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Clinical characteristics and antifungal susceptible patterns of yeast-like fungal isolates during 2004-2009 in Japan

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A B S T R A C T

The frequency of invasive mycoses due to opportunistic fungal pathogens has increased dramatically past two decades and Candida species ranks as the major common cause of nosocomial infections. However, the pattern of these fungal isolates has not been elucidated in mid-Japan. Our aim was to characterize the yeast-like fungi isolated from aseptic site in hospitalized patients at the Nagoya City University Hospital. A retrospective analysis was conducted from 2004 to 2009 by reviewing medical records of patients from Nagoya City University Hospital. The total of 74 yeast-like fungi was analyzed. They were obtained from blood sample 69 (91.5%), ascites 2 (2.7%), spinal cord fluid 1 (1.3%), and miscellaneousness 2 (4.5%). The following species were *Candida albicans* 27 (36.5%), *Candida parapsilosis* 17 (23%), *Candida glabrata* 10 (13.5%), *Candida krusei* 8 (10.8%), *Candida guilliermondi* 3 (4%), *Cryptococcus laurentii* 2 (2.7%), and other yeast like fungi 6 (9.5%). All *Candida albicans* isolates were susceptible for antifungal drug. The numbers of amphotericin-B-, flucytosine-, fluconazole-, itraconazole-, and micafungin- resistant *Candida non-albicans* isolates were 4, 3, 7, 24, and 1, respectively. Appropriate usage of antifungal drug based on local antifungal pattern can certainly help the physician in reducing the mortality rate of yeast-like fungal infection.

Introduction

Yeast is the term for a fungus that exists as a single-celled organism rather than as hyphae and yeast-like fungi are called because they exist as yeast for part of their life cycle, but can be hyphal for a significant portion of it. *Candida* and Cryptococcus species are known as human pathogenic yeast-like fungi (Skinner *et al.*, 1947).

Candida species accounts for almost 80% of fungal pathogens causing nosocomial infections (Cortés *et al.*, 2014). The incidence of candidemia has increased more than five-fold in the last decade (Bassetti *et al.,* 2011). They are important in clinical setting because the mortality rate associated with candidemia is very high (Morii *et al.,* 2014). Candidemia is also associated with prolonged hospital stay, increased costs (Asmundsdottir *et al.,* 2013). Although many hospitals lack suitable facilities for investigating fungal assays of *Candida* species, empirical therapy is used because it is not possible to delay treatment until the result of fungal culture (Morii *et al.,* 2014). Delaying empirical therapy for more than a half day is associated with high mortality (Morrell *et al.,* 2005).

Although the importance of yeast-like fungi from aseptic site is recognized, little is known about the clinical characteristics and antifungal susceptible patterns associated with yeast-like fungi in Japan. In this study, we determined the clinical characteristics and antifungal susceptible patterns of yeast-like fungi from aseptic site in hospitalized patients at the Nagoya City University Hospital using data collected during 2004-2009.

Material and Methods

Strains and clinical data collection

A total of 74 yeast-like fungi isolates were obtained from clinical specimens at aseptic site at Nagoya City University Hospital in Japan from 2004 to 2009. We also used medical records appended to clinical species for the analysis of clinical feature at Nagoya City University Hospital. We considered several isolates from the same region of the same patient as one isolate per one patient for the analysis in this study. All fungal isolates were identified by standard conventional biochemical methods or the VITEK2 system (bioMe'rieux, Durham NC, USA). Our experimental design was approved by the ethics committee at Nagoya City University.

Antimicrobial susceptibility analysis

Yeast-like fungi isolates were examined for 5 antifungal susceptibilities as follows; amphotericin-B, flucytosine, fluconazole, itraconazole, and micafungin.

Antifungal susceptibility of 65 *Candida* species was determined by theVITEK2 system (bioMe'rieux). These results were interpreted according to the Clinical Laboratory Standard Institute (CLSI) criteria M27-A3 (Clinical and Laboratory Standards Institute, 2008) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria version 7(http://www.eucast.org/ast_of_fungi/).

Statistical analysis of the data

We conducted the statistical analysis with the chi-squared test or Fisher's exact test when appropriate. Differences were considered significant when p was< 0.05.

Result and Discussion

First of all, we evaluated the relationship between clinical patients' features and yeast-like fungi. The total number of male patients was about two times as same as that of females in this study (Figure 1).

The age range was categorized every 10 years old in Figure 2. The number of 60 - 70 years patients was largest in this study. The number of 0 - 10 years patients was second largest for 5 years. Totally, about fifty percent of yeast-like fungi were isolated from patients of over 60 years.

The relationship between clinical department and yeast-like fungi revealed that pediatrics (21.6%) was the most frequent clinical department(Figure 3). Approximate nineteen percent of yeast-like fungi were isolated from respiratory medicine and general medicine, respectively.

We also represented that the relationship between biological sources and yeast-like fungi (Figure 4). The biological sources of yeast-like fungi isolated most was blood (93.2%) for six years. Two yeast-like fungi were isolated from ascites in six years.

Most of the yeast-like fungi isolates were *Candida albicans* (28/ 37.8%) followed by *Candida parapsilosis* (17 / 23%), *Candida glabrata* (10 / 13.5%), *Candida krusei* 8 (10.8%), *Candida guilliermondi* 3 (4%), *Cryptococcus laurentii* 2 (2.7%), and other yeast like fungi 6 (9.5%).

Furthermore, we analyzed the antifungal susceptibilities of *Candida albicans*, *Candida glabrata*, *Candida guilliermondii*, *Candida krusei*, and *Candida parapsilosis*in this study. All *Candida albicans* isolates were susceptible for antifungal drug. One *Candida glabrata* and three *Candida krusei* ($p< 0.05$) had significant amphotericin-B-resistant activity compared to *Candida albicans* (Table 1). Three *Candida krusei* ($p< 0.05$) and one *Candida parapsilosis* had significant flucytosine- resistant activity compared to *Candida albicans* (Table 2). Seven *Candida krusei* ($p< 0.05$) had significant fluconazole- resistant activity compared to *Candida albicans* (Table 3). Nine *Candida glabrata* ($p< 0.05$), three *Candida guilliermondii* ($p< 0.05$), six *Candida krusei* ($p< 0.05$), and four *Candida parapsilosis* ($p< 0.05$) had significant itraconazole- resistant activity compared to *Candida albicans* (Table 4). One *Candida parapsilosis* had micafungin- resistant activity (Table 5). The numbers of amphotericin-B-, flucytosine-, fluconazole-, itraconazole-, andmicafungin-resistant *Candida* isolates were four, three, seven, twenty-four, and one, respectively.

In this study, we described the clinical characteristics and antifungal susceptible patterns of yeast-like fungal isolates during 2004-2009 in Japan.

With respect to gender group, yeast-like-fungi were isolated more from male patients than female patients. Although previous research showed that there was no significant difference in ratio of gender (Yamamoto *et al.*, 2013), another research demonstrated that the numbers of male patient with candidemia was more than female (Laupland *et al.*, 2005; Hirai *et al.*, 2014; Wang *et al.*, 2014). Our results are consistent with the latter results.

Our results revealed that the very young and elderly were at highest risk for invasive *Candida* species. They are consistent with Canadian study and other Japanese study (Laupland *et al.*, 2005; Yamamoto *et al.*, 2013; Hirai *et al.*, 2014). However, in China, the distribution of *Candida* species did not differ between elderly and younger patients (Wang *et al.*, 2014).

In the analysis of clinical department, we found that department where most patients with yeast-like fungi were detected was pediatrics. This result is consistent with the risk factors of age. In our result, ICU is second popular department for fungal isolation (18.9%). Another Japanese study showed that about fifteen percent of patients with fungemia were admitted in ICU in (Yamamoto *et al.*, 2013). Our results are almost consistent with this result. However, Chinese study showed that thirty percent of patients with fungemia were admitted in ICU.

Table.1 Anti-amphotericin-B susceptibilities of *Candida* species

(µg/mL)	0.12	0.25	0.5	1	2	MIC_{90}
Candia albicans	0	7	16	4	0	1
Candida glabrata	0	2	3	4	1	1
Candida guilliermondii	0	2	1	0	0	0.5
Candida krusei	0	0	1	4	3	2
Candida parapsilosis	0	2	13	2	0	1
Total	0	13	34	14	4	2

Table.2 Anti-flucytosine susceptibilities of *Candida* species

(µg/mL)	<0.12	0.12	0.25	0.5	1	2	4	8	16	32	64	MIC_{90}
Candida albicans	4	13	6	2	2	0	0	0	0	0	0	0.5
Candida glabrata	5	5	0	0	0	0	0	0	0	0	0	0.12
Candida guilliermondii	0	0	2	1	0	0	0	0	0	0	0	0.5
Candida krusei	0	0	0	0	0	0	0	0	5	2	1	64
Candida parapsilosis	0	5	2	8	1	0	0	0	0	0	1	0.5
Total	9	23	10	11	3	0	0	0	5	2	2	16

Table.3 Anti-fluconazole susceptibilities of *Candida* species

(μg/mL)	0.12	0.25	0.5	1	2	4	8	16	32	64	>64	MIC_{90}
Candida albicans	4	15	5	3	0	0	0	0	0	0	0	1
Candida glabrata	0	0	0	0	0	0	2	4	4	0	0	32
Candida guilliermondii	0	0	0	0	0	0	2	1	0	0	0	16
Candida krusei	0	0	0	0	0	0	0	0	1	3	4	>64
Candida parapsilosis	0	2	2	2	7	4	0	0	0	0	0	4
Total	4	17	7	5	7	4	4	5	5	3	4	64

Table.4 Anti-itraconazole susceptibilities of *Candida* species

(μg/mL)	≤0.03	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	MIC_{90}
Candida albicans	4	6	6	7	4	0	0	0	0	0	0	0.25
Candida glabrata	0	0	0	0	0	1	3	2	2	2	0	16
Candida guilliermondii	0	0	0	0	0	0	2	1	0	0	0	2
Candida krusei	0	0	0	0	0	2	4	2	0	0	0	2
Candida parapsilosis	0	0	1	3	4	5	4	0	0	0	0	1
Total	4	6	7	10	8	8	13	5	2	2	0	2

Table.5 Anti-micafungin susceptibilities of *Candida* species

(μg/mL)	≤0.03	0.03	0.06	0.12	0.25	0.5	1	2	MIC_{90}
Candida albicans	1	12	8	4	1	1	0	0	0.12
Candida glabrata	1	3	5	1	0	0	0	0	0.12
Candida guilliermondii	0	0	0	0	1	1	1	0	1
Candida krusei	0	0	0	1	5	2	0	0	0.5
Candida parapsilosis	0	1	0	0	2	2	11	1	1
Total	2	16	13	6	9	6	12	1	1

Figure.1 Gender distribution of yeast-like fungi

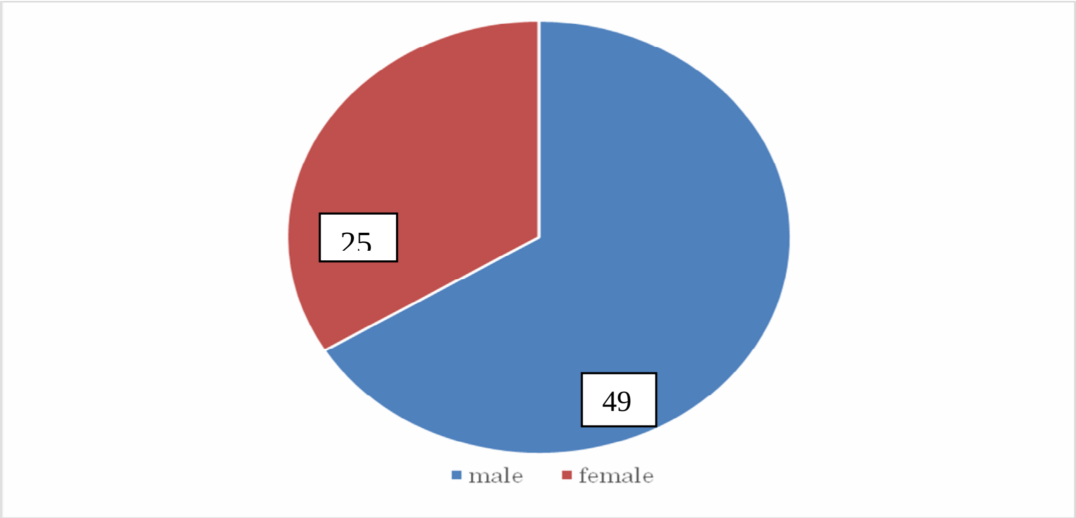


Figure.2 Age distribution of yeast-like fungi

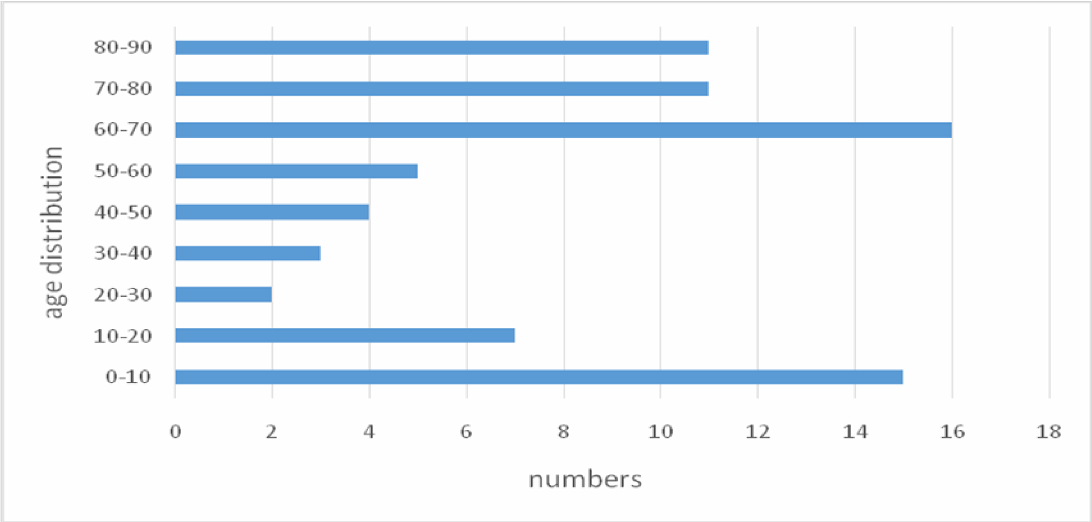


Figure.3 Clinical department distribution of yeast-like fungi

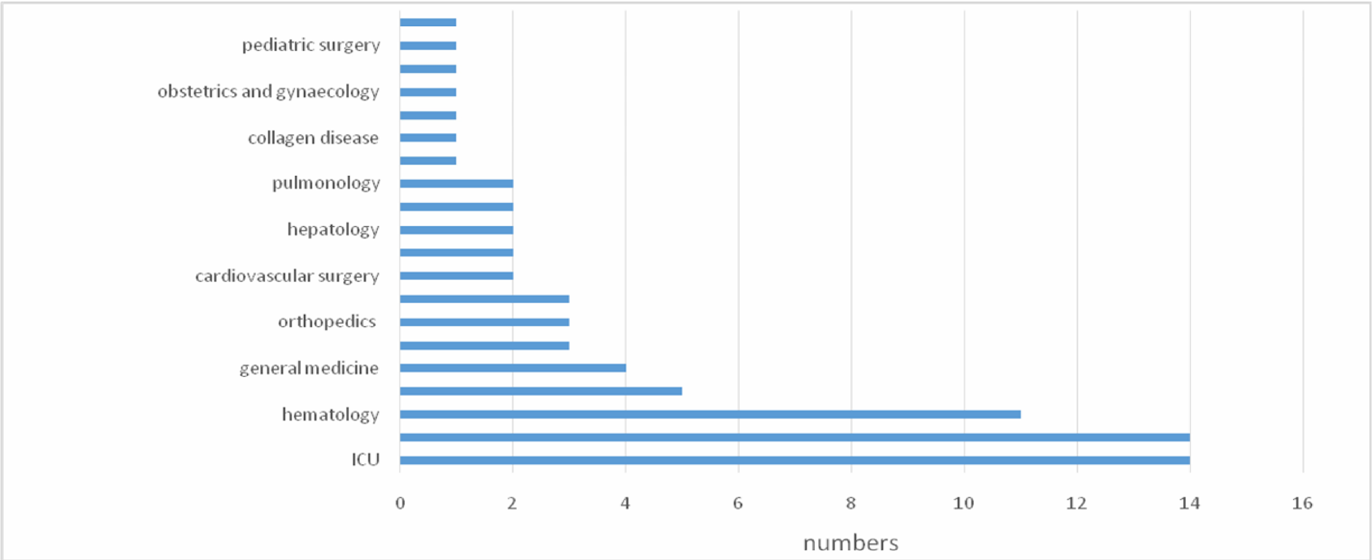


Figure.4 Biological source distribution of yeast-like fungi

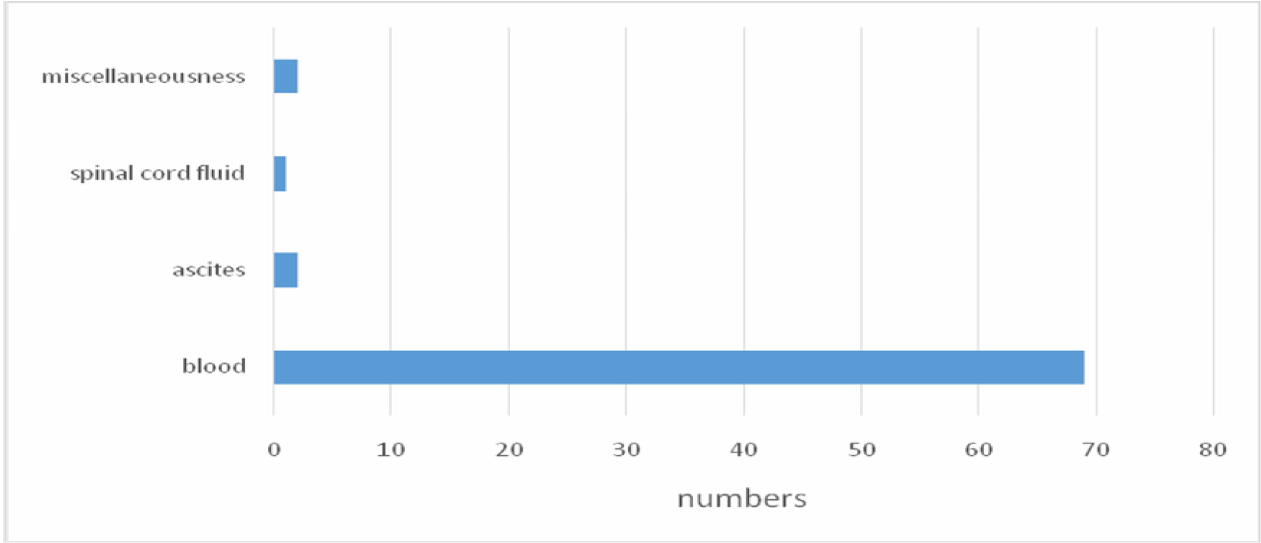
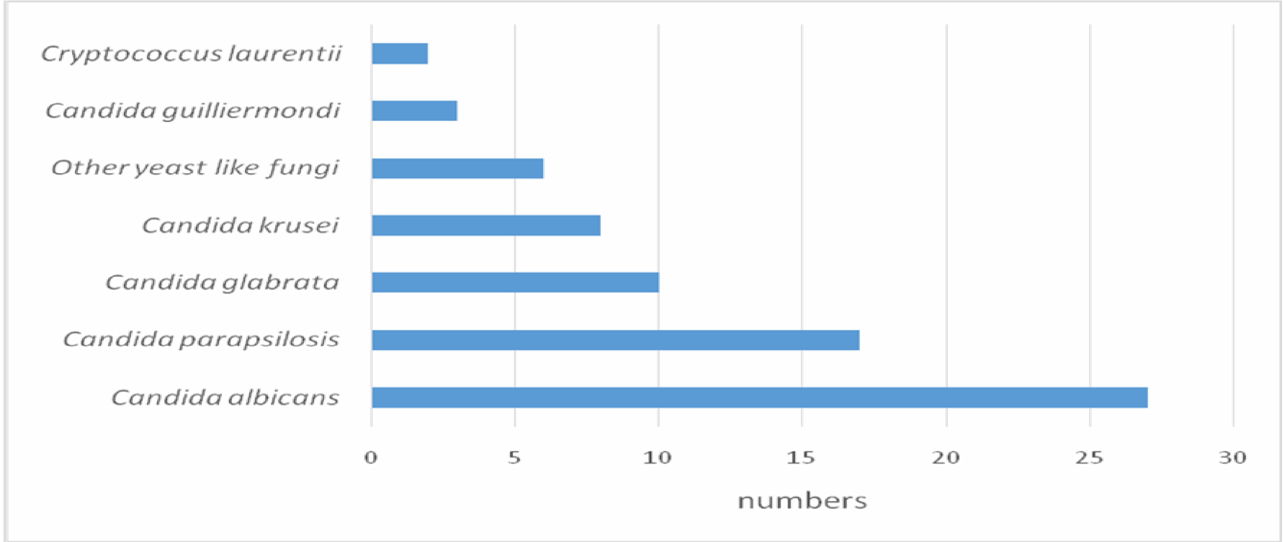


Figure.5 Distribution of yeast-like fungi



The distribution of *Candida* species varies geographically. Canadian report showed that the most common species isolated were *Candida albicans*, *Candida glabrata* and, *Candida parapsilosis* in order (Laupland *et al.*, 2005). Norwegian report revealed that *Candida albicans* was dominant, followed by *Candida tropicalis*, *Candida parapsilosis*, and *Candida glabrata* (Berdal *et al.*, 2014).Another report from Latin America found that *Candida albicans* was the dominant *Candida* species followed by *Candida parapsilosis*, *Candida tropicalis* (Cortés *et al.*, 2014). In Indian reports, *Candida tropicalis* was the most common, followed by *Candida parapsilosis*, *Candida albicans*, *Candida glabrata* (Prakash *et al.* 2012; Tak *et al.*, 2014).Chinese study showed that *Candida* species isolated were as follows: *Candida albican*s, *Candida tropicalis*, *Candida Parapsilosis*, *Candida glabrat*a, and *Candida krusei*in order (Wang *et al.*, 2014). Previous early report in Japan demonstrated that *Candida albicans* was the most commonly isolated species, followed by *Candida tropicalis*, *Candida palapsilosis*, *Candida krusei*, and *Candida glabrata* (Kazama *et al.*, 2003). Other Japanese study showed that *Candida albicans* was the dominant *Candida* species followed by *Candida parapsilosis*, *Candida glabrata*, *Candida tropicalis*, and *Candida Krusei* (Morii*et al.*, 2014). Our result showed that *Candida albicans* is the dominant *Candida* species followed by *Candida parapsilosis*, *Candida glabrata*, and *Candida krusei* in order. Although we did not find *Candida tropicalis* in this study, our results were almost consistent with the results in other Japanese and Norwegian studies.

This diversity of fungal distribution is considered to be important because of affecting the choice of empirical antifungal therapy (Morii *et al.*, 2014).

In our study, all *Candida albicans* isolates were susceptible for antifungal drug, several *Candida non-albicans* isolates had antifungal activity, especially itraconazole (37%). Both *Candida glabrata* and *Candida krusei* are considered to be azole-resistant species (Spellberg *et al.* 2006). Amphotericin B- and fluconazole- resistant isolates were about three percent of all fungal isolates in Indian trauma care (Tak *et al.*,2 014). Thirteen percent of fungal isolates demonstrated reduced susceptibility to fluconazole in Canadian study (Laupland *et al.*, 2005). Chinese study showed that about three percent of *Candida albicans* and thirty percent of *non-Candida albicans* were fluconazole-resistant (Wang *et al.*, 2014).In early Japanese study, percentages of fluconazole- resistance were about two percent for *Candida albicans*, about one percent for *Candida parapsilosis*, about five percent for *Candida glabrata* (Takakura *et al.*, 2004). Other *Candida non-albicans* antifungal study in Japan showed that *Candida parapsilosis* was susceptible to micafungin and fluconazole (Hirai *et al.*, 2014).However, *Candida non-albicans* species were gradually more resistant to fluconazole and, itraconazole than *Candida albicans* in Japanese study (Shigemura *et al.* 2014). Further continuous investigation will be necessary to elucidate the antifungal resistant patterns.

Conclusion

This study showed the characteristics and antifungal susceptibilities of most common yeast-like fungal isolates in mid-Japan. These observations are important for patient management and the development of antifungal treatment guidelines. Appropriate usage of antifungal drug based on local antifungal pattern can certainly help the physician in reducing the mortality rate of yeast-like fungal infection

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References

Asmundsdottir, L.R.,Erlendsdottir, H., Gottfredsson, M. 2013. Nationalwide study of candidemia, antifungal use, and antifungal resistance in Iceland 2000 to 2011. *J. Clin. Microbiol.*, 51:841-848.

Bassetti, M.,Taramasso, L., Nicco, E., Molinari, M.P., Mussap, M., Viscoli, C. 2011. Epidemiology, species distribution, antifungal susceptibility and outcome of nosocomial candidemia in a tertiary care hospital in Italy. *PLos ONE*, 6:e24198.

Berdal, J.E.,Haagensen, R., Ranheim, T., Bjørnholt, J.V. 2014. Nosocomial candidemia; risk factors and prognosis revisited; 11 years' experience from a Norwegian secondary hospital. *PLos ONE*, 9(7):e103916.

Clinical Laboratory Standards Institute. 2008. Reference method for broth dilution antifungal susceptibility testing of yeast: approved standard M27-A3. CLSI. Wayne, PA, USA.

Cortés, J.A., Reyes, P., Gómes, C.H., Cuervo, S.I., Rivas, P. *et al.* 2014. Clinical and epidemiological characteristics and risk factors for mortality in patients with candidemia in hospitals from Bogotá, Colombia. *Braz. J. Infect. Dis.*, 18(6):631-637.

Hirai, Y.,Asahata, S., Ainoda, Y., Goto, A., Fujita, T. *et al.* 2014. Nosocomial *Candida* parapsilosis*Candida*emia: risk factors, antifungal susceptibility and outcome. *J. Hospital. Infect.*, 87:54-58.

Kazama, I., Furukawa, K. 2003. A study for candidemia during the six year period from 1993 to 1999 in St. Luke's International Hospital. *J. Jp. Assoc. Infect. Dis.*, 77(3):158-166.

Laupland, K.B.,Gregson, D.B., Church, D.L, Ross, T., Elsayed, S. 2005. Invasive *Candida* species infections: a 5 year population-based assessment. J. Antimicrob. *Chemothr.*, 56:532-537.

Morrell, M., Fraser, V.J., Kollef, M.H. 2005. Delaying the empiric treatment of *Candida* blood stream infection until positive blood culture results are obtained: a potential risk factor for hospital mortality. Antimicrob. Agents. *Chemother.*, 49:3640-3645.

Morii, D., Seki, M., Bionongo, J.N., Ban, R., Kobayashi, A., *et al.* 2014. Distribution of *Candida* species isolated from blood cultures in hospitals in Osaka, Japan. *J. Infect, Chemother.*, 20:558-562.

Prakash, G.,Bhargav, P., Swati, V., Zina, Hussain. 2012. Prevalence of *Candida* in sputum of patients with respiratory disease in Jamnagar region. *Int. J. Sci. Res.*, 1(7):129-130.

Sigemura, K.,Osawa, K., Jikimoto, T., Yoshida, H., Hayama, B., *et al.* 2014. Comparison of the clinical risk factors between *Candida* albicans and *Candida* non-albicans species for bloodstream infection. *J. Antibiot.*, 67(4):311-314.

Skinner, C.E. 1947. The yeast-like fungi: *Candida* and brettanomyces. *Bacteriol. Rev.*, 11(4):227-274.

Spellberg, B.J., Filler, S.G., Edwards, Jr. J.E. 2006. Current treatment strategies for disseminated candidiasis. *Clin. Infect. Dis.*, 42:244-251.

Tak, V., Mathur, P., Varghese, P., Gunjiyal, J., Xess, I., *et al.* 2014. The epidemiological profile of candidemia at an Indian trauma care center. *J. Lab. Physicians*, 6(2):96-101.

Takakura, S., Fujihara, N., Saito, T., Kudo, T., Iinuma, Y. *et al.* 2004. National surveillance of species distribution in blood isolates of *Candida* species inJapan and their susceptibility to six antifungal agents including voriconazole and micafungin. *J. Antimicrob. Chemoter.,* 53: 283-289.

Wang, H., Liu, N., Yin, M., Han, H., Yue, J. *et al.* 2014. The epidemiology, antifungal use and risk factors of death in elderly patients with candidemia: a multicenter retrospective study. *BMC. Infect. Dis.,* 14:609.

Yamamoto, M., Takakura, S., Hotta, G., Matsumura, Y., Matsushima, A., *et al.* 2013. Clinical characteristics and risk factors of non-*Candida* fungaemia. *BMC infect. Dis.*, 13:247.