out its high pathogenicity in immunocompromised personnel, so it is necessary that laboratory workers would be fully vaccinated and use of safety precautions. As studies indicate robust VACV-specific immune responses in humans following vaccination, pre-exposure vaccination[4] with VACV may prevent laboratory workers. The Advisory Committee on Immunization Practices recommends routine vaccination with live smallpox vaccine for laboratory personnel who directly work with this virus or other orthopoxviruses that infect humans to avoid possible transmission.[5]

This kind of vaccinia lesions have good prognosis although resolution is slow and a scar can persist. Treatment is usually symptomatic and the main aim is avoid complications as bacterial superinfections.

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Use of isavuconazole in cryptococcal meningitis in a cirrhotic patient

Uso de isavuconazol en la meningitis criptocócica de un paciente cirrótico

Cryptococcal meningoencephalitis (CM) is a serious disease that causes 181,100 deaths worldwide each year.[1] The main causative species are *Cryptococcus neoformans* and *Cryptococcus gattii*. It is frequently associated with HIV infection in a situation of advanced immunosuppression or with other types of immunodeficiencies (solid organ transplant, cancers and patients undergoing chemotherapy or on immunosuppressive drugs). However, up to 20% of cases occur in immunocompetent patients, with the prevalent varieties being *C. neoformans grubii* and *C. gattii*.[2] We present an autochthonous case of CM due to *C. gattii* resistant to fluconazole with a favourable response to isavuconazole.

The subject is a 59-year-old man with a history of poly-drug addiction, ischaemic stroke with right hemiparesis and Child-Pugh B cirrhosis due to C virus with sustained viral response. He went to the Emergency Care Service with a three-week history of unsteadiness in gait, sensation of spinning objects and fever. Examination revealed upward gaze, fundus without papillary oedema, and no alterations in the rest of the physical and neurological examination.

Head CT was without findings and lumbar puncture (LP) showed cloudy cerebrospinal fluid (CSF) with normal pressure and biochemistry suggestive of bacterial meningitis (glucose 8 mg/dl, total proteins 383.7 mg/dl, leucocytes 8297 cells, 92% polymorphonuclear); yeasts compatible with *Cryptococcus* spp. were found in the Gram and India ink staining (Fig. 1), confirming the detection of

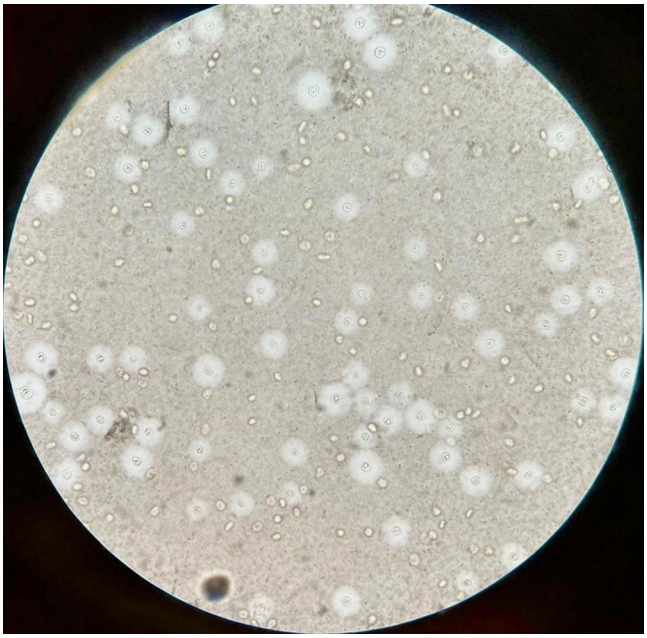

Fig. 1. Direct study of the patient's CSF using India ink technique in which yeast-like structures with a capsule are observed, some of them large (halo or clear-transparent halo, with a central element) on the dark background.

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DNA for *C. gattii/neoformans* using a molecular technique based on multiplex PCR nucleic acids (Biomérieux, FilmArray™). *C. gattii was identified in the culture.* Blood cultures were negative and the baseline titre of cryptococcus antigen in CSF (latex) was >1/10,000.

Brain MRI showed cerebellar cryptococcomas. Immunoglobulin subclasses, complement, lymphocyte populations and HIV serology were determined and occult neoplasms were ruled out. Splenomegaly was detected on CT without signs of portal hypertension.

For the treatment of central nervous system (CNS) infection due to *C. gattii*, data on *C. neoformans* were extrapolated,[2] and it was decided to start treatment, according to WHO recommendations,[3] with liposomal amphotericin B (AmB-L) 10 mg/kg in single intravenous dose + flucytosine (5-FC) 100 mg/kg/day orally in four doses, followed by fluconazole 1200 mg orally every 24 h for two weeks.

Despite this treatment, the patient continued to show a decreased level of consciousness and the growth of the fungus persisted in successive CSF cultures. Finally, he was switched to the IDSA[4] scheme with AmB-L 3–4 mg/kg/day and 5-FC. After six weeks of treatment, yeast continued to be isolated in the CSF with antigen titres >1/10,000. We therefore decided to combine an azole with the previous regimen.

At Spain's National Microbiology Centre, using the broth microdilution method, the MIC of the different azoles were: fluconazole > 4 μg/ml; voriconazole 2 μg/ml; and isavuconazole 1 μg/ml.

The patient's liver disease limited the use of voriconazole so, due to its better liver profile and based on data from the literature,[5] we opted for IV isavuconazole (IVZ) at 200 mg/8 h for 48 h and 200 mg every 24 h as maintenance.

Trough levels of isavuconazole after six doses were determined by liquid chromatography with mass spectrometry, both in plasma and CSF, being 5 μg/ml and 0.66 μg/ml respectively.

After two weeks with this regimen (AmB-L/5-FC/IVZ) CSF cultures were negative and the cryptococcal antigen titre dropped to 1/4000 the first week and 1/2000 the second week, associated with neurological improvement. The subject completed six weeks with this last regimen.

In Spain, C. *gattii* has been isolated as a coloniser in trees on the Mediterranean coast[6] and autochthonous cases have been described in patients without HIV infection.[7]

In CM, in both immunosuppressed and immunocompetent patients, the CSF shows a white blood cell count of less than 50 cells/μl with mononuclear predominance, mild protein morrachia, and low or normal glucose. Therefore, our case had an atypical presentation that forced us to consider other aetiologies.

Treatment of infection by *Cryptococcus* spp. is based on the use of polyenes, azoles and nucleoside analogues (pyrimidines).[1] Clinical experience with azoles other than fluconazole is limited. Possible resistance to antifungals should be considered when negative CSF cultures cannot be achieved. For isolates not identified as *C. neoformans*, the Clinical and Laboratory Standards Institute (CLSI) defines the breakpoint for fluconazole as 16 μg/ml, classifying strains with MIC > 16 μg/ml as non-wild phenotype or with possible resistance mechanisms. For *C. gattii*, EUCAST has not proposed breakpoints for fluconazole, but it does establish them for amphotericin B (0.5 μg/ml) and posaconazole (1 μg/ml).[8] In our case we chose isavuconazole, as opposed to voriconazole or posaconazole, due to its lower interaction profile, lower hepatorenal toxicity and good diffusion to the CNS.[9] There are trials supporting the efficacy of isavuconazole with a loading dose of 200 mg/8 h for two days, followed by 200 mg daily as maintenance for lung and meningeal disease.[10] Kohno et al. obtained a response rate with isavuconazole as high as 90%.[5] In our case, although the plasma concentration of isavuconazole exceeds the MIC, in CSF it was lower than expected and lower than the MIC of the yeast, suggesting that the response was due to the combination of antifungals.

Isavuconazole could be a safe and effective azole as part of the treatment regimen for cryptococcosis when the use of fluconazole or voriconazole is not possible.

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All the authors made substantial contributions to each of the following: 1) data collection; 2) the critical review of the intellectual content; and 3) final approval of the version submitted.

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Usefulness of decentralized sequencing networks on antimicrobial resistance surveillance

Utilidad de las redes de secuenciación descentralizadas en la vigilancia de la resistencia antibiótica

The increase of bacterial antimicrobial resistance is one of the biggest challenges to modern medicine, being associated with almost five million deaths and 1.2 million attributable deaths in 2019.[1] One of the key lessons learned from SARS-CoV-2 or mpox is that highly interconnected world that we live in, allows the rapid spread of new variants and diseases to almost every continent.[2] Therefore, even if carbapenemase producing Enterobacteriaceae (CPE) are not prevalent at a certain location currently this may change due to the introduction of a new CPE clone rapidly.[3]

Moreover, in recent years health systems are getting increasingly complicated with more intricate relationship between different hospitals and health-centres. Consequently, the introduction of clonal CPE may no longer be a local problem and represent a regional problem, causing a multihospital outbreak.[4,5]

To tackle this problem, the characterization of CPE is needed to recognize the appearance of regional outbreaks. Until now the most common strategy has been to develop hierarchical networks with core laboratories that conducted most of the molecular characterization of CPE at regional or national level. However, the appearance of new sequencing platforms such as Oxford Nanopore (ONT) sequencers coupled with the flongle flowcells[6] represent the perfect opportunity to develop new nodal networks. In this case, instead a central laboratory performing all analysis, each laboratory of the network can produce their own sequences that can be later shared between them.

In our area the most common CPE are *Klebsiella pneumoniae* and *Escherichia coli*.[7] However, other less common CPE such as *Citrobacter freundii* group have also been described.[8] Therefore, the simultaneous first isolation of two carbapenem resistant *C. freundii* isolates on different hospitals in the same region raised many alarms.

The isolates were identified as *C. freundii* with the MALDI Biotyper MBT smart system (Bruker Daltonics, Germany). The antibiotic susceptibility was conducted with the Phoenix BD system (Becton Dickinson, USA) or the MicroScan Walkaway system (Beckman Coulter, USA). Both strains were carbapenem resistant and tested positive for New-Dehli-metalobetalactamase (NDM) with the CARBA-5 assay (NG Biotech, France). After swift coordination between both microbiology laboratories both were selected for a more thorough analysis by whole genome sequencing (WGS).

The DNA extraction was conducted with the MagNA Pure Bacteria 2.0 Protocol (Roche, Switzerland) and DNA concentration was quantified using the Qubit4 fluorometer (Thermo-Fisher Scientific, USA), while quality assessment was performed using the NanoDrop One spectrophotometer (Thermo-Fisher Scientific, USA). The library was prepared with 400 ng of DNA following the ONT protocol for flongles and loaded on a FLO-MIN106 flongle (ONT, United Kingdom). This approach to the library synthesis requires a hands-on time of around half an hour. Genome assembly was performed using flye[9] and medaka.

One isolate belonged to the ST22 and harboured a bla_{NDM-5} and a IncX3 incompatibility complex on the same contig, while the other isolate was an ST18 with a bla_{NDM-1} and a IncR on the same contig, thus ruling out the hypothesis of an interhospitalary outbreak.

To the conclusion of this study no new bla_{NDM}-producing Enterobacteriaceae has been isolated in any of both hospitals besides an ST214 *Klebsiella grimonti* with a bla_{NDM-5} and a IncX3 isolated from a rectal swab of the first patient. Therefore, even if no specific plasmid analysis was conducted these findings highlight the possibility of these worrying resistant genes on transmissible elements.

All in all, we think that this is a good example of a good cooperation between nearby centres and where the current state of the sequencing technologies at clinical microbiology laboratories. Such strategies will not undermine the role of said central laboratories as they will keep a vital role in the establishment of laboratory networks capable of performing WGS, coordinating the efforts of the network and advising nodal laboratories. Meanwhile, this approach enables a swift response as performing this analysis on a local base enables the nodal centres to take the necessary measures more quickly. Moreover, in our opinion this will improve the engagement of nodal laboratories in the efforts of AMR surveillance, and we firmly believe that the WGS no longer is a technology reserved for central reference laboratories but rather an important diagnostic that should be considered in almost every clinical microbiology laboratory.

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