INTRODUCTION

Burns are a common trauma. Burn patients have a high risk of infection due to many different agents such as bacteria, fungi ... Up to now, infection is still one of the leading causes of death in burn patients.

Fungal infections in burn patients have different degrees such as fungus growing on the lesion surface or deeply invading healthy tissue or sepsis. *Candida* is the most common cause in addition to some types of mould such as *Aspegillus*, *Fusarium*, *Mucor*…. There is emerging of resistance to antifungal drugs which occur in different species of causative fungi and different antifungals.

Every year in Vietnam there are thousands of patients who suffer from burns and hundreds of them have to be treated in the Intensive Care Unit (ICU). However fungal infections in burn patients have received little attention so far. Therefore, we carry out the thesis "*Study on epidemiological characteristics and treatment results of fungal infections in patients with severe burns at the National Hospital of Burn (2017 - 2019)*" with 3 objectives:

1. *Determine the prevalence and associated factors of fungal infection in patients with severe burns (March 2017 - December 2019).*

2. Determine the fungal species composition in patients with severe burns by morphological and molecular biology methods.

3. Evaluate the sensitivity of the isolated strains to some antifungal drugs and results of treatment of fungal infections in severely burned patients.

*** NOVELTY AND SCIENTIFIC SIGNIFICANCE OF THE THESIS**

1. This is the first study in Vietnam to determine the prevalence of colonization and invasive fungal infections as well as related factors in patients with severe burns.

2. Applying morphological methods and molecular biology techniques to determine the fungal species composition isolated from burn patients.

3. Evaluate the sensitivity of *Candida* species to antifungal drugs commonly used in treatment.

4. Develop treatment regimens and evaluate the results of antifungal therapy in burn patients.

THESIS STRUCTURE

The thesis consists of 120 pages divided into the following sections: Introduction (2 pages), literature review (33 pages), study subjects and methods (22 pages), study results (32 pages), discussions (28 pages), conclusions (2 pages), and recommendations (1 page). There are 167 references, 33 tables and 19 figures.

Chapter 1. LITERATURE REVIEW

1.1. The prevalence and related factors of fungal colonization and infection in burn patients

1.1.1. Definition, classification

1.1.1.1. Definition and characteristics of burn injury

A burn is a type of injury/ wound caused by non-mechanical factors but by elements of heat (heat/cold), chemistry, radiation (ionization, non-ionization ...). Burns caused by heat compose most of the patients. A

burn is considered a disease with different stages including acute reaction, infection - intoxication with complications and recovery phase.

1.1.1.2. Definition and medical role of fungus

Fungi are eukaryotic, cell-walled, heterotrophic, and spore-reproducing organisms. Fungi can cause fungal diseases of the skin or subcutaneous and systemic disease that can affect the blood or deep-seated, visceral.

1.1.1.3. Definition, classification of fungal infections in burn patients

Fungal colonization (FC) was defined as fungi isolated from a non-sterile body sites ((respiratory fluids, faeces, urine ...).

Fungal wound/burn infection (FWI) was defined as the presence of fungus in the healthy layer of the wound.

Fungemia is the bloodstream infection caused by fungus.

Invasive fungal infections (IFI) include FWI and fungemia.

1.1.2. Prevalence of fungal infection in patients with burns

1.1.2.1. Prevalence of fungal colonization

An Italian study on patients with ICU found that 92.3% of patients had a fungal infection on the admission of the ICU.

1.1.2.2. Prevalence of fungal wound infection

Prevalence of fungal wound infection ranges from 2 - 20%.

1.1.2.3. Prevalence of fungemia

Prevalence of fungemia ranges from 3 - 5% among burn patients.

1.1.3. Factors related to fungal infection

1.1.3.1. Factors of burn pathology

Post-burn pathological changes associated with fungal infection. Heat-induced skin damage and impaired local and systemic immune so burn patients are at risk of infection. Bacterial penetration of the burn occurs

early. Fungal infections usually begin to appear in the second week and get the highest prevalence in the third and fourth week after the burn.

1.1.3.2. Factors of burn treatment

Burn patients usually undergo multiple interventions such as surgeries, blood transfusions, total parenteral nutrition (TPN) and kidney replacement which weaken the patient's immune system thereby facilitating fungal infections after burns.

1.1.3.3. Factors of environment

The risk of fungal infection from the polluted surrounding environment of patients in burn care units

1.2. Fungal identification techniques and species composition in burn patients

1.2.1. Fungal identification techniques

1.2.2.1. Fungal identification techniques based on morphological characteristics

- Identification of yeast: the occurrence of the spores; germ tube, biochemical reactions or the use of a chromogenic medium. There are several automated identification systems like Vitek II.

- Identification of mould: based on macroscopic and microscopic morphological features with the help of commonly used keys.

1.2.2.2. Identification of fungi by molecular biology

Previously, many techniques were applied to identify fungal species such as RFLP, RAPD ... Nowadays the sequencing of internal transcribed spacer regions (ITS1 / ITS2) or other regions is applied.

1.2.2. Fungal species composition in burn patients

Common fungal agents causing colonization are *Candida, Aspergillus, Fusarium, Mucor ... C. albicans* is the most common but the rate of non-albicans tends to increase.

FWI: yeast is the leading cause of FWI followed by some moulds like *Aspergillus*, *Syncephalestrum* and *Fusarium*.

Fungemia: The five most common species that cause IFI are *C. albicans, C. tropicalis, C. glabrata, C. parapsilosis,* and *C. krusei*. *Candida* species composition is different in different geographical location, the patient population as well as a history of antifungal drugs.

Some studies on the composition of fungal species in Vietnam

Some studies at the National Hospital of Burn found that *C. albicans* is the causative agent among all burn patients with fungemia.

1.3. The sensitivity of the fungus to some antifungal agents and results of fungal infection treatment in burn patients

1.3.1. Antifungal agents

1.3.1.1. Polyene: amphotericin B

1.3.1.2. Azole: fluconazole, voriconazole.

1.3.1.3. Echinocandins: caspofungin, micafungin

1.3.1.4. Flucytosine (5-FC): flucytosine.

1.3.2. The sensitivity of the fungus to some antifungal agents

1.3.2.1. Methods for antifungal sensitivity testing

The two available reference technique for antifungal sensitivity testing are CLSI (US) and (EUCAST) (Europe). There are some commercial kits such as Etest, Vitek 2, Sensititre Yeast One which have shown consistent results with the reference methods.

1.3.2.2. Susceptibility of common fungi to some antifungals

- The sensitivity of *Candida* to antifungals

C. albicans is usually sensitive to azoles and amphotericin B while *C. glabrata* is less susceptible to azoles and amphotericin B. *C. krusei* is less susceptible to all drugs.

Echinocandins are effective against *Candida*, including azole-resistant strains and echinocandins resistance among *Candida* strains are very rare

+ Sensitivity of *Aspergillus*: azole resistance already exists.

Fusarium is usually resistant to amphotericin B.

1.3.3. Treatment of fungal infections in burn patients

1.3.3.1. Treatment strategies

Prophylactic: an antifungal agent is administered to patients at risk for IFI but in the absence of any attributable signs and symptoms. Empiric treatment is defined as an antifungal treatment in patients at risk of IFI and with established clinical signs and symptoms. Pre-emptive treatment is defined as an antifungal treatment in patients at risk of IFI and a diagnostic workup have yielded results suspicious of IFI. Targeted treatment may be applied if diagnostic criteria allow for definite pathogen identification.

+ Treatment of invasive candidiasis: amphotericin B or caspofungin and de-escalation with an azole.

+ Treatment of invasive aspergillosis: triazole, echinocandin (caspofungin, micafungin) or amphotericin B.

+ Treatment of invasive fusariosis: voriconazole

1.3.3.2. Evaluate the results of fungal infection treatment in burn patients

Routine clinical and subclinical parameters, fungal tests, the occurrence of severe complications, mortality or survival.

Chapter 2 STUDY SUBJECTS AND METHODS

2.1. Subject and method for objective 1: Prevalence and related factors of fungal infection in patients with severe burns

2.1.1. Subjects

- Severely burned patients due to heat at the National Burn Hospital.

2.1.2. Time and site of the study

- Study time: From March 2017 to December 2019.
- Site: ICU, NHB; mycological laboratory, Department of Parasitology, Vietnam Military Medical University.

2.1.3. Methods

2.1.3.1. Study design: Descriptive, analytic, prospective research.

2.1.3.2. Sample size

According to the formula for a ratio, the calculated sample size was 385 patients. The thesis collected information of 400 patients

2.1.3.3. Sampling method

All patients who met the study criteria were selected.

2.1.3.4. Research content

Determine the prevalence of colonization/infection and related factors in patients with severe burns.

2.1.3.5. Methods of determining and measuring variables

2.1.3.6. Variables

- Prevalence of FC, FWI and fungemia.

Colonization index (CI), heavy colonization: when a CI index is ≥ 0.5.

- Factors related to fungal colonization/infection in burn patients.

2.1.3.7. The techniques applied in the study

- Examination, evaluation and description of burn injuries.

- Collection of samples: weekly according to routine procedures.
- Detection of fungi in collected samples: direct examination, staining, culture.
- Techniques to determine factors related to fungal infection.

2.1.3.8. Materials

- Medical record.
- Tools for collecting specimens, examination and isolation of fungi.

2.1.3.9. Data analysis and processing: according to biomedical statistics by SPSS 16.0 software.

2.2. Subject and method for objective 2: Determine the fungal species composition in patients with severe burns

2.2.1. Subjects

- Fungi: isolated from the burn patients involved in objective 1.

2.2.3. Methods

2.2.3.1. Study design: an experimental study.

2.2.3.2. Sample size: All fungal isolation from the burn patients involved in objective 1.

2.2.3.3. Sampling method

- All isolated strains were subjected to identification based on morphological and biochemical features.
- Identification by molecular techniques: strains isolated from sterile samples, identified as rare yeast or representatives of common species.

2.2.3.6. The techniques applied in the study

- Identification of fungi by histopathological examination and morphological characteristics with the help of common keys. Yeasts were identified by Brilliance Candida Agar medium and Vitek 2 system.

- Molecular biology technique: amplification of ITS region with primers ITS1, ITS4, sequenced and compared with those in GenBank.

2.3. Subject and method for objective 3

2.3.1. Evaluate the sensitivity of the isolation to some antifungals

2.3.1.1. Subjects

- 184 *Candida* strains isolated from the burn patients.

2.3.1.5. The techniques applied in the study

Antifungal susceptibility testing (AST) was performed with Vitek2Compact (Biomerieux, France).

2.3.1.6. Variables

- Levels of susceptibility (S (susceptible), I (intermediate) and R (resistant) according to species-specific clinical breakpoints (CBP) established by the Clinical and Laboratory Standards Institute (CLSI).

2.3.2. Evaluation of treatment outcomes for fungal infections in patients with severe burns

2.3.2.1. Subjects:

- Severely burned patients due to heat at the National Burn Hospital.

2.2.2.3. Methods

- Study design: evaluate the effectiveness of the therapy regime.
- Sample size: All patients who indicated antifungal drugs.
- Treatment indication

+ Fungal-oriented treatment: patients at risks for IFI, have fever, heavy colonization; Ostrosky-Zeichner prediction rules, Candida score> 2.5.

+ Fungal-targeted treatment: patients with definite diagnose of IFI.

- Treatment regimen: fluconazole, caspofungin for patients infected with yeast species and voriconazole for those with mould.

Chapter 3 RESULTS

3.1. Results of the prevalence and related factors of fungal infections in patients with severe burns

3.1.1. Information about the subjects

The average age was 29.74 years old. The age group with the highest percentage is children ≤ 10 years old and who from 31-40 years old. Men were more affected than women (male / female = 3.49 / 1).

The overall burn area is 44.39%; 17.72% of deep burns.

The average length of hospital stay was 36.73 days, ICU stay was 18.39 days. The subjects had many serious consequences and treatment interventions. The mortality rate was 19.50%.

3.1.2. Prevalence of fungal colonization and infection

Table 3.4. The prevalence of fungal colonization/infection

Fungal colonization/infection				n	Percentage (%)
Yes (360)	FC (309)			309	77.25
	FC and IFI (51)	FWI	Yeasts	22	5.50
			Molds	11	2.75
			Yeasts and moulds	3	0.75
		Fungemia		12	3.00
		Fungemia + FWI		3	0.75
No (40)				40	10.00

There were 90% of patients with fungal including 12.75% of patients with IFI. All patients with IFI had fungal colonization.

The prevalence of fungal colonization/infection in male and female patients was not statistically significant.

Table 3.6. Prevalence of fungal colonization/ infection by age group

Age groups	n	Colonization		Infection	
		n1	%	n2	%
1 - 15 (1)	113	97	85.84	6	5,31
16 – 65 (2)	267	244	91.39	42	15,73
66 – 94 (3)	20	19	95.00	3	15,00
Total	400	360	100	51	100
p		0,192		0.019	

The rate of IFI in the 1-15-year-old group was lower ($p < 0.05$).

3.1.3. Factors related to fungal infection

3.1.3.1. Factors related to fungal colonization

Table 3.9. Multivariate analysis of factors related to fungal colonization

Factors \ FC		Yes	No	OR (CI 95%)	p
Severe infection	Yes	306	27	1.620 (0.701 - 3.746)	0.259
	No	54	13		
Hyperglycemia	Yes	162	9	1.355 (0.539 - 3.406)	0.518
	No	198	31		
Hemodialysis	Yes	59	1	4.758 (0.594 - 38.139)	0.142
	No	301	39		
TPN	Yes	277	23	1.402 (0.626 - 3.138)	0.412
	No	83	17		

On multivariate analysis, no factors made the patients a higher risk of fungal colonization.

The rate of FC has little change over time.

3.1.3.2. Factors related to invasive fungal infection

Table 3.14. Multivariate analysis of factors related to invasive fungal infection

Factors \ IFI		Yes	No	OR (CI 95%)	p
Severe infection	Yes	49	284	0.250 (0.035 - 1.787)	0.167
	No	2	65		
Prolonged ICU stay	Yes	41	166	2.572 (1.098 - 6.023)	**0.030**
	No	10	183		
Renal failure	Yes	21	57	0.899 (0.352 - 2.293)	0.823
	No	30	292		
Hyperglycemia	Yes	45	126	4.067 (1.333 - 12.408)	**0.014**
	No	6	223		
Catheter	Yes	48	228	0.976 (0.219 - 4.350)	0.974
	No	3	121		
Hemodialysis	Yes	23	38	2.257 (0.892 - 5.709)	0.086
	No	28	311		
TPN	Yes	50	250	4.588 (0.389 - 54.155)	0.226
	No	1	99		
Mechanical ventilation	Yes	30	94	0.738 (0.305 - 1.789)	0.502
	No	21	255		
Immunosup-pressive therapy	Yes	45	165	2.398 (0.832 - 6.913)	0.106
	No	6	184		
Heavy colonization	Yes	45	197	2.790 (1.071 - 7.265)	**0.036**
	No	6	152		

Hyperglycemia, prolonged ICU stay and heavy *Candida* spp. colonization was found to be independently predictive of IFI on the multivariate model. The rate of IFI increase with time.

3.2. Results on fungal species composition in burn patients

3.2.1. Composition of species causing fungal colonization

Table 3.15. Composition of yeast and mould in burn patients

Yeast/mould	n	(%)/colonized (n=360)	(%)/total of patients (n=400)
Yeast	360	100	90
Mould	28	7.78	7.00
Combination	28	7.78	7.00

100% of colonized patients had yeast. 7.78% of patients infected with a combination of yeast and mould.

Table 3.16. Distribution of yeast species isolated

Species	n	%
C. albicans	151	41.94
C. tropicalis	164	45.56
C. parapsilosis	23	6.39
C. lusitaniae	4	1.11
C. glabrata	5	1.39
C. dubliniensis	4	1.11
C. famata	3	0.83
C. ciferrii	2	0.56
C. krusei	2	0.56
C. tropicalis + *C. duobushaemulonii*	1	0.28
C. parapsilosis + *Kodemaea ohmeri*	1	0.28

Among 11 isolated species, *C. tropicalis* (45.56%) accounted for the highest proportion, followed by *C. albicans* (41.94%).

Table 3.18. Distribution of mould species isolated

Species	n	(%)/colonized (n=360)	(%)/colonized with mould (n=28)
A. fumigatus	11	3.06	39.29
A. oryzae	6	1.67	21.43
A. flavus	6	1.67	21.43
A. chevalieri	2	0.56	7.14
A. nomius	2	0.56	7.14
F. solani	1	0.28	3.57

Aspergillus accounted for the main proportion (27/28 cases = 96.43%). Besides, *Fusarium solani* was also encountered.

A. fumigatus species is most common among mould (39.29%).

3.2.2. Composition of species causing an invasive fungal infection

Table 3.20. Distribution of yeast species causing fungal wound infection

Species	n	Percentage (%)	
		Among those with FWI (n=40)	Among those with FWI caused by yeast (n=29)
C. tropicalis	20	50.0	68.97
C. albicans	7	17.5	24.14
C. parapsilosis	1	2.5	3.45
K. ohmeri	1	2.5	3.45

The most common yeast causing FWI was *C. tropicalis* (50,0%), *C. albicans* (17,5%).

Table 3.21. Distribution of mould species causing fungal wound infection

Species	n	Percentage (%)	
		Among those with FWI (n=40)	Among those with FWI caused by mould (n=14)
A. fumigatus	6	15.00	42.86
A. flavus	3	7.50	21.43
A. oryzae	2	5.00	14.29
A. nomius	1	2.50	7.14
A. chevalieri	1	2.50	7.14
F. solani	1	2.50	7.14

Aspergillus was the main cause of FWI caused by mould (13/14 cases), in addition to *F. solani* (1/14 cases). The most common *Aspergillus* species were *A. fumigatus* (15%), *A. flavus* (7.5%).

Table 3.23. Distribution of species causing fungemia

Species	N	Percentage (%)
C. tropicalis	9	64.29
C. albicans	3	21.43
C. parapsilosis	2	14.29
Tổng	14	100

The most common etiologies of fungemia was *C. tropicalis* (64.29%), *C. albicans* (21.43%). *C. parapsilosis* was also encountered.

3.3. Results of the sensitivity of the fungi and treatment of fungal infections in severely burned patients

3.3.1. Results of the sensitivity of the fungal isolation

Table 3.24. Susceptibility of *Candida* species to antifungal drugs

Antifungal	n*	Sensitivity		Intermediate		Resistant	
		n_1	%	n_2	%	n_3	%
Fluconazol	180	150	83.33	9	5.00	21	11.67
Voriconazol	181	163	90.06	7	3.87	11	6.08
Caspofungin	181	177	97.79	3	1.66	1	0.55
Micafungin	181	180	99.45	1	0.55	0	0.00
Amphotericin B	183	177	96.72	0	0.00	6	3.28
Flucytosin	182	172	94.51	6	3.30	4	2.20

The echinocandin drugs (caspofungin and micafungin) had the highest sensitivity. The lowest susceptibility rate was azole drugs: fluconazole (83.33%), followed by voriconazole (90.06%). Flucytosine (94.51%) and amphotericin B (96.72%) had a relatively high rate of sensitivity.

C. albicans was not resistant to echinocandin. The rate of sensitivity to fluconazole was low (86.49%), The rate of sensitivity to flucytosine, voriconazole and amphotericin B was about 95%.

The rate of *C. tropicalis* resistant to azoles was high, and resistant to echinocandin and amphotericin B was low.

Other fungi were not resistant to echinocandin.

3.3.2. Results of the treatment of fungal infections in severely burned patients

Candida score were all above 3. The change between groups was not significant ($p > 0.05$). CI index after treatment decreased

compared to that before treatment.

The average clearance time in patient's biopsy tissue and blood was 12.71 days (7 to 23 days) and 8.11 days (4 to 12 days) respectively.

Table 3.32. The outcome of treatment of invasive fungal infection

Diagnosis	n	Survive		Mortality	
		n	%	n	%
FWI	31	24	77.42	7	22.58
Fungemia	7	2	28.57	5	71.43
FWI + Fungemia	3	1	33.33	2	66.67
Total	41	27	65.85	14	34.15

Among those with IFI and antifungal treatment, there were 65.85% of the patients had succeeded in treatment.

Table 3.33. Comparison of mortality and duration of fungal treatment in patients with invasive fungal infections

Parameters			**Mortality**	**Survive**	**total**	**p**
Strategies	Fungal-oriented	n	3	16	19	0.048
		%	15.79	84.21	100	
	Fungal-targeted	n	11	11	22	
		%	50.00	50.00	100	
Time of treatment	Yearly	n	4	13	17	0.383
		%	23.53	76.47	100	
	Lately	n	10	14	24	
		%	41.67	58.33	100	

The outcome of treatment depended on the treatment strategies but not related to the time of treatment.

Chapter 4. DISCUSSION

4.1. Prevalence and related factors of fungal colonization and infections in burn patients

4.1.1. Prevalence of fungal colonization and infections

4.1.1.1. Prevalence of fungal colonization

There were 90% of patients colonized or infected with fungi, among them 77.25% had colonization and 12.75% had a combined IFI.

4.1.1.2. Prevalence of fungal wound infection

The prevalence of FWI was low (9.75%) and consistent with other reports. The low prevalence of FWI was also concordance with the low rate of isolation of fungi on the wound surface.

4.1.1.3. Prevalence of fungemia

The prevalence of fungemia was 3.75% and in line with other studies. The prevalence of fungemia in the current study is higher compared to the prevalence reported at Cho Ray hospital, Ho Chi Minh city (2.09%). However, in the present study, only those with severe burn and treated at ICU were involved so that they may have a higher risk of fungemia.

4.1.2. Factors related to fungal colonization/infection in burn patients

4.1.2.1. Factors related to fungal colonization

The univariate analysis presented severe infection, prolonged ICU stay, hyperglycemia, dialysis and TPN as significant predictors of FC. However, the multivariate analysis showed that there were no factors predicted FC in burn patients. This would be reasonable because most colonized patients having isolation on the ICU admission and the rate of FC was nearly steady over the time those patients were in the ICU. This result is consistent with some other studies.

4.1.2.2. Factors related to fungal invasive infection

Several factors were found to be associated with IFI on univariate analysis, nevertheless, on multivariate analysis only hyperglycemia, prolonged ICU stay and heavy *Candida* spp. colonization was independently predictive of IFI.

4.2. Species composition of fungal isolation

4.2.1. Species composition causing fungal colonization

All colonized patients were colonized with yeast and some colonized with both yeast and mould. This result is also consistent with most reported studies.

4.2.1.1. Species composition of yeast

10 species of *Candida* were discovered, of which *C. tropicalis* was the most common (45.56%) followed by *C. albicans* (41.94%). *C. albicans* is still a common species but not accounts for over 50%; This result follows the trends of the decreasing rate of *C. albicans* and the increasing rate of non-albicans *Candida* in the world and Vietnam specifically.

4.2.1.2. Species composition of mould

There were 28 patients (7%) or infected with mould including *Aspergillus* (accounted for the most) and *Fusarium* species. Among *Aspergillus* species, *A. fumigatus* accounted for the majority (2.75%) along with some other species such as *A. oryzae, A .flavus ...*

4.2.2. Species composition causing an invasive fungal infection

4.2.2.1. Species composition causing fungal wound infections

- Composition of yeast and mould: Yeast is the leading cause of FWI that is consistent with some other authors. The high incidence of FWI caused

by mould contradicted to the low rate of FC by mould emphasize the importance of protecting wounds from the contaminated environment.

- Yeast species composition: The most common responsible agent of FWI was *C. tropicalis* (43.59%), followed by *C. albicans* (17.95%) that was comparable to previous reports.

- Mould species composition: *Aspergillus* was still the most common cause of FWI that was consistent with other studies and demonstrated high virulence of *Aspergillus* among moulds. A patient infected with *F. solani*, the *Fusarium* species most commonly encountered in humans

4.2.2.2. Species composition causing fungemia

Among agents responsible for fungemia *C. tropicalis* accounted for the main proportion (64.29%), followed by *C. albicans* (21.43%) and *C. parapsilosis*. This result is in agreement with some of the reports in Vietnam showing that *C. albicans* and *C. tropicalis* were the most common *Candida* isolated from blood.

4.3. The sensitivity of the isolated strains to some antifungals and results of treatment of fungal infections in severely burned patients

4.3.1. The sensitivity of the isolated strains to some antifungals

- Except for micafungin, the isolated strains showed some degrees of resistance to the tested drugs. The lowest susceptibility was to fluconazole, followed by voriconazole, flucytosine, amphotericin B. The echinocandin drugs had a high susceptibility rate.

+ The lowest rate of sensitivity to fluconazole was and consistent with some studies showing that azoles, especially fluconazole, had a high rate of reduced response and drug resistance. In Vietnam, the high rate of *Candida* resistance against fluconazole has been reported (57.7%).

+ Voriconazole: 90.06% of *Candida* strains were sensitive to voriconazole. A study on 271 clinical strains in Singapore found that the rate of susceptibility to voriconazole was relatively low (86.9%).

+ Echinocandin: has a very high sensitivity rate (98.90% to caspofungin and 99.45% to micafungin). At present, an echinocandin is considered the first choice in the treatment of invasive candidiasis.

+ Amphotericin B: the sensitivity rate to amphotericin B was 96.72%. Resistance to amphotericin B is still rare, possibly due to the fungicidal nature of the drug that limits the mutation.

+ Flucytosin: 94.51% of the Candida strains were sensitive to flucytosine which complied with some other reports.

+ Susceptibility of *C. albicans*: *C. albicans* strains were not resistant to echinocandin. This result is this follow the trend described previously that most strains of *C. albicans* are sensitive to common antifungals, however, some strains show the resistance, especially against fluconazole and flucytosine.

+ Susceptibility of *C. tropicalis*: the rate of *C. tropicalis* resistant to echinocandin was low but to azole were high (fluconazole 15.66%, and voriconazole 9.88%). Our results seem to highlight that antifungal resistant rate among *C. tropicalis* is higher than that of *C. albicans*.

+ Susceptibility of other *Candida* species: other *Candida* species had a high rate of resistance to antifungals, especially to azoles.

4.3.2. Evaluate the results of treatment of fungal infection in burn patients

4.3.2.1. Regimen for treatment of fungal infections in burn patients

Most patients took the fungal-oriented regimen which was consistent with other researches. The rate of taking targeted regime was low.

4.3.2.2. Changing of fungal assay

- Candida score: All patients had Candida score above 3 at the all time-point of evaluation and there was no change of statistical significance.

- CI: CI tended to decrease after antifungal treatment. Even so, fungi were still isolated from non-sterile samples of patients taking antifungals.

- The time to clear the fungus in the blood was similar to that of some other reports.

4.3.3.4. Result of treatment

Among 41 patients with IFI receiving antifungals, the survival rate was 65.85% that was equivalent to other reports. The approach is more important than the timing of treatment. Patients taking fungal-oriented therapy have a lower mortality rate than fungal-targeted treatment, while the early treatment does not reduce mortality compared with the late treatment group.

CONCLUSION

1. Prevalence and associated factors of fungal colonization/infection in patients with severe burns

- The prevalence: 90% of the patients are colonized with fungi including 12.75% having an invasive fungal infection (9.25% have fungal wound infections, 2.75% have fungal infections). The prevalences are not different by age and sex, except that the prevalence of invasive fungal infection in children (1-15 years old) is lower than that of the other age groups.

- Associated factors of fungal colonization/infection

Patients with severe burns have a high rate of fungal colonization on hospital admission and no factors made the patients a higher risk of fungal colonization.

Hyperglycemia (OR = 4,067), prolonged ICU stay (OR = 2,790) and heavy *Candida* spp. colonization (OR = 2,572) is independently predictive of invasive fungal infection.

2. Fungal species composition in patients with severe burns

Colonization: 100% of colonized patients have yeast isolation, 7.78% have both yeast and mould. The isolated fungi belong to 17 species, including 11 species of yeast and 6 species of mould. Among yeast species, *Candida tropicalis* accounts for the highest proportion (45.56%), followed by *Candida albicans* (41.94%). *Aspergillus* is the most common mould (in which *Aspergillus fumigatus* accounts for 39.29%).

Fungal wound infections: 72.5% of cases are caused by yeasts and the most common agents are *Candida tropicalis* (50.0%), *Candida albicans* (17.5%). 35% are caused by mould such as *Aspergillus fumigatus* (15%), *Aspergillus flavus* (7.5%), *Fusarium solani* (2.5%).

Fungemia: *Candida tropicalis* accounts for the most (64.29%), followed by *Candida albicans* (21.43%), *Candida parapsilosis* (14.29%).

3. The sensitivity of the isolated strains to some antifungals and results of treatment of fungal infections

The echinocandin drugs (caspofungin and micafungin) have a high rate of sensitivity while azole drugs have the lowest sensitivity.

Candida albicans is not yet resistant to echinocandin but 5.19% of strains are resistant to fluconazole.

The *Candida* non- *albicans* yeast has a high rate of resistance to azole and amphotericin B but susceptibility to echinocandin.

- Evaluation of treatment results

Over 67 patients taking antifungals: Candida score is little changed and colonization index tends to decrease. The average clearance time in the biopsy tissue was 12.71 days (7 to 23 days); in the blood is 8.11 days (4 to 12 days).

Over 41 patients with invasive fungal infection and take antifungals: The cure rate is 65.85%. The approach of treatment is more important than the timing of treatment. Patients taking fungal-oriented therapy have a lower mortality rate than fungal-targeted treatment, while the early treatment does not reduce mortality compared with the late treatment group

RECOMMENDATION

1. Screen for fungi in patients with severe burns, especially among those with hyperglycemia, prolonged ICU stay.

2. Use of fungal-oriented treatment regimen for patients who have risk factors (severe burns, hyperglycemia), unexplained fever despite broad-spectrum antibiotic therapy, heavy colonization (Colonization index ≥ 0.5), Candida score> 2.5, clinical conditions that meet criteria for Ostrosky-Zeichner' clinical prediction rule.

3. Test the susceptibility to antifungals of fungi isolated from patients with fungal wound infection or fungemia caused by *Candida* to select suitable antifungals.

4. Monitor the changing of species composition and susceptibility to antifungals for appropriate treatment recommendations.