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Investigation of a healthcare associated *Candida tropicalis* candidiasis cluster in a haematology unit and a systematic review of nosocomial outbreaks

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ABSTRACT

Background: Non-albicans *Candida spp.* are an emerging cause of hospital-bloodstream infections, associated with high mortality due to the challenges in diagnosis and delayed treatment.

Objectives: We aimed to investigate a cluster of healthcare associated invasive candidiasis caused by *C. tropicalis* and review the literature of healthcare associated outbreaks or clusters caused by *C. tropicalis*.

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Methods: An investigation was performed to determine clinical presentation, treatment outcomes and the factors contributing to *C. tropicalis* candidemia occurrence. We searched the Medline database via PubMed and Ovid using the keywords of "Candida tropicalis" combined with "outbreak" or "clustering" or "clusters", and we limited the search to studies conducted from January 1989 to January 2019

Results: We report two related cases of *C. tropicalis* candidemia among patients with AML following a period of neutropenia, who had erythematous skin rash as a first manifesting sign of candidiasis. *C. tropicalis* was isolated from blood and skin cultures of both patients, which were identical by pulsed-field gel electrophoresis typing. Our systematic review of outbreaks caused by *C. tropicalis* suggests that (1) most reported outbreaks have occurred in neonatal and adult ICUs, (2) patients who receive total parenteral therapy, antibiotics and those who have indwelling catheters and recent surgery are at high risk of infection, (3) environmental and healthcare personnel surveillance suggest that cross-contamination is a major risk factor.

Conclusion: Control of nosocomial outbreaks caused by *C. tropicalis* should include better infection control measures, education of healthcare professionals especially working in adult and neonatal intensive care and haematology units.

Keywords: candidemia, *Candida tropicalis,* candidiasis, cross-contamination, outbreak, cluster.

Introduction

Fungemia is a significant cause of hospital-acquired bloodstream infections, which often leads to septic shock and is associated with 80% attributable mortality in some settings.[1,2] *Candida* spp. remains the most common cause of nosocomial invasive fungal infections accounting for 98% of fungaemia and associated adverse outcomes among haemato-oncological patients during prolonged neutropenia.[3,4] Candidemia incidence has dramatically increased over the last 3 decades due to the frequent use of immunomodulatory agents, prolonged broad spectrum antimicrobials and central vascular catheters, now accounting for more fungemia cases than invasive aspergillosis.[5]

While *C. albicans* is the leading cause of nosocomial candidemia, the emergence of *C. tropicalis* has repeatedly been reported among patients with acute leukemia[6-8] and allogeneic hematopoietic stem cell transplant as a cause of invasive candidiasis.[3,9-11] C. *tropicalis* is often found

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in intensive care units (ICU) patients accounting for approximately 5-10% of yeast infections in ICU[12]. But it is also found in patients with indwelling catheters, malignancy and neutropenia.[3,9,13] Notably, during neutropenia and mucositis *C. tropicalis* has been shown to have high virulence compared to *C. albicans*,[14,15] predominantly due to its high ability to develop biofilms and azole resistance[16,17] and increase capillary permeability, manifesting atypically with haemorrhagic syndrome or skin rash.[18,19]

Despite the improvements in diagnosis, treatment and prevention of invasive candidiasis, early recognition and diagnosis remain a challenge due to unspecific presentation.[18,20] Given the limitations in diagnoses, true epidemiology and incidence of invasive candidiasis are not well understood. Surveillance is not routinely performed in the Balkan region and there are also no published studies from this region precluding the understanding of nosocomial candidemia epidemiology caused by non-*albicans* species.

This study aimed at investigating a cluster of healthcare associated invasive candidiasis caused by *C. tropicalis* in a haematology unit. A systematic review of the literature including clusters or outbreaks caused by *C. tropicalis* was also performed to understand the epidemiology, source of outbreaks and important infection control measures.

Methods

Identification of a cluster

A nosocomial cluster was defined as a group of related isolates obtained from 2 or more patients in the same unit. An investigation was performed to determine the factors contributing to its occurrence. This investigation included establishing a case definition, performing case ascertainment, describing the clinical characteristics of cases, and performing a literature review. Cases were defined as patients with at least one blood culture positive for *C. tropicalis* in the Haematology unit at the Clinical Centre of Serbia, Belgrade. Case ascertainment was performed by reviewing microbiology laboratory results and medical records.

Pulsed-field gel electrophoresis typing

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Pulsed-field gel electrophoresis (PFGE) is a highly discriminative molecular typing technique that is an affordable method for small laboratories[21]. This method is based on immigration of large DNA fragments in an electrical field of alternating polarity.

A systematic review of the clusters or outbreaks caused by C. tropicalis

All published papers that reported outbreak investigation or nosocomial clusters that included 2 or more related *C. tropicalis* cases were included to provide an overview of the literature and understand the location, patient characteristics, source of outbreaks and important measures of infection control. We searched the Medline database via PubMed and Ovid using the key words of "Candida tropicalis" combined with "outbreak" or "clustering" or "clusters", and we limited the search to studies conducted from January 1989 to January 2019. Papers with limited information, review papers or articles reporting outbreaks caused by other *Candida* pathogens, clusters with less than a single *C. tropicalis* case or papers that only included microbiological data without related outbreak or cluster investigation were excluded. We extracted following variables from each paper: the first author, the year of publication, location of study, number of patients with *C. tropicalis* infection, localisation of the infection, treatment and clinical outcome details, outbreak investigation, surveillance findings, and infection control measures implemented. Due to the nature of our research question, we report the findings qualitatively only.

Results

Investigation of related cases of healthcare associated C. tropicalis candidiasis

Two related cases of healthcare associated *C. tropicalis* fungemia were identified in the haematology unit. The first case was a 53-year-old male patient with acute myeloid leukaemia (AML) with maturation. Bone marrow aspirate was hypocellular with 30% blasts, some positive for myeloperoxidase. The patient received "3+7" induction chemotherapy (cytosine-arabinoside 2x 180mg/d i.v. day 1-7, anthracycline 80 mg/d i.v. day 1-3, fluconazole 200 mg day 1-7) as per local practice. Nine days post-chemotherapy, he became febrile and developed a rash. On examination, he had nonspecific, widespread, diffuse erythematous papular lesions of all skin regions. Abdominal examination revealed splenomegaly. Ultrasound and CT scan of the abdomen revealed splenomegaly with multifocal splenic and renal abscesses. *C. tropicalis* was isolated from the skin biopsy as well as

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the blood cultures. Immediately after the *C. tropicalis* isolation, antifungal therapy with caspofungin 70 mg loading dose followed by 50 mg/day and amphotericin B (AmB) deoxycholate 1 mg/kg per day was commenced according to the local haematology guidelines at the time. According to the susceptibility testing by EUCAST methodology, both *C. tropicalis* isolates were susceptible to fluconazole (MIC50 = 0.25mg/l and MIC90 = 1mg/l), caspofungin (MIC50 and MIC90 = 1mg/l), AmB (MIC50 = 0.5mg/l and MIC90 = 1mg/l) and itraconazole (MIC50 = 0.016mg/l and MIC90 = 0.031 mg/l), however, showed intermediate sensitivity to miconazole (MIC50 and MIC90 = 4mg/l and voriconazole (MIC50 = 2mg/l and MIC90 = 4mg/l). One week after the initiation of the treatment, the clinical condition had improved. Blood cultures became negative at day 10. The patient was treated with a 3-weeks course of caspofungin and AmB combination achieving culture conversion. After one month of therapy initiation abdominal ultrasound and CT scan showed no pathological finding. Patient was followed-up for 2 years.

The second case was a 54-year-old male diagnosed with AML subtype M2 and chronic kidney disease (stage 2). Induction chemotherapy was started according to the "3+7" protocol in combination with antifungal prophylaxis (fluconazole 200 mg/d, day 1-7) as per local guidelines. Seven days after the completion of chemotherapy, the patient became febrile with productive cough and developed generalized nonspecific diffuse erythematous papular rash, mostly on extremities. Chest radiography showed findings suggestive of pneumonia. Given the suspicion for invasive candidiasis, antifungal therapy with caspofungin 70 mg loading dose followed by 50 mg/day and voriconazole initially 6 mg/kg IV every 12 hrs for 2 doses, then 3–4 mg/kg every 12 hrs were commenced. Despite the initiation of treatment, the patient developed pleural effusion and transient acute on chronic renal failure, requiring multiple diagnostic and therapeutic pleural aspirations. Skin biopsy together with pleural fluid and blood culture were sent for mycological examination. *C. tropicalis* was isolated from blood culture and skin biopsy, while the pleural fluid culture was sterile. Susceptibility results were identical to the first patient. Based on susceptibility results, voriconazole was switched to AmB 1 mg/kg per day. Blood cultures became negative on day 8. Two weeks after initiation of the treatment, the clinical condition improved, and subsequent blood cultures were negative. In total, he received 14 days of treatment after symptom resolution and culture conversion. In addition, a bone marrow aspirate showed complete remission.

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The genetic relatedness of *C. tropicalis* isolates obtained from both patients was analysed by pulsed-field gel electrophoresis (PFGE) with modifications, and clonal nature of isolates were identical.

Environmental or healthcare screening was not performed. Both cases were hospitalized in the same room and fungaemia occurred over a 10 days interval. None of the patients had a central venous catheter or received parenteral nutrition prior to candidemia. After this investigation, as an infection prevention control measure, patients in the haematology unit were not allowed to have family visits within two-weeks after of chemotherapy to protect patients during this vulnerable period.

Systematic review of nosocomial clusters or outbreaks caused by Candida tropicalis

The systematic search identified 117 potentially relevant articles. After initial screening based on title, abstract and removal of duplicates, 15 were retrieved for eligibility. The number of selected papers at each step of the screening and eligibility are reported in the flow diagram (Fig. 1). We identified ten published outbreaks or clusters caused by *C. tropicalis* (Table 1). In an outbreak described in 1989, *C. tropicalis* was isolated from the sternal wounds of eight postoperative coronary bypass patients, all isolates were identical by PFGE and healthcare personnel was identified as a source of the outbreak.[22] An outbreak of *C. tropicalis* peritonitis in five peritoneal dialysis patients was reported associated with three deaths. The strains recovered from affected patients were identical to those recovered from the metal grid of water baths. [23] In a neonatal ICU, six neonates with *C. tropicalis* fungemia were identified in an outbreak. The same organism was also isolated from fingernail samples taken from the ward housekeeper and an asymptomatic nurse.[7] Further cases of *C. tropicalis* fungemia were reported in different neonatal ICUs, involving two related cases[24] and 16 neonates, and cross-contamination was the suspected cause of these outbreaks.[25] In a surgical ICU, an outbreak involving 34 patients with candiduria caused by *C. tropicalis* was identified, and improper disposal of infectious medical waste was thought to be the source of cross-transmission.[26] Investigation of two outbreaks of *C. tropicalis* candiduria among 11 patients revealed the presence of *C. tropicalis* on the hands of a healthcare worker. The risk factors identified were the presence of urinary catheters, broad-spectrum antibiotic therapy and diabetes.[27] In an unselected hospital population in Iceland, 19%–40% of candidemia episodes were attributed to nosocomial clusters,

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mainly caused by *C. albicans* followed by *C. glabrata* and *C. tropicalis* [28]. Another cluster investigation performed in Canada suggested that 33% of the patients with candidemia were caused by nosocomial clusters and inter- and intra-ward clusters were identified.[29] A detailed outbreak investigation of neonates in a Brazilian NICU identified 7 infants with candidemia, out of 12 positive blood cultures, 4 yielded *C. tropicalis* and 10 yielded *C. albicans*.[30] Risk factors included premature birth, very low birth weight, central venous catheter, TPN and ventilatory support in those with *C. tropicalis*. Further details of the outbreaks including patient characteristics, source of the outbreak and measures of infection control provided are described in Table 1.

DISCUSSION

We investigated two related cases of nosocomial invasive candidiasis caused by *C. tropicalis* in a haematology unit. Fungaemia with *C. tropicalis* occurred following first chemotherapy cycle in a period of prolonged neutropenia and mucositis supporting the available evidence that *C. tropicalis* is more virulent during prolonged neutropenia.[14,15,31] Diffuse maculopapular rash was the first presenting sign in both cases, which raised the suspicion of invasive candidiasis. Our literature review of outbreaks caused by *C. tropicalis* suggests that (1) most reported outbreaks of *C. tropicalis* candidemia have occurred in neonatal and adult ICUs, (2) patients who receive total parenteral therapy, antibiotics and those who have indwelling catheters and recent surgery are at high risk of infection with *C. tropicalis*, (3) environmental and healthcare personnel surveillance suggest that *C. tropicalis* is found on the hands of healthcare workers and can be present in the environment, therefore, cross-contamination is a possible cause of these outbreaks.

The emergence of *C. tropicalis* is linked to increasing populations of susceptible hosts attributed to rise in malignancy, aggressive chemotherapeutic agents and immunosuppressive treatments.[3,5,32] *C. tropicalis* has been associated with haematological malignancies, especially shown to be virulent during neutropenia.[14,15,31] In a recent study, Protein kinase A in *C. tropicalis* was found to drive virulence regulating drug tolerance and disease burden.[33] While early mortality could be prevented with factors such as prompt antifungal therapy and early removal of central venous catheters, late mortality is often associated with host factors.[2,34]

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The diagnosis of *C. tropicalis* infection is often delayed or overlooked because of nonspecific clinical manifestations, which is also directly correlated with delay in early initiation of appropriate antifungal therapy.[35-37] Current available diagnostic methods are often limited to culture methods. Until improved diagnostics are in place, especially in countries like Serbia, knowledge of candidemia risk factors in at risk populations and awareness about atypical clinical signs would facilitate prompt identification of patients with candidiasis. Both patients in this investigation presented with a maculopapular rash, which raised the suspicion of invasive candidiasis. The characteristic maculopapular skin lesions occur in approximately 10-13% of haematological patients with systemic candidiasis and are often associated with *C. tropicalis.*[18,38,39] In a study by Bae et al, of 53 documented systemic candidiasis cases in a haematology unit, 19 (35%) had characteristic skin lesions and 86% of those had *C. tropicalis* detected in blood cultures[38]. A recent systematic review included 33 studies reporting 100 acute candidiasis cases with skin lesions in neutropenic patients. Skin lesions were most commonly seen with *C. tropicalis* (68%) followed by *C. krusei* (15%).[40] Diffuse maculopapular lesions were more common in *C. tropicalis* cases in comparison to a nodular rash seen with *C. krusei*. Therefore, skin lesions in haematology patients with neutropenic sepsis may be a much more important early sign of *C. tropicalis* candidiasis than is currently recognised. This may assist prompt initiation of antifungal therapy in at risk population before the availability of culture results.

Cross-contamination in adult and paediatric patient populations has been reported in all outbreaks, suggesting that hand-carriage by the healthcare personnel may be an important source of *C. tropicalis*. In one of the outbreak investigations, personnel surveillance indicated that *C. tropicalis* was found in the oral cavity (10%), stool (15%), and vagina (10%) of healthy personnel.[22] Further surveillance suggested that *C. tropicalis* was present on hands and nails of asymptomatic healthcare workers.[22,23] This emphasises the need for better infection control measures including strict hand hygiene and education of healthcare professional especially in neonatal and haematology units. In our haematology unit, a visitor restriction policy was followed, although this is a controversial measure in preventing outbreaks. According to our literature review of *C. tropicalis* outbreaks, strict infection prevention measures to prevent cross-contamination between healthcare workers and patients in haematology units is required.

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Increasing resistance to azoles, polyenes, and echinocandins stands as another major problem in *C. tropicalis*.[41-44] *C. tropicalis* has been shown to constitute moderate resistance to fluconazole, requiring higher doses to achieve adequate plasma concentrations.[13] While some reports suggests that azole resistance can be as high as 34% in *C. tropicalis*, this does not correlate with overall mortality and MICs of isolates suggesting that azole-based antifungal treatment could still be effective regardless of fluconazole MICs.[45] We have not observed antifungal resistance among these two cases, although susceptibility testing revealed intermediate results for voriconazole. Recovery of white blood cells after complete remission may have contributed to the successful outcome.

In conclusion, prevention of nosocomial outbreaks or clusters caused by *C. tropicalis* can only be achieved with better infection control measures and education of healthcare professionals especially those working in neonatal and haematology units. Continuous surveillance data assessing the epidemiology of nosocomial candidemia caused by non-*albicans* species could improve monitoring incidence and distribution of species and antifungal resistance. In addition, significant attention should be given to atypical but indicative signs such as disseminated maculopapular rash, which could be an early sign of *C. tropicalis* candidemia in neutropenic haematology patients.

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Table 1: Summary of *C. tropicalis* associated outbreaks and clusters in the literature

Studies	Patient characteristics	Localisation of infection	Department	Treatment	Outcome	Source of outbreak	Measures of infection control
Isenberg et al. 1989	8 post-operative coronary bypass patients	Sternal wound	Cardiothoracic Surgery	Not reported in the publication	All recovered	Healthcare personnel – confirmed	• Exclusion of the nurse from the cardiac team terminated the cluster outbreak
Yuen et al. 1992	5 dialysis patients	Peritonitis	Medical wards	All received intravenous AmB (0,5 mg per kg/ day)	Three patients died despite removal of the Tenckhoff catheter, 2 from infection, and one 2 months later from 'dialysis encephalopathy'	Potential cross-contamination via healthcare personnel Metal grid of water baths	• Prohibition of wet-warming of peritoneal dialysate in the hospital • Frequent handwashing and the use of gloves for the handling of dialysate bags advised
Finkelstein et al. 1993	6 neonates	Blood stream	Neonatal ICU	Not reported in the publication	Not reported in the publication	Healthcare personnel - confirmed	• Emphasized the importance of hand washing and compliance with guidelines for preventing nosocomial

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							infections
Roilides et al. 2003	2 neonates	Blood stream	Neonatal ICU	AmB, 1 mg/kg	All recovered	Potential cross-contamination Of 593 neonates 17 (24%) were colonised with *C. tropicalis*	• Emphasized the importance of hand washing and compliance with guidelines for preventing nosocomial infections
Chowdhary et al. 2003	16 neonates Affected infants were admitted in adjacent rooms with single beds	Blood stream	Neonatal ICU	All received AmB deoxycholate (10–30 mg/kg)[1] total cumulative dose)	2 neonates had persistent fungemia despite antifungals and died 14–28 days after onset	Potential cross-contamination Blankets and mattresses used for neonates	• Strict hand washing and the use of gloves were stressed

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Rho et al. 2004	11 patients	Urine	Surgical ICU	No treatment received	Candiduria	Healthcare personnel - confirmed	• Catheters of all the patients involved in the outbreaks were inserted or exchanged within 2 weeks • Emphasizing hand washing procedures • Education about nosocomial transmission
Jang et al. 2005	34 patients 81.5% of patients had undergone major surgery, 100% were receiving antibiotic therapy and 74.1% had been intubated	Urine	Surgical ICU	No treatment received	None of the patients developed candidemia; all candiduria improved without antifungal therapy after removal of the urinary catheter	Potential cross-contamination Improper disposal of infectious material	• Better urine disposal system • Emphasizing hand washing procedures • Education about nosocomial transmission

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Asmundsdottir et al 2008	4 patients	Blood stream	NICU ICU	N/A	N/A	Cross contamination	• Hand washing • Improved skin disinfection, • Removal of unnecessary catheters
Maganti et al. 2011	4 patients	Blood stream	NICU ICU	N/A	N/A	Cross contamination	N/A
de Oliveira et al. 2014	7 neonates	Blood stream	NICU	AmB deoxycholate	2 deaths	Environmental contamination	• Reinforced hand hygiene education • Adequate sterilization protocols were also implemented

Abbreviations: AmB, amphotericin B; ICU, intensive care unit; NICU, neonatal intensive care unit.

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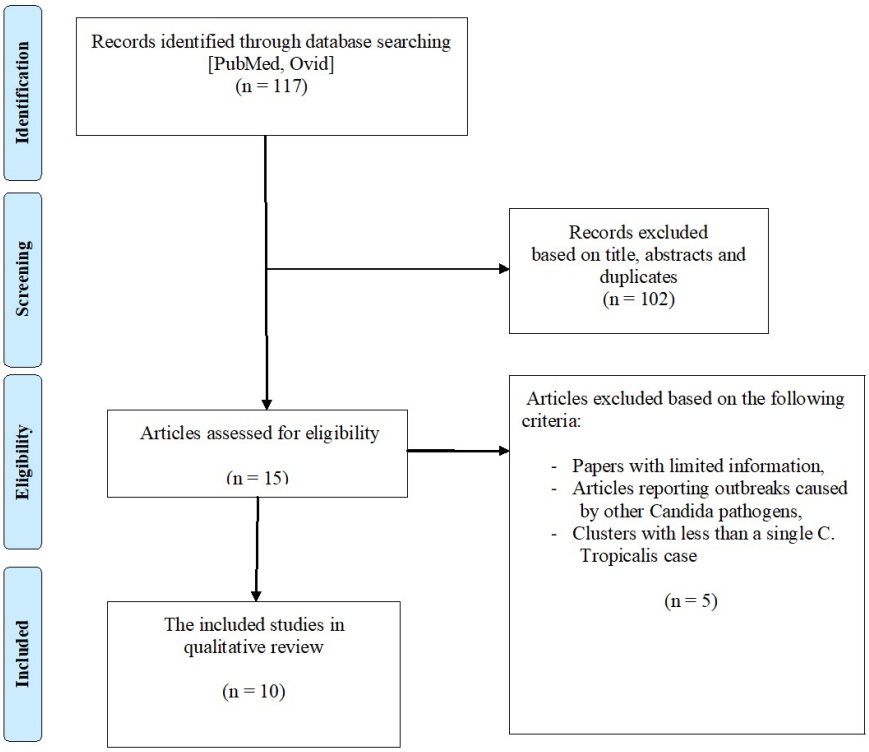
Figure legends

Figure 1. Flowchart describing the study design process



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