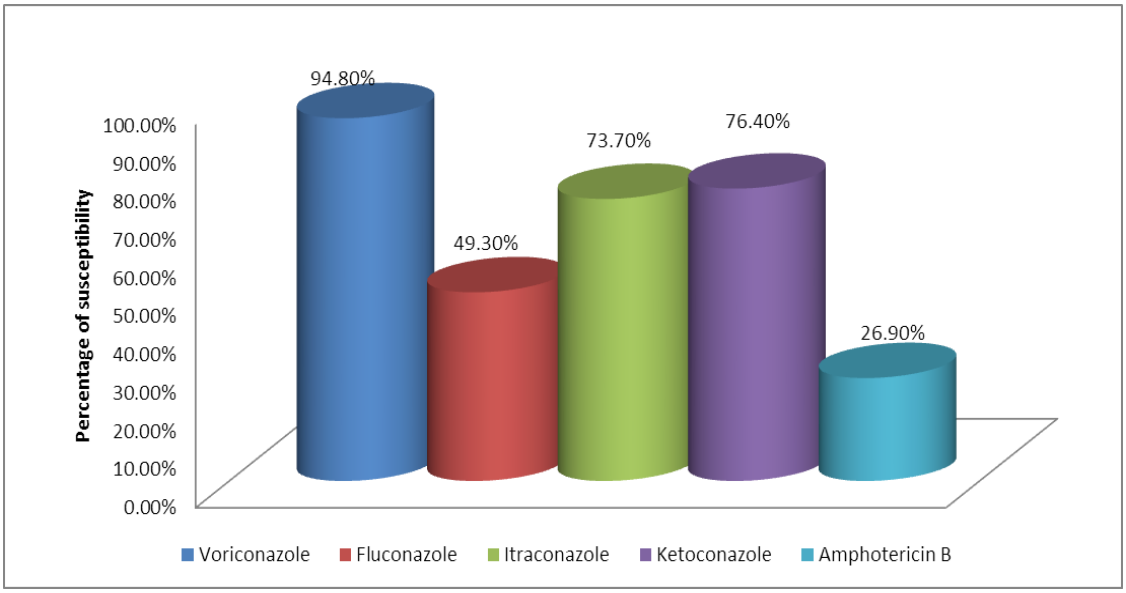
BACTERIAL SPECIES	NO. OF ISOLATES (N=204)	PERCENTAGE (%)	FUNGAL SPECIES	NO. OF ISOLATES (N= 156)	PERCENTAGE (%)
GRAM POSITIVE ORGANISMS			*Candida albicans*	40	25.6
Staphylococcus aureus	56	27.4	*Candida parapsilosis*	10	6.4
Enterococcus faecalis	10	4. 9	*Candida glabrata*	06	3.8
Coagulase negative Staphylococci	02	1	*Candida tropicalis*	04	1.9
GRAM NEGATIVE ISOLATES			*Candida dubliniensis*	02	1
Pseudomonas aeruginosa	44	21.5	*Fusarium keratoplasticum*	34	21.8
Klebsiella pneumoniae	30	14.7	*Fusarium falciforme*	12	7.7
Escherichia coli	20	9.8	*Trichophyton mentagrophytes*	10	6.4
Acinetobacter spp	14	6.8	*Trichophyton violaceum*	08	5.1
Proteus mirabilis	14	6.8	*Trichophyton rubrum*	08	5.1
Enterobacter aerogenes	10	4.9	*Trichophyton tonsurans*	06	3.8
Citrobacter koseri	04	1.9	*Trichophyton verrucosum*	04	2.6
Serratia marcescens	02	1	*Fusarium sacchari*	04	2.6
			Acremonium spp	04	2.6
			Geotrichum spp	03	1.9
			Epidermophyton floccosum	02	1.3
			Rhodotrula spp	02	1.3
			Aspergillus niger	02	1.3

The difference between positive and negative KOH mount result for prediction of subsequent culture result was statistically significant with a *p value of 0.005* and a positive predictive value of 91 % as represented in table-III.

Table III: Correlation between KOH mount and fungal growth

KOH MOUNT	CULTURE POSITIVE	CULTURE NEGATIVE	TOTAL
Positive	142	14	156
Negative	14	6	20
Total	156	20	Grand total -176

Out of 156 fungal isolates, *Candida spp* 62 (39.7 %) and *Fusarium spp* 50 (32 %) were the most common isolated yeast and mould respectively and 38 (24.3 %) dermatophyte species were isolated with *T. mentagrophytes* being the commonest, as summarised in table-II. Antifungal susceptibility to determine the MIC of all the fungal isolates was performed by E-test for amphotericin B, voriconazole, itraconazole, ketoconazole and fluconazole, results of which are represented in graph-I.



Graph -I: MIC of fungal isolates determined by E-test.

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A representative sample (15 numbers) of *Fusarium* isolates were subjected to species identification by Conventional PCR and MIC determination by broth microdilution test. *Fusarium keratoplasticum* was the most common species identified.

MIC for amphotericin B, voriconazole, posaconazole, itraconazole and caspofungin was determined and was found to be constantly high > 64 for itraconazole in 15/15 (100 %) isolates, least MIC was seen for voriconazole and amphotericin B as shown in table - IV.

Table IV: Comparison of MIC determined by E test and broth dilution method for fusarium isolates

S.No	Species Isolated	E test results in µg/ml					Broth micro dilution method results in µg/ml				
		AP	VRC	ITR	FLC	KET	AP	VRC	PSC	ITR	CSP
01.	*Fusarium keratoplasticum*	8	1	>32	>64	>32	4	1	32	>64	2
02.	*Fusarium falciforme*	4	1	>32	>64	>32	2	4	8	>64	16
03.	*Fusarium falciforme*	2	2	>32	>64	>32	2	2	4	>64	8
04.	*Fusarium keratoplasticum*	2	2	>32	>64	>32	4	4	16	>64	32
05.	*Fusarium keratoplasticum*	1	2	>32	>64	>32	1	2	8	>64	8
06.	*Fusarium keratoplasticum*	1	2	16	>64	>32	0.5	2	1	>64	2
07.	*Fusarium Sacchari*	1	1	16	>64	>32	0.5	2	1	>64	2
08.	*Fusarium keratoplasticum*	6	1	16	>64	>32	8	1	4	>64	8
09.	*Fusarium keratoplasticum*	2	2	8	>64	>32	2	4	8	>64	16
10.	*Fusarium keratoplasticum*	2	2	>32	>64	>32	1	4	32	>64	32
11.	*Fusarium Sacchari*	2	2	8	>64	>32	2	2	8	>64	16
12.	*Fusarium keratoplasticum*	8	4	16	>64	>32	8	4	>64	>64	8
13.	*Fusarium falciforme*	8	4	8	>64	>32	8	8	32	>64	32
14.	*Fusarium falciforme*	2	4	>32	>64	>32	1	4	32	>64	16
15.	*Fusarium keratoplasticum*	2	2	16	>64	>32	2	2	16	>64	16

AP-Amphotericin B, VRC- Voriconazole, ITR- Itraconazole, FLC- Fluconazole, KET- Ketoconazole, PSC- Posaconazole, CSP-Caspofungin

A comparison was made between the two methods and it was found that the results of both the methods were almost comparable and most of the isolates remained susceptible to voriconazole and showed an MIC of < 4, results depicted in table-IV.

Discussion

Web space infections of the toe are intriguing because they are common and they are difficult to treat as they mimic other non-infectious etiologies, moreover there is lack of enough substantial studies on the microbial profile of these infections. Hence, a study was undertaken to resolve the void in the web space profile.

In our study, maximum number of cases were seen in the age group of 41-50 years 58/200 (29%) which is in the

range of a study by *Krishna et al*[2] (21-60 yrs) and study by *Ahmad et al*[7] (21-50 yrs).The economically productive age group fall in this age group involving mostly men wearing shoe and women mostly house wives involved in frequent contact with water during household chores could probably explain a higher incidence of web space infections in this age group.

The incidence of web space infections was slightly higher among males 118/200 (59 %) in our study, similar to study by *Jing-Yi Lin et al* [8] and *Aste et al*.[9]

There were 48/200 (24%) diabetic patients. A significant number of diabetic patients 43/48 (89.5 %) had involvement of interdigital spaces of both the feet especially involving the fourth web space along with the third web space and infrequently even the second space. Superficial dermatoses caused by dermatophytes and *Candida spp* are commonly seen in diabetic patients as they have a decreased capacity to resist infections and delayed healing as suggested in a study by *Raghunath S et al* [10] and *Atzori et al*. [11]

In an earnest attempt to determine the bacterial and fungal profile of the samples, we found that web space lesions had a polymicrobial niche with co- existing bacterial and fungal pathogens 170/200 (85%). *S. aureus* was the most common gram positive bacterial isolate 56/204 (27.4 %). *P. aeruginosa* 44/204 (21.5%) was the most common gram negative bacteria isolated followed by *K. pneumoniae*, similar to a study by *Krishna et al* [2], *Atzori et al*[11] and *Kates et al.*[12]

The interdigital space is colonised by polymicrobial flora. Dermatophytes erode the stratum corneum and produce

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substances with antibiotic properties. However, gram negative bacteria evade these antibiotic like substances and proliferate, hence substantiating the co-existence of bacterial and fungal growth in the web space infections.[8] Among the fungal yeast isolates we found that *C. albicans* 40/156 (25.6%) was the most common isolate, *Fusarium spp* 50/156 (32 %) was the most common mould isolated and 38/156 (24.3 %) dermatophytes (*T. mentagrophytes, T. violaceum and T. rubrum*) were isolated, similar findings were seen in a study by *Krishna et al*[11] and study by *Ahmad et al*[7] which showed *C. albicans* as predominant isolate, however other studies like *Ingordo et al*[14] have shown dermatophytes like *T. rubrum* and *T. mentagrophytes* as predominant isolate.

There has been increasing interest and evidence in the study and isolation of non-dermatophytes like *Fusarium spp*, *Aspergillus* and dematiaceous fungi but there is still a lacuna in the substantial evidence for these pathogens. Hence, this calls for more interest and research in this regard.[13]

There was no growth in 02/200 (1 %) probably explained by the fact that there was mechanical damage and initial infection had set without any secondary bacterial infection in or probably a small number of patients were already empirical antifungal treatment. In such cases a histopathological examination of a biopsy from the site of infection would be ideal.

In our study, we found that only 34/204 (16.7%) of the gram positive isolates were susceptible to erythromycin, which correlates with a study by Krishna et al[2] who showed that maximum number of gram positive isolates were resistant to erythromycin. Among the gram negative isolates, there was an increasing trend of resistance. Only 18/204 (8.8%) and 54/204 (26.4 %) of the gram negative isolates were susceptible to amoxyclav and cefuroxime respectively. The increasing use of steroids as empirical therapy and over the counter misuse of antibiotics has led to increase in resistance in organisms.

Antifungal susceptibility to determine the MIC of all the fungal isolates was performed by E-test for amphotericin B, voriconazole, itraconazole, ketoconazole and fluconazole, results of which are represented in graph-I. There has been increase in resistance to commonly used fluconazole and itraconazole, however, voriconazole is a promising agent in the treatment of fungal pathogens, also suggested in a review by *Patrick et al.* [15]

A representative sample of *Fusarium* isolates was subjected for further species confirmation by conventional PCR and MIC was determined by broth microdilution for voriconazole, itraconazole, amphotericin B, posaconazole and caspofungin. The results were comparable and we found that voriconazole and amphotericin B showed lower MIC compared to itraconazole. Broth microdilution is the prescribed method by CLSI and remains the standard for antifungal testing but it is tedious and time consuming. In comparison, E-test strips are easy to interpret less time consuming and produce comparable results but are expensive to be adopted as a routine testing protocol.

Conclusion

Web space infections are a common condition especially in middle aged diabetic and non-diabetic patients, frequently affecting third and fourth web space of both the feet and have a slight male preponderance. Interdigital infection can be confused with eczema and psoriasis and is often complicated with secondary bacterial infection. Hence, our study urges to promote further studies on etiological diagnosis of interdigital infections, to determine antibacterial and antifungal susceptibility to ensure better early response to treatment for the benefit of the patient.

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