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Short report

Emergence of *Candida auris* in Russia

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SUMMARY

This paper reports the emergence of *Candida auris* infections in an intensive care unit at a hospital in Moscow. Forty-nine cases were diagnosed in 2016–2017, and the risk factors and antifungal susceptibilities are described. The 30-day all-cause mortality for 19 bloodstream infections in patients who did not receive appropriate antifungal therapy was 42.1%. Phylogenetic analysis of the internal transcribed spacer and D1–D2 regions and K143R substitution in the *ERG11* gene indicated that the studied *C. auris* strains were of South Asian origin. This first reported series of *C. auris* infections in Russia demonstrates the rapid dissemination of this species, and the need for international surveillance and control measures.

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Introduction

Candida auris is an emerging fungal pathogen, often associated with nosocomial infections. This yeast was first recognized in 2009, and has since been detected on five continents [1–4]. *C. auris* frequently demonstrates resistance to fluconazole and other widely used antifungal agents, and has caused outbreaks of hospital-acquired infections, often with high levels of mortality [4,5]. This paper reports the emergence of *C. auris* infection in an intensive care unit (ICU) for patients with severe trauma at a general hospital in Moscow, Russia.

Methods

C. auris was isolated from routine samples of inpatients hospitalized on an ICU using Sabouraud dextrose agar (Oxoid, Basingstoke, UK) and chromogenic agar (Liofilchem, Roseto degli Abruzzi, Italy). Identification to species level was performed using matrix-assisted time-of flight (MALDI-TOF) mass

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spectrometry using MALDI Biotyper software (Bruker, Billerica, MA, USA). Susceptibility to widely used antifungal preparations was determined with Sensititre YeastOne AST plates (Thermo Fisher Scientific, Waltham, MA, USA).

Studies of the hospital environment were performed with NRSII Transwab (Medical Wire and Equipment Co. Ltd, Corsham, UK). Isolates from the positive samples were cultured on Columbia agar (Oxoid), Sabouraud dextrose agar (Oxoid) and chromogenic agar (Liofilchem) plates. Swabs from the hands of healthcare workers were examined similarly. Identification of the isolated micro-organisms was performed with MALDI-TOF mass spectrometry using MALDI Biotyper software.

Molecular studies of *C. auris* strains included sequencing of the internal transcribed spacer (ITS) region and the D1–D2 large subunit ribosomal DNA region, as well as the *ERG11* gene. Sequencing was performed by the Sanger method with an ABI Prism 3130 Genetic Analyser (Applied Biosystems, Foster City, CA, USA). The D1–D2 domain and the ITS region were amplified and sequenced using NL1/NL2 and ITS1/ITS4 primers, respectively [6,7]. Sequences were compared with the NCBI GenBank sequence database examples using the BLAST algorithm. Consensus sequences were aligned using ClustalW alignment. Reference-based phylogenetic analyses were conducted using the Bio neighbor-joining (BioNJ) algorithm in Seaview 4 software.

The *ERG11* gene was amplified and sequenced using three primer sets – ERG11-1F,5′-TCTCAGAAAAGACAGAGCTC-3′ and ERG11-1R, 5′-CTTCACGCCATCTTTATACG-3’; ERG11-2F, 5′-GTTAGGAAAAGTTATGACGG-3′ and ERG11-2R, 5′-TTGGTGACTTTACCAAACCC-3’; and ERG11-3F, 5′-AGATCTCTGCTACCTACATG-3′ and ERG11-3R, 5′-GATTTCTGCTGGCTCCATTG-3’ – which separated the ERG11 coding region into three parts and yielded the polymerase chain reaction products from 126 to 1542 bp of the *ERG11* gene. The construction of primers was based on the *C. auris* ERG11 gene sequence (GenBank accession no. KY410388.1). The sequence was evaluated for amino acid substitutions, which were compared with the known types, associated with different clades of *C. auris* [8].

Results and discussion

The first detection of *C. auris* at the study hospital was in October 2016, isolated from a urine sample of a 40-year-old male who was a temporary labour migrant from one of the post-Soviet Middle Asian counties. The patient was hospitalized in the ICU due to severe traffic trauma and alcohol intoxication. This index patient did not have any travel history, except the recent migration to Russia, and had not been treated in any other hospitals over the last 5 years. Routine microbiological sampling of urine was performed weekly, and *C. auris* was only detected once. This strain of *C. auris* was resistant to fluconazole – the only antifungal agent the patient received during his hospital stay – with a minimum inhibitory concentration (MIC) of 256 mg/L.

Subsequently, 49 patients in the ICU were diagnosed with *C. auris* infection between October 2016 and December 2017. The patients’ ages ranged from 17 to 87 years [mean 52.4, standard deviation (SD) 2.4 years], and 40 patients were male. The underlying condition in all patients was severe multi-trauma.

C. auris was isolated from urine in 27 (55.1%) patients; from blood and urine in 11 (22.4%) patients; from blood, urine and tracheal aspirate in five (10.2%) patients; from blood in two (4.0%) patients; from urine and tracheal aspirate in two (4.0%) patients; from blood and tracheal aspirate in one (2.0%) patient; and from tracheal aspirate in one (2.0%) patient.

The risk factors commonly associated with *C. auris* infection [2,5] were evaluated as follows: ICU stay preceding isolation of *C. auris* from patient samples of 2–78 days (mean 23.6, SD 2.7 days); 39 (79.6%) patients underwent surgical operations, including neurosurgery in 37 (75.5%) patients; and diabetes mellitus was confirmed in eight (16.3%) cases. All patients received broad-spectrum antibiotics, 32 (65.3%) patients received fluconazole and 14 (28.6%) patients received corticosteroids prior to *C. auris* isolation. Intravenous lines and urinary catheters were present in all cases.

The data on MICs to antifungals for non-duplicate *C. auris* isolates are shown in Table I.

None of the patients with *C. auris* received any antifungal preparation except fluconazole (400 mg/day) after isolation of the fungus, although only one isolate was susceptible to this agent *in vitro*. The patients did not receive appropriate antifungal treatment as Russia currently has no standards for the management of *C. auris* infection, and their condition was not clearly deteriorating due to infection with *C. auris*: the majority of the patients had multi-trauma with severe head injury and poor prognosis, as well as simultaneous pulmonary, surgical site, bloodstream or urinary tract infections caused by other micro-organisms.

Candidaemia due to *C. auris* was diagnosed in 19 (38.8%) patients. The 30-day all-cause mortality rate for these cases was 42.1%. A high level of mortality from *C. auris* bloodstream infections has been reported previously: a large study from Spain recorded a 30-day mortality rate of 41.4% in patients receiving appropriate treatment, although a series from the UK revealed no attributable mortality [4,5,9].

Microbiological studies of the hospital environment were performed monthly and included floor, walls, beds and devices. In total, 450 swabs were cultured. *C. auris* was not isolated from any of these sites, or from swabs from the hands of healthcare workers. Precautions that were introduced included isolation of patients; hand hygiene with alcohol-containing or other appropriate disinfection solutions; and wearing disposable gloves, medical coats and masks when examining patients or performing any invasive or non-invasive procedures. The environmental disinfectants that were previously

Table I
Minimum inhibitory concentration (MIC) results for *Candida auris* isolates

Antifungal agent	MIC, range (mg/L)	MIC_{50} (mg/L)	MIC_{90} (mg/L)
Amphotericin B	≤0.12–8	2	4
Fluconazole	4–256	256	256
Itraconazole	0.06–16	0.5	16
Voriconazole	0.12–8	2	8
Posaconazole	0.03–8	0.25	2
Anidulafungin	0.03–1	0.12	1
Caspofungin	0.03–8	0.12	1
Micafungin	0.015–2	0.12	1
5-Fluorocytosine	≤0.06–64	0.12	0.5

used in the ICU were ammonium-containing preparations, which are known to have insufficient effect on *C. auris* [10]. Hence, a change in disinfection procedures, involving introduction of chlorine-containing agents for environmental procedures, was made at the beginning of 2017. Studies of in-vitro activities of chlorine-containing agents showed their superiority against *C. auris* compared with oxygen or ammonium-containing preparations [10]. However, in the previously reported cases [5,9], these agents were not effective in eliminating *C. auris* and preventing the spread of infection. This mirrors the present experience, with new *C. auris* cases continuing to be diagnosed later in 2018.

Four isolates (one from blood and three from urine) were available for molecular genetic testing, which was performed at Almazov National Medical Research Centre, Saint Petersburg, Russia. The number of strains available for further study was limited due to restricted capability for storage and investigations of the yeasts in the non-teaching hospital where *C. auris* was routinely isolated from clinical samples. Analysis of DNA fragments covering the nuclear 28S rDNA D1—D2 domain and ITS region was performed, and all four samples showed identical sequences in the D1—D2 region and the ITS region. Sequences were compared with the NCBI GenBank sequence database examples using the BLAST algorithm, and showed 100% identity with other *C. auris* sequences in the database. The 11 sequences from each region demonstrating maximum identity with the present isolates were selected from GenBank to construct a phylogenetic tree (Figure 1).

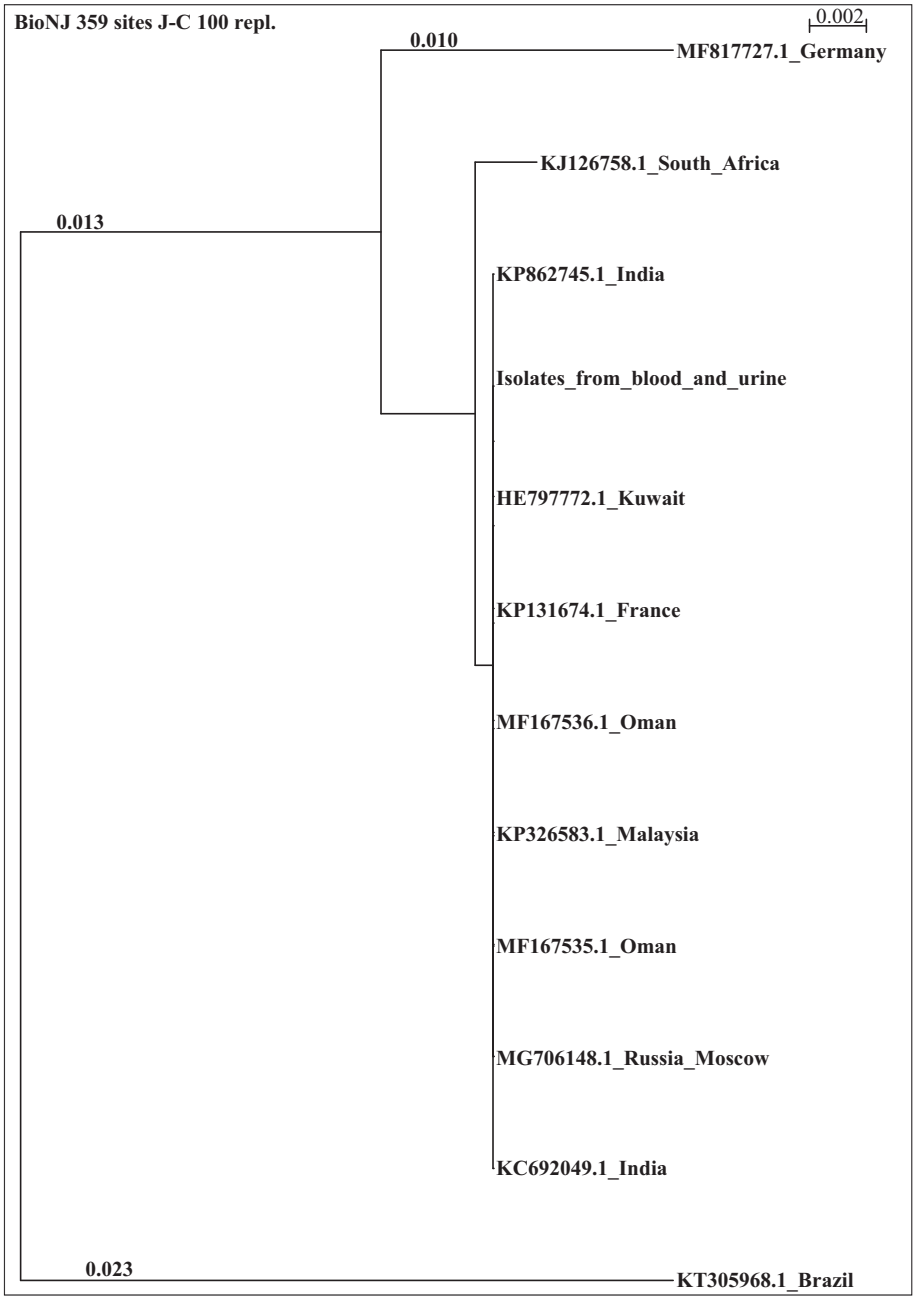

Figure 1. Phylogenetic tree of the internal transcribed spacer region of *Candida auris* isolates from blood and urine.

The phylogenetic trees of both the ITS and D1—D2 regions revealed that all four Russian *C. auris* isolates clustered with *C. auris* isolates that originated from Asia (Malaysia, India). While this work was ongoing, the sequence of the ITS region from a *C. auris* isolate from a patient in another hospital in Moscow was published (MG706148.1); this was similar to that reported here, suggesting an Asian origin for these first studied *C. auris* strains from Russia. To date, four clades of different geographic origin of *C. auris* have been described: East Asian, South Asian, South American and African. Each clade has characteristic substitutions in the *ERG11* gene associated with azole resistance [8]. Evaluation of sequences of the *ERG11* gene in four *C. auris* isolates from the present series for amino acid substitutions at spot mutation points revealed a K143R substitution previously described as typical for isolates from India and Pakistan belonging to the South Asian clade [8].

To the best of the authors' knowledge, this is the first reported series of *C. auris* infection in Russia. The findings demonstrate that the number of countries where *C. auris* has been detected in hospital specimens is increasing, and this species is likely to become even more widespread. A high level of suspicion should be maintained. International surveillance and infection control measures are needed to prevent its further spread.

Conflict of interest statement
None declared.

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