Enterococcus raffinosus sinusitis post-*Aspergillus flavus* paranasal infection, in a patient with myelodysplastic syndrome: report of a case and concise review of pertinent literature

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Enterococcus raffinosus sinusitis post-*Aspergillus flavus* paranasal infection, in a patient with myelodysplastic syndrome: report of a case and concise review of pertinent literature

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ABSTRACT

A case of *Enterococcus raffinosus* nosocomial sinusitis which appeared to complicate a previous *Aspergillus flavus* paranasal infection is presented. This uncommon enterococcal species is rarely responsible for human diseases, and has never previously been associated with sinusitis.

CASE REPORT

Three months after admission to the Department of Hematology, a 59-year-old female neutropenic (500/mm^3 white blood cells) patient with myelodysplastic syndrome developed nasal obstruction, thick and purulent discharge from the right nasal cavity, tenderness, swelling and local pain over the right maxillary and the frontal sinuses areas, headache, and raised temperature (38.5°C). Acute sinusitis was diagnosed,[1] involving ethmoidal, sphenoidal, frontal and right maxillary sinuses; CT showed scan thickening of the above mentioned sinuses, which was compatible with fungal infiltrates. Mould infection was expected, as the patient had been treated with cytotoxic compounds and corticosteroids during hospitalisation. Gram staining of the mucopurulent secretion (obtained from the right nasal cavity) showed the presence of polymorphonuclear leucocytes and fungal hyphae. Finally, the Platelia test for detection of *Aspergillus* galactomannan, performed on serum, showed an increased titre of 3.56 (reference index <0.5).

Material was placed onto blood sheep agar (Biolife), mannitol salt agar (Biolife) and MacConkey agar (Biolife), which were incubated in air. A Sabouraud plate (Biolife) was incubated at 36°C; a second one was kept at 25°C. Two further blood agar plates were incubated in air supplemented with 5% CO_2, and anaerobically, respectively. After 24 h of incubation, *Aspergillus flavus* (about 30 CFU/plate) was grown as a single organism; bacteria (aerobic, microaerophilic or anaerobic) or fungi other than *A flavus* were not collected. An Etest was carried out (antifungal strips provided by AB BIODISK), which showed a MIC of 0.5 μg/ml for voriconazole, whereas no antifungal activity appeared to be exerted by itraconazole (MIC ≥32 μg/ml) and amphotericin B (MIC 6 μg/ml).[2 3]

Surgical curettage of the paranasal cavities was performed, and parenteral voriconazole was started, leading to slow and gradual clinical improvement during the following 2 months. *Aspergillus* was no longer grown from cultures and CT showed absence of progression of the fungal infection, except for sinus thickening which persisted.

During the third month of treatment, the patient developed nasal obstruction, mucopurulent discharge from the right nasal cavity, local pain over the right and frontal sinuses areas, headache and fever (38.5°C). CT showed worsening of sinus thickening, compatible with absence of *Aspergillus* infiltrates. Purulent material was collected and cultured. Unexpectedly, blood agar plates yielded numerous (>200 CFU/plate) α-haemolytic, smooth colonies, around 1 mm in diameter. The organism was identified as *Enterococcus raffinosus* with 99% certainty by Vitek2 system (bioMérieux); identification was then confirmed by 16S rRNA sequencing. *E raffinosus* was grown as a single organism, as no aerobic, microaerophilic or anaerobic organisms other than *E raffinosus* were isolated. Gram staining of secretion showed the presence of numerous Gram positive cocci, forming pairs and short chains, mostly inside polymorphonuclear leucocytes (phagocytosis), so confirming the pathogenic role of *E raffinosus* in the disease case studied (mould images were no longer present). The isolate was not motile at 36°C, and belonged to none of Lancefield group A, B, C, D, F and G groups (SLIDEX Strepto Plus, bioMérieux). Antibiotic susceptibility testing was performed by agar diffusion method (using Liofilchem disks).[4] The organism showed resistance to penicillin, ampicillin, amoxicillin/clavulanate, ampicillin/sulbactam, piperacillin/tazobactam, imipenem, meropenem, gentamicin, ciprofloxacin, clindamycin, erythromycin and tetracycline, but was found to be susceptible to vancomycin and teicoplanin. Pending results of cultures, antifungal treatment was stopped while empirical therapy with teicoplanin was started, but the patient died 2 days later as a result of non-infectious complications of the underlying haematological malignancy.

DISCUSSION

E raffinosus was labelled as a new species in 1989. It is a Gram positive, catalase-negative, motile or non-motile bacterium, forming smooth, α/γ-haemolytic colonies on blood agar plates. It has also been

occasionally found to belong to Lancefield group D, and has been described as part of the commensal flora of domestic cats, besides being cultured from human rectal swabs. The organism has a facultatively anaerobic metabolism; in particular, raffinose utilisation differentiates it from the phenotypically similar *Enterococcus avium*.[5–7] *E raffinosus* is not present in the database for API 20 Strep (bioMérieux) and API 32 STREP (bioMérieux), so that misidentification by these instruments is possible. Instead, correct identification can be provided by Rapid ID 32 Strep (bioMérieux), the BD Phoenix system and Vitek2 (bioMérieux).[5 6]

E raffinosus has been rarely known to cause nosocomial infections, most of which are related to long-term hospitalisation, urinary catheterisation, antimicrobial agents administration, surgical procedures, urinary tract instrumentation, venous stasis, alcoholic liver disease, rheumatoid arthritis, wounds and cancer.[6 7] In particular, urinary tract infections, wounds and stasis dermatitis ulcer infections, vaginitis, Bartolin glands abscesses, biliary infections, peritonitis, visceral abscesses (including myocardial), vertebral osteomyelitis, endocarditis, sepsis, and a case of haematoma infection by *E raffinosus* have been reported.[5–9] Interestingly, data on haematological hosts infected by *E raffinosus* are poorly reported in the literature. Samuel *et al* investigated a cluster of 17 patients harbouring strains of this species in the enteric tract. These were cultured from stool samples during a routine GRE (glycopeptide-resistant enterococci) screening undertaken on the haematology ward; all showed glycopeptide-resistance. Finally, they were shown to represent a single *E raffinosus* strain by PFGE. Aiming to recognise the route of nosocomial spread of this clone, an environmental screening of bath tubs and hoists, sinks, taps, toilets, beds, bed railings and an exercise machine was performed, but these were all negative. Therefore, the source of the studied strain diffusion remained unknown.[10] To our knowledge, sinus infection by this organism has never been described, previously, neither in immunocompetent patients nor in compromised (such as haematological) hosts.[11–15]

Prevalence of *E raffinosus* resistance to antimicrobials is increasing; in particular, resistance to penicillin, ampicillin, ampicillin–sulbactam, ciprofloxacin, carbapenems, erythromycin, clindamycin, tetracycline, minocycline, fosfomycin and glycopeptides has been found in clinical isolates. Lack of sensitivity to both gentamicin alone and gentamicin plus streptomycin has also been observed. Also, resistance to the only streptomycin, as well as to rifampin, was found to involve isolates from an aquatic environment in Greece.[7 16–18]

This case confirms the role of immunosuppression and surgical procedures as risk factors for *Enterococcus* disease. Further, previous *Aspergillus* infection and antifungal treatment seemed to play a role in predisposing to colonisation and infection by *E raffinosus*. Damaged epithelium could expose mucosal receptors for enterococci, or predispose to biofilm formation by the latter, but these hypotheses are just speculative and need confirmation. In conclusion, we have reported a case of *E raffinosus* paranasal infection, and discussed the emerging role of this organism as a nosocomial opportunistic difficult-to-treat agent.

Competing interests None.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

Take-home messages

- *Enterococcus raffinosus* is an uncommon human pathogen, infections by which are poorly discussed in the literature.
- Resistance expression to antimicrobials by *E raffinosus* has been reported, but epidemiology and clinical pathogenicity of this rare species still have to be partly clarified.
- The first case of paranasal infection by this bacterial agent is presented; the sinusitis episode followed previous *Aspergillus flavus* infection.
- Results further confirm that immunosuppression and surgical procedures are potential risk factors for *Enterococcus* disease. In addition, previous fungal infection and antifungal treatment seemed to predispose to colonisation and infection by enterococci.
- *E raffinosus* may be considered an emerging agent of disease in hospitalised, compromised hosts.

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