www.jpnim.com Open Access eISSN: 2281-0692
Journal of Pediatric and Neonatal Individualized Medicine 2014;3(2):e030212
doi: 10.7363/030212
Received: 2014 May 31; revised: 2014 Jul 22; accepted: 2014 Jul 23; published online: 2014 Oct 23

Original article

Fungal sepsis in a Level III Neonatal Intensive Care Unit: a 10-year retrospective analysis

Rita Silva[1], Marta Grilo[1,2], Susana Pissarra[1,2], Hercília Guimarães[1,2]

[1]Faculty of Medicine, University of Porto, Portugal

[2]Division of Neonatology, Department of Pediatrics, Centro Hospitalar de São João, Porto, Portugal

Abstract

Objective: Neonatal sepsis caused by fungal agents entails great mortality and morbidity. The goals of this study are to characterize local epidemiology, to analyze the presence of risk factors, laboratorial findings, and to describe therapeutics performed and their effectiveness.

Method: In a retrospective study performed at the neonatal intensive care unit of "Centro Hospitalar São João", Porto, Portugal, patients who had positive blood cultures for fungus between 2003 and 2012 were selected.

Results: From a total population of 3,933 patients, 15 (3.8 in every 1,000) had sepsis caused by fungal agents. Eleven (73.3%) patients were very-preterm and 9 (60%) were extremely low birth weight. All patients had central venous catheters, received parenteral nutrition and broad-spectrum antibiotics, and most were invasively ventilated (n = 14, 86.7%). *Candida spp.*, namely *C. albicans* (n = 7, 43.8%) and *C. parapsilosis* (n = 9, 56.3%), were the identified agents. Patients were treated with liposomal amphotericin B alone (n = 9, 60%) or with the addition or replacement of flucytosine, caspofungin, or fluconazole. First choice treatment was effective in 7 patients (46.7%). Mortality occurred in 46.7% (n = 7).

Conclusions: Although the incidence of fungal sepsis was in accordance with what is described in the literature, mortality was higher. Rates of *C. albicans* and *C. parapsilosis* were also similar to other reports. Well-known modifiable risk factors for fungal sepsis could be identified in most cases. These data must be considered in the prevention of fungal sepsis in neonatal intensive care units.

Keywords

Sepsis, fungemia, *Candida*, newborn, infant, premature, low birth weight.

Corresponding author

Rita Silva, Departamento de Pediatria da Faculdade de Medicina da Universidade do Porto, Alameda Professor Hernâni Monteiro 4200-319, Porto, Portugal; mobile: +351 912441142; fax: +351 225513600; email: rita.silva.027@gmail.com.

How to cite

Silva R, Grilo M, Pissarra S, Guimarães H. Fungal sepsis in a Level III Neonatal Intensive Care Unit: a 10-year retrospective analysis. J Pediatr Neonat Individual Med. 2014;3(2):e030212. doi: 10.7363/030212.

Introduction

Sepsis is a systemic infectious disease which frequently affects infants in neonatal intensive care units (NICUs) all around the world (6.3 in 1,000 admissions) [1], mainly in pre-term and low birth weight (LBW) neonates. In some studies, 21% to 36% of admitted patients had either bacterial or fungal sepsis [2, 3]. Although less common than bacterial sepsis, fungal sepsis (with incidences between 0.3% and 6.7%) [4-8] entails great mortality (19% to 36%) [2-6, 8, 9] and great morbidity (57.2%) [5]. In one study of extremely low birth weight (ELBW) infants, death or neurodevelopmental impairment were observed in 73% of infected patients [5].

Neonatal fungal sepsis occurs in immunologically immature or very ill patients because of individual susceptibility and of health care with all the devices needed and invasive procedures. Therefore, most cases of fungal sepsis are health care related infections. *Candida spp.* are the most frequently isolated agents (93% to 99%) [7, 10].

It is often difficult to establish the diagnosis of fungal sepsis because there are no easy, reliable and rapid tests, and so doctors have to attend to clinical signs and various laboratory findings. Although blood cultures have low sensitivity, they are still the "gold standard" to confirm the diagnosis of fungal sepsis. There are current investigations on non-culture diagnostic methods by polymerase chain reaction (PCR) test, but they are not fully standardized, nor have enough commercial assays yet [11].

Prevention and therapeutic strategies depend on knowledge of local epidemiology, so it is crucial to have data concerning local units.

The goals of this study are: to calculate the incidence of neonatal fungal sepsis, in the NICU of "Centro Hospitalar São João", Porto, Portugal, over the last 10 years; to identify the presence of risk factors; to recognize the most frequent clinical, laboratory and microbiologic features; to analyze treatment options, the presence of comorbidities, and clinical outcome.

Methods

Among patients admitted to "Centro Hospitalar São João" (a Level III hospital in Porto, Portugal) NICU between 1st January 2003 and 31st December 2012 (n = 3,933), those with the diagnosis of fungal sepsis confirmed by blood cultures within their stay (n = 15) were selected and included for analysis.

Information was obtained by accessing the department's digital database and automatic statistics (to globally characterize the patients admitted in the unit); by consulting microbiology laboratory reports of blood cultures and patient's discharge reports to identify the patients to include; and by consulting their complete hospitalization file to collect data from the patients included in the study, their fungal infection and outcomes. The retrieved variables were: sex, gestational age (GA), birth weight, antibiotic therapy, mechanical ventilation, central catheters, analytic abnormalities, age at diagnosis, surgery before diagnosis, isolated agents, antifungal used, duration of therapy, therapeutic effectiveness, length of NICU stay and clinical outcome.

In compliance with the WHO, patients were classified according to GA as late preterm (LPT; GA 32 to < 37 weeks), very preterm (VPT; GA 28 to < 32 weeks) and extremely preterm (EPT; GA < 28 weeks). ICD 10 was used to consider birth weight less than 2,500, 1,500 and 1,000 grams as definition of LBW, very low birth weight (VLBW) and ELBW. Low Apgar was defined as an Apgar score lower than 7 at the 5th minute of life.

Third or 4th generation cephalosporin and carbapenem were considered to be "broad-spectrum" antibiotics.

We considered elevated C-reactive protein if it was > 10 mg/L [12], thrombocytopenia if platelets were < 100 x 10^9/L (100,000/μL); severe thrombocytopenia if platelets were < 50 x 10^9/L (50,000/μL); and leukocytosis if white blood cells were > 15 x 10^9/L (15,000/μL).

Throughout the period studied, the blood specimens were collected to the BACTEC™ broth culture bottles (Becton-Dilckinson): BACTEC™ Peds/Plus F Culture Vial or BACTEC™ Mycosis IC/F Culture Vial. The automatic system BACTEC™ 9240 or 9000MB (Becton-Dilckinson) was used to detect growth from the blood specimens. All the positive cultures were examined by Gram stain and subcultured onto horse blood agar medium. The isolates

were identified using the germ tube test and the VITEK2™ system technology (Biomerieux), with the yeast identification Card (YST).

We considered "therapy failure" when cultures remained positive after 7 days of antifungal therapy; or the patient died before having any negative blood culture but had at least 24 hours of therapy; or another antifungal was necessary, as in other studies [4, 5].

We used simple mortality rate, rather than sepsis attributable mortality rate, because in these critical patients it is very difficult to assess the cause of death, which is extremely subjective, as it was described by other authors [13].

We used *IBM SPSS® Statistics for Windows, Version 21.0* (Armonk, NY: IBM Corp.) to do descriptive statistics: frequencies, means, medians, range, and standard deviation.

This study was approved by our Hospital's Ethics Committee.

Results

Between January 2003 and December 2012, 3,933 patients were admitted to our NICU (**Tab. 1**). Mean length of NICU stay was 12.0 days. Five hundred fifty three (14.1%) were VLBW and 336 (8.5%) were VPT. Eight hundred eighteen patients (20.8%) underwent surgery within their stay in the NICU.

There were 549 (13.9%) patients with clinical or confirmed sepsis (either bacterial or fungal). Fifteen of which (2.7%) had confirmed fungal sepsis on blood cultures (**Tab. 2** and **Tab. 3**). In this period of 10 years, the cumulative incidence of neonatal fungal sepsis (confirmed on blood cultures) was 3.8 per 1,000 NICU admissions.

Patients with fungal sepsis had a mean (standard deviation [SD]) length of NICU stay of 70.4 days (± 44.5). Nine (60%) patients were male. Median (range) GA was 27 (23-39) weeks. Two (13.3%) were LPT, 2 (13.3%) were VPT, and 9 (60%) were EPT. Median (range) of birth weight was 845 (450-2,340) grams. Fifteen (100%) had LBW, 13 (86.7%) had VLBW and 9 (60%) had ELBW. Ten (66.7%) patients were born by vaginal delivery. Low Apgar score was attributed to 7 (46.7%) patients.

Thirteen (86.7%) underwent invasive mechanic ventilation before diagnosis of fungal sepsis.

Ten (66.7%) patients underwent surgery before diagnosis, 5 (50%) abdominal surgeries (for duodenal atresia, intestinal volvulus, abdominal

Table 1. NICU admitted patients characteristics (n = 3,933).

	n (%)
LPT	1,198 (30.5)
VPT	336 (8.5)
EPT	185 (4.7)
LBW	1,718 (43.7)
VLBW	553 (14.1)
ELBW	260 (6.6)
Surgery within NICU stay	818 (20.8)
Clinical or confirmed sepsis, either bacterial or fungal	549 (13.9)
Confirmed fungal sepsis on blood cultures	15 (0.38)

NICU: neonatal intensive care unit, LPT: late preterm; VPT: very preterm; EPT: extremely preterm; LBW: low birth weight; VLBW: very low birth weight; ELBW: extremely low birth weight.

Table 2. Study results (n = 15).

	n (%)	Mean/median (SD/range)
Male	9 (60)	–
Gestational age	–	27 (23-39) weeks
LPT	2 (13.3)	–
VPT	2 (13.3)	–
EPT	9 (60)	–
Birth weight	–	845 (450-2,340) grams
LBW	15 (100)	–
VLBW	13 (86.7)	–
ELBW	9 (60)	–
Low Apgar Score	7 (46.7)	–
Parenteral nutrition (BD)	15 (100)	26.4 (± 14.2) days
Invasive MV (BD)	13 (86.7)	17.4 (± 12.7) days
Broviac® CVC (TD)	8 (53.3)	18.1 (± 14.4) days
PICC (TD)	8 (53.3)	11 (1-30) days
Surgery (BD)	10 (66.7)	–
Abdominal surgery (BD)	5 (33.3)	–
Broad spectrum antibiotics (BD)	13 (86.7)	–
Cefotaxime (BD)	12 (80)	15.5 (± 7.0) days
Meropenem (BD)	3 (20)	7.3 ± 4.2 days
Age (TD)	–	31.9 (± 17.5) days
C. albicans	6 (40)	–
C. parapsilosis	8 (53.3)	–
C. albicans* and then *C. parapsilosis	1 (6.7)	–
Antifungal therapy	–	20 (5-94) days
Liposomal amphotericin B	15 (100)	18.4 (± 7.2) days
Liposomal amphotericin B as the only antifungal	9 (60)	15.2 (± 6.8) days
Flucytosin	3 (20)	10.7 (7-14) days
Caspofungin	2 (13.3)	25.5 (25-26) days
Fluconazol	2 (13.3)	29 (28-30) days
Therapy failure	8 (53.3)	–
NICU stay	–	70.4 (± 44.5) days
Mortality	7 (46.7)	–

SD: standard deviation; LPT: late preterm; VPT: very preterm; EPT: extremely preterm; LBW: low birth weight; VLBW: very low birth weight; ELBW: extremely low birth weight; BD: before diagnosis of fungal sepsis; MV: mechanical ventilation; CVC: central venous catheter; TD: at the time of diagnosis; PICC: peripherally inserted central catheters; NICU: neonatal intensive care unit.

Table 3. Study sample characteristics.

ID	GA	BW	BR	PICC	IMV	PN	Surg	ATB	Inf. Date	Blood culture	ATF	Outcome
1	27	845	0	0	0	21	Y	14	Dec 2004	*C. albicans*	Amphotericin B	Improved
2	26	770	0	13	12	19	Y	11	Aug 2004	*C. parapsilosis*	Amphotericin B + flucytosine	Improved
3	23	535	5	0	34	30	N	25	Apr 2005	*C. parapsilosis*	Amphotericin B	Deceased
4	38	2,340	22	0	37	35	Y	33	Jul 2005	*C. albicans*	Amphotericin B + flucytosine	Deceased
5	28	1,150	20	0	0	49	N	20	Oct 2005	*C. albicans*	Amphotericin B + flucytosine	Improved
6	39	1,980	3	0	7	12	N	11	May 2006	*C. albicans*	Amphotericin B	Deceased
7	25	790	0	13	16	16	Y	11	Feb 2007	*C. parapsilosis*	Amphotericin B	Deceased
8	32	1,360	0	10	14	7	Y	0	Nov 2007	*C. parapsilosis*	Amphotericin B	Deceased
9	26	780	0	11	5	7	N	6	May 2008	*C. albicans*	Amphotericin B + caspofungin	Improved
10	24	570	0	3	18	18	Y	0	Sep 2009	*C. albicans*	Amphotericin B	Deceased
11	34	1,385	28	30	25	31	Y	16	Dec 2009	*C. parapsilosis*	Amphotericin B, caspofungin, fluconazole	Improved
12	27	520	6	11	27	37	Y	25	Feb 2010	*C. parapsilosis*	Amphotericin B	Improved
13	31	1,360	46	0	7	42	Y	26	May 2010	*C. parapsilosis*	Amphotericin B + fluconazole	Improved
14	26	850	15	0	38	51	Y	20	Aug 2010	*C. parapsilosis*	Amphotericin B	Improved
15	24	450	0	1	21	21	N	10	Aug 2012	*C. albicans* + *C. parapsilosis*	Amphotericin B	Deceased

ID: identification number; GA: gestational age (weeks); BW: birth weight (grams); BR: Broviac® CVC (days) before diagnosis; PICC: peripherally inserted central catheters (days) before diagnosis; IMV: invasive mechanical ventilation (days) before diagnosis; PN: parenteral nutrition (days) before diagnosis; Surg: surgical intervention before diagnosis; Y: yes; N: no; ATB: broad-spectrum antibiotics (days) before diagnosis; Inf. Date: date of the first positive blood culture for fungus; ATF: antifungal used.

hernia, and necrotizing enterocolitis), 2 non-cardiac thoracic surgeries (for esophageal atresia and tracheoesophageal fistula) and 3 surgical repairs of patent ductus arteriosus. All the surgical repairs of patent ductus arteriosus occurred less than 48 hours before the first positive blood culture for fungus.

All patients (n = 15, 100%) had at least one central venous catheter (CVC) implanted during hospitalization, 9 (60%) had it for more than 21 days, and 4 (26.7%) for more than 28 days. Mean (range) duration of umbilical arterial or venous catheter placement was 8.3 (1-18) days.

At the time of the first positive blood culture for fungus, 1 patient had no CVC, six had Broviac® CVC, 6 had peripherally inserted central catheters (PICC), and 2 had a Broviac® CVC simultaneously with a PICC. The majority of patients (5 out of 8) with Broviac® CVC had the catheter for more than 10 days. Mean duration of catheter placement was 18.1 (± 14.4) days. Only 2 out of 8 patients had Broviac® CVC for more than 21 days. Among patients with PICC, the majority (5 out of 8 patients) had the catheter for more than 10 days, but only 1 had it for more than 21 days. Mean duration of catheter placement was 11 (1-30) days.

After diagnosis, prompt catheter removal (less than 2 days after diagnosis) was performed in 4 out of 8 PICC and in 1 out of 8 Broviac® CVC.

All patients (n = 15, 100%) had parenteral nutrition before diagnosis and 7 (46.7%) had it for more than 21 days.

Thirteen (86.7%) patients had previous treatment with broad-spectrum antibiotics, mainly cefotaxime (n = 12, mean [SD] duration 15.5 [± 7.0] days) and meropenem (n = 3, mean [SD] duration 7.3 [± 4.2] days). Four (30.8%) patients were treated for more than 21 days.

At the time of diagnosis, 5 (33.3%) patients were treated with topical clotrimazole and/or oral nystatin for mucocutaneous candidiasis (1 of them generalized in the patient body).

First positive blood culture for fungus occurred at a mean (SD) age of 31.9 (± 17.5) days of life.

Elevated C-reactive protein at the time of antifungal prescription occurred in 12 (80%) patients; 9 (60%) had thrombocytopenia (severe in 5 of them); and 7 (46.7%) patients presented leukocytosis.

Only *Candida spp.* were identified on blood cultures. Eight (53.3%) patients had isolates of *C. parapsilosis*, 6 (40%) had *C. albicans*,

and 1 (6.7%) had *C. albicans* first, and then *C. parapsilosis*.

All patients (n = 15, 100%) were treated with liposomal amphotericin B, either in monotherapy (n = 9, 60%), or in combination with flucytosin (n = 3), with caspofungin (n = 1) or with fluconazole (n = 1). Replacement of liposomal amphotericin B by caspofungin and then fluconazole occurred in 1 patient. Mean (SD) duration of treatment with liposomal amphotericin B was 18.4 (± 7.2) days, median (range) duration of treatment was 10.7 (7-14) days for flucytosin, 25.5 (25-26) days for caspofungin and 29 (28-30) days for fluconazole. In 3 (20%) cases, antifungal treatment was introduced before blood cultures results. First choice treatment was ineffective in 8 (53.3%) patients. In our NICU, investigation for end-organ localizations (such as eye, liver, etc) is routinely performed in every confirmed fungal sepsis, but we found no reports of that sort of findings. Death before discharge occurred in 7 (46.7%) patients. Among the remaining, at the age of 18 months, 4 (50%) patients had neurodevelopment impairment (deafness, psychomotor delay, or cerebral palsy).

Discussion

Incidence, as well as fungal agents identified in our study, were similar to those reported in other low incidence series [4, 5, 8, 10].

In accordance with several other studies, newborns that developed fungal sepsis are mainly VPT and VLBW [2, 3, 5, 13], and we have actually a large portion of ELBW with fungal sepsis. They almost always have several serious comorbidities (cardiac, lung, intestinal diseases), and are mechanically ventilated, have CVCs and parenteral nutrition. The previous use of meropenem, which is not first line therapy, suggests previous serious infections in some patients.

The fact that most of our patients underwent surgery (mainly abdominal) before diagnosis suggests the possibility that entering the circuit of the operating room, out of the NICU, and being exposed for long periods to demanding surgeries may contribute to infection. Surgery has already been described in the literature as a predisposing factor [10, 14].

Most of our cases had well-known risk factors for fungal sepsis, namely previous use of broad spectrum antibiotics and PICC exposure [6, 15], some of them potentially modifiable. Since reduction of the exposure to some of these risk factors in these critically ill patients may reveal difficult, clinicians use this knowledge to assess the probability of fungal sepsis, which is also influenced by non-modifiable risk factors such as gestational age and birth weight.

First choice treatment was found to be ineffective in 53.3%. This could be due to the criteria used to define effectiveness, to great immunologic immaturity or the severe comorbidities, rather than to liposomal amphotericin B resistance itself, as it has not been described great resistance in recent literature [16]. In our setting, laboratory tests for *in vitro* antifungal susceptibility are not available which precludes our knowledge regarding antifungal resistance.

Our unit conducts preventive measures to fungal infections, and in fact it does not have high prevalence, with some years (2003 and 2011) without any fungal sepsis, which suggests their effectiveness. Even the patients infected in the same year were at least 2 months apart, so it suggests effectiveness of isolation measures.

Systemic fluconazole prophylaxis is not performed in every VLBW or ELBW newborns, as it is suggested by some authors [17, 18] because our NICU does not qualify as a high prevalence unit, and there are not enough data to support that practice as it is shown in a 2013 Cochrane review [19] and in more recent data [20]. Systemic fluconazole prophylaxis is only performed in selected cases, after individual risk assessment. This study and further investigations may contribute to improve this risk assessment.

Morbidity observed was equivalent to other studies [5]. Mortality in our unit (n = 7, 46.7%) was higher than in other studies (23.8% to 36%) [2, 5, 6], which can be explained by different inclusion criteria (we included all the patients and did not exclude patients with congenital abnormalities) and by the diversity of NICU characteristics (the low incidence lead us to think that only the very immature or the very ill patients were infected, and they are, from the outset, more likely not to survive), and because we calculated simple mortality rate rather than sepsis attributable mortality rate.

We identify some study limitations, of which the small number of cases included, the retrospective nature of the study, and the fact that the studied population is not representative of all national NICUs are, in our opinion, the most important.

In future investigations, some limitations could be overcome by a multicenter study improved by

matching selected cases with controls, in a case-control study.

These results alert clinicians to one of the risks associated with prolonged use of PICC and broad spectrum antibiotics, highlighting the need to objectify NICU policies (creating strict written protocols concerning the judicious use of central lines and broad spectrum antibiotics) and to institute effective monitoring of nosocomial infection.

Because this is a Level III unit, with many patients with severe comorbidities, including surgical, a great number of patients are critically ill and so it is difficult to reduce exposure to some of those risk factors. Knowledge of local data is essential to permanently adjust surveillance and prevention measures in order to reduce the occurrence of this often fatal complication.

Acknowledgements

We thank the support of the Division of Microbiology of the Clinical Pathology Laboratory of "Centro Hospitalar São João", for the information about how blood specimens are analyzed, for the access to laboratory's database and search of pediatric positive blood cultures for fungal agents.

We thank all NICU nursing and medical staff for generous assistance, shared knowledge and expertise and for the access to such detailed records.

Declaration of interest

The Authors declare that there is no conflict of interest.

References

1. Cohen-Wolkowiez M, Moran C, Benjamin DK, Cotten CM, Clark RH, Benjamin DK Jr, Smith PB. Early and late onset sepsis in late preterm infants. Pediatr Infect Dis J. 2009;28(12):1052-6.
2. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, Lemons JA, Donovan EF, Stark AR, Tyson JE, Oh W, Bauer CR, Korones SB, Shankaran S, Laptook AR, Stevenson DK, Papile LA, Poole WK. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. Pediatrics. 2002;110(2 Pt 1): 285-91.
3. Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR, Walsh MC, Hale EC, Newman NS, Schibler K, Carlo WA, Kennedy KA, Poindexter BB, Finer NN, Ehrenkranz RA, Duara S, Sánchez PJ, O'Shea TM, Goldberg RN, Van Meurs KP, Faix RG, Phelps DL, Frantz ID 3rd, Watterberg KL, Saha S, Das A, Higgins RD; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. Pediatrics. 2010;126(3):443-56.
4. Ascher SB, Smith PB, Watt K, Benjamin DK, Cohen-Wolkowiez M, Clark RH, Benjamin DK Jr, Moran C. Antifungal therapy and outcomes in infants with invasive Candida infections. Pediatr Infect Dis J. 2012;31(5):439-43.
5. Benjamin DK Jr, Stoll BJ, Fanaroff AA, McDonald SA, Oh W, Higgins RD, Duara S, Poole K, Laptook A, Goldberg R; National Institute of Child Health and Human Development Neonatal Research Network. Neonatal candidiasis among extremely low birth weight infants: risk factors, mortality rates, and neurodevelopmental outcomes at 18 to 22 months. Pediatrics. 2006;117(1):84-92.
6. Benjamin DK Jr, Stoll BJ, Gantz MG, Walsh MC, Sanchez PJ, Das A, Shankaran S, Higgins RD, Auten KJ, Miller NA, Walsh TJ, Laptook AR, Carlo WA, Kennedy KA, Finer NN, Duara S, Schibler K, Chapman RL, Van Meurs KP, Frantz ID 3rd, Phelps DL, Poindexter BB, Bell EF, O'Shea TM, Watterberg KL, Goldberg RN; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Neonatal candidiasis: epidemiology, risk factors, and clinical judgment. Pediatrics. 2010;126(4):e865-73.
7. Clerihew L, Austin N, McGuire W. Prophylactic systemic antifungal agents to prevent mortality and morbidity in very low birth weight infants. Cochrane Database Syst Rev. 2007(4):CD003850.
8. Montagna MT, Lovero G, De Giglio O, Iatta R, Caggiano G, Montagna O, Laforgia N; AURORA Project Group. Invasive fungal infections in neonatal intensive care units of Southern Italy: a multicentre regional active surveillance (AURORA project). J Prev Med Hyg. 2010;51(3):125-30.
9. Rodriguez D, Almirante B, Park BJ, Cuenca-Estrella M, Planes AM, Sanchez F, Gene A, Xercavins M, Fontanals D, Rodriguez-Tudela JL, Warnock DW, Pahissa A; Barcelona Candidemia Project Study Group. Candidemia in neonatal intensive care units: Barcelona, Spain. Pediatr Infect Dis J. 2006;25(3):224-9.
10. Peman J, Cantón E, Linares-Sicilia MJ, Roselló EM, Borrell N, Ruiz-Pérez-de-Pipaon MT, Guinea J, García J, Porras A, García-Tapia AM, Pérez-Del-Molino L, Suárez A, Alcoba J, García-García I. Epidemiology and antifungal susceptibility of bloodstream fungal isolates in pediatric patients: a Spanish multicenter prospective survey. J Clin Microbiol. 2011;49(12):4158-63.
11. White PL, Hibbitts SJ, Perry MD, Green J, Stirling E, Woodford L, McNay G, Stevenson R, Barnes RA. Evaluation of a commercially developed semi-automated PCR-SERS assay for the diagnosis of invasive fungal disease. J Clin Microbiol. 2014 Jul 16. [Epub ahead of print].
12. Hofer N, Zacharias E, Muller W, Resch B. An update on the use of C-reactive protein in early-onset neonatal sepsis: current insights and new tasks. Neonatology. 2012;102(1):25-36.
13. Clerihew L, Lamagni TL, Brocklehurst P, McGuire W. Candida parapsilosis infection in very low birthweight infants. Arch Dis Child Fetal Neonatal Ed. 2007;92(2):F127-9.

14. Leibovitz E. Strategies for the prevention of neonatal candidiasis. Pediatr Neonatol. 2012;53(2):83-9.
15. Cotten CM, McDonald S, Stoll B, Goldberg RN, Poole K, Benjamin DK Jr; National Institute for Child Health and Human Development Neonatal Research Network. The association of third-generation cephalosporin use and invasive candidiasis in extremely low birth-weight infants. Pediatrics. 2006;118(2):717-22.
16. Pammi M, Holland L, Butler G, Gacser A, Bliss JM. Candida parapsilosis is a significant neonatal pathogen: a systematic review and meta-analysis. Pediatr Infect Dis J. 2013;32(5):e206-16.
17. Kaufman D, Boyle R, Hazen KC, Patrie JT, Robinson M, Donowitz LG. Fluconazole prophylaxis against fungal colonization and infection in preterm infants. N Engl J Med. 2001;345(23):1660-6.
18. Manzoni P, Stolfi I, Pugni L, Decembrino L, Magnani C, Vetrano G, Tridapalli E, Corona G, Giovannozzi C, Farina D, Arisio R, Merletti F, Maule M, Mosca F, Pedicino R, Stronati M, Mostert M, Gomirato G; Italian Task Force for the Study and Prevention of Neonatal Fungal Infections; Italian Society of Neonatology. A multicenter, randomized trial of prophylactic fluconazole in preterm neonates. N Engl J Med. 2007;356(24):2483-95.
19. Austin N, McGuire W. Prophylactic systemic antifungal agents to prevent mortality and morbidity in very low birth weight infants. Cochrane Database Syst Rev. 2013;4:CD003850.
20. Benjamin DK Jr, Hudak ML, Duara S, Randolph DA, Bidegain M, Mundakel GT, Natarajan G, Burchfield DJ, White RD, Shattuck KE, Neu N, Bendel CM, Kim MR, Finer NN, Stewart DL, Arrieta AC, Wade KC, Kaufman DA, Manzoni P, Prather KO, Testoni D, Berezny KY, Smith PB; Fluconazole Prophylaxis Study Team. Effect of fluconazole prophylaxis on candidiasis and mortality in premature infants: a randomized clinical trial. JAMA. 2014;311(17):1742-9.