Case Report

First report of vaginal infection caused by *Enterococcus raffinosus*

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The authors have reported the first case of vaginal infection caused by *Enterococcus raffinosus*. The latter is a rarely identified species, but some of the infections described in the literature should direct some attention to this, often opportunistic pathogen, and its emerging multidrug resistance.

Case report

During the second month of hospitalization by the Department of Haematology, a 50-year-old immunocompromised patient with acute leukaemia developed severe vulvovaginal itching and burning. At speculum examination, erythema and discharge were observed. Vaginal pH was 5.2, the amine test was negative and microscopy (Gram stain) showed the presence of numerous Gram-positive cocci and leukocytes. We obtained swab specimens from the middle third of the vagina and placed them on Sabouraud dextrose agar, sheep blood agar, MacConkey agar and Mannitol salt agar (all from Biolife). The plates were incubated in air. A second blood agar plate was inoculated and incubated anaerobically, whilst a third blood agar plate and a Thayer-Martin plate were inoculated and incubated in an atmosphere of 5% CO₂. All cultures were kept at 37 °C and examined after 24 and 48 h. After 24 h of incubation, smooth and α -haemolytic colonies (>150 c.f.u.), about 1 mm in diameter, were observed on all blood agar plates. The organism was found to be a Gram-positive coccus that formed pairs and short chains, and non-motile at 36 °C. None of Lancefield group A, B, C, D, F and G antisera produced a positive reaction (SLIDEX Strepto Plus; bioMérieux) (Facklam & Collins, 1989) and the catalase test was negative. No other organisms of known vaginal pathogenicity were isolated. The isolate was identified as *Enterococcus raffinosus* by using the VITEK 2 system (card GP; bioMérieux) with 99% certainty, and the identification was confirmed by using the API system (Rapid ID 32 Strep; bioMérieux). Antibiotic susceptibility testing was performed with brain heart infusion agar (Biolife) using the disc diffusion method and was interpreted as per Clinical and Laboratory Standards

Institute guidelines (Chirurgi *et al.*, 1991; NCCLS, 2000; Prakash *et al.*, 2005). The organism was found to be resistant to penicillin, ampicillin, amoxicillin/clavulanate, ampicillin/sulbactam, piperacillin/tazobactam, imipenem, meropenem, ciprofloxacin, clindamycin, erythromycin, cotrimoxazole, tetracycline, amikacin, gentamicin, netilmicin and tobramycin, and susceptible only to vancomycin, teicoplanin and rifampicin. Discs were provided by Oxoid.

Pending the results from cultures, the patient was treated empirically and unsuccessfully with oral amoxicillin/clavulanate (1000 mg, every 12 h). Instead, the vaginal symptoms disappeared suddenly within the second day of teicoplanin therapy that the patient had received empirically because of the outbreak of a Gram-positive cocci bacteraemia (data not shown). One week after glycopeptide treatment, vaginal examination and cultures were repeated but they did not show signs of inflammation or *E. raffinosus* colonies, respectively. Further, Gram stain showed the restoration of a physiological vaginal environment.

Discussion

Enterococcus species are one of the main causes of nosocomial infections. Although *Enterococcus faecalis* and *Enterococcus faecium* represent 90% of clinical isolates, the incidence of enterococcal species other than *E. faecalis* and *E. faecium* is increasing (Prakash *et al.*, 2005). In particular, uncommonly *E. raffinosus* has been isolated from hospitalized patients. *E. raffinosus* is a facultative, motile or non-motile, non-encapsulated, non-sporulating organism. The cells are Gram-positive and are arranged singly, in pairs or short chains. On blood agar with 5% sheep blood, colonies are smooth, grey, α -haemolytic or non-haemolytic and ≤ 1 mm

in diameter. This species is characterized by the presence of pyrrolidonylarylamidase and leucine aminopeptidase activities, hydrolysis of aesculin and utilization of mannitol, sorbitol, raffinose, sucrose and L-arabinose, and the absence of catalase activity. Further, *E. raffinosus* occasionally presents Lancefield Group D antigen (Facklam & Collins, 1989; Freyaldenhoven *et al.*, 2005; Sandoe *et al.*, 2001).

The species *E. raffinosus* was recognized in 1989, when the bacterium was distinguished from the phenotypically similar *Enterococcus avium* by the ability of the former to utilize raffinose. Misidentification is possible by using API 20 Strep and API 32 Strep (bioMérieux), as *E. raffinosus* is not included in the database for these products. Instead, VITEK 2, Rapid ID 32 Strep and the BD Phoenix system include this species in the Gram-positive identification database (Freyaldenhoven *et al.*, 2005; Grayson *et al.*, 1991; Sandoe *et al.*, 2001).

E. raffinosus is associated with urinary tract infections, wounds, ulcers and plague infections, intra-abdominal and inguinal abscesses, Bartolin gland abscesses, biliary and peritoneal infections, vertebral osteomyelitis, endocarditis and bacteraemia (Chirurgi *et al.*, 1991; Sandoe *et al.*, 2001). A case of haematoma infection has been reported recently (Freyaldenhoven *et al.*, 2005). Isolation of *E. raffinosus* is usually related to long-term hospitalization, antibiotic prophylaxis and therapies, urinary catheterization and surgical procedures (Chirurgi *et al.*, 1991; Prakash *et al.*, 2005; Sandoe *et al.*, 2001). It is difficult to provide a standard susceptibility pattern for *E. raffinosus*, and its emerging multidrug resistance is increasing in importance (Gordon *et al.*, 1992), as resistance to penicillin, aminoglycosides, fluoroquinolones, carbapenems and glycopeptides has been described in the literature. In particular, resistance to penicillin is mediated mostly by mutations in penicillin-binding proteins. Further, gentamicin resistance is often associated with resistance to erythromycin, tetracycline and minocycline (Chirurgi *et al.*, 1991; Grayson *et al.*, 1991; Sapico *et al.*, 1989; Straut *et al.*, 1996; Tanimoto *et al.*, 2006; Wilke *et al.*, 1997).

To date, little has been published concerning *E. raffinosus*. In the case described here, the patient had received parenteral piperacillin–tazobactam, imipenem and vancomycin during the first month of admission to hospital, due to the onset of bacteraemia caused by *Escherichia coli*, and of a catheter-related *Staphylococcus epidermidis* bloodstream infection (data not shown). Other factors or underlying diseases able to increase the risk of acquiring such an enterococcal infection were not present. *E. raffinosus* is known to only inhabit the oropharynx of domestic cats, as part of the commensal flora; nothing else is known about its natural habitat (Sandoe *et al.*, 2001). It is not known whether the patient in this case kept any pets.

To our knowledge, this report describes the first case of vaginitis caused by *E. raffinosus*, confirming the role of this organism as a cause of nosocomial infections. Further, it focuses on the emerging occurrence of antimicrobial

resistance in this uncommon species. Hence, the authors re-emphasize the importance of the implementation of infection control measures, in order to limit the nosocomial spread of unusual and atypical bacteria, and the emergence of multidrug resistance among them.

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References

- Chirurgi, V. A., Oster, S. E., Goldberg, A. A., Zervos, M. J. & McCabe, R. E. (1991). Ampicillin-resistant *Enterococcus raffinosus* in an acute-care hospital: case-control study and antimicrobial susceptibilities. *J Clin Microbiol* **29**, 2663–2665.
- Facklam, R. R. & Collins, M. D. (1989). Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. *J Clin Microbiol* **27**, 731–734.
- Freyaldenhoven, B. S., Schlieper, G., Lütticken, R. & Reinert, R. R. (2005). *Enterococcus raffinosus* infection in an immunosuppressed patient: case report and review of the literature. *J Infect* **51**, e121–e124.
- Gordon, S., Swenson, J. M., Hill, B. C., Pigott, N. E., Facklam, R. R., Cooksey, R. C., Thornsberry, C., Jarvis, W. R. & Tenover, F. C. (1992). Antimicrobial susceptibility patterns of common and unusual species of enterococci causing infections in the United States. Enterococcal Study Group. *J Clin Microbiol* **30**, 2373–2378.
- Grayson, M. L., Eliopoulos, G. M., Wennersten, C. B., Ruoff, K. L., Klimm, K., Sapico, F. L., Bayer, A. S. & Moellering, R. C., Jr (1991). Comparison of *Enterococcus raffinosus* with *Enterococcus avium* on the basis of penicillin susceptibility, penicillin-binding protein analysis, and high-level aminoglycoside resistance. *Antimicrob Agents Chemother* **35**, 1408–1412.
- NCCLS (2000). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7–A5. Wayne, PA: National Committee for Clinical Laboratory Standards.
- Prakash, V. P., Rao, S. R. & Parija, S. C. (2005). Emergence of unusual species of enterococci causing infections, South India. *BMC Infect Dis* **5**, 14.
- Sandoe, J. A. T., Witherden, I. R. & Settle, C. (2001). Vertebral osteomyelitis caused by *Enterococcus raffinosus*. *J Clin Microbiol* **39**, 1678–1679.
- Sapico, F. L., Canawati, H. N., Ginunas, V. J., Gilmore, D. S., Montgomerie, J. Z., Tuddenham, W. J. & Facklam, R. R. (1989). Enterococci highly resistant to penicillin and ampicillin: an emerging clinical problem? *J Clin Microbiol* **27**, 2091–2095.
- Straut, M., De Cespèdes, G. & Horaud, T. (1996). Plasmid-borne high-level resistance to gentamicin in *Enterococcus hirae*, *Enterococcus avium*, and *Enterococcus raffinosus*. *Antimicrob Agents Chemother* **40**, 1263–1265.
- Tanimoto, K., Nomura, T., Maruyama, H., Tomita, H., Shibata, N., Arakawa, Y. & Ike, Y. (2006). First VanD-Type vancomycin-resistant *Enterococcus raffinosus* isolate. *Antimicrob Agents Chemother* **50**, 3966–3967.
- Wilke, W. W., Marshall, S. A., Coffman, S. L., Pfaller, M. A., Edmund, M. B., Wenzel, R. P. & Jones, R. N. (1997). Vancomycin-resistant *Enterococcus raffinosus*: molecular epidemiology, species identification error, and frequency of occurrence in a national resistance surveillance program. *Diagn Microbiol Infect Dis* **29**, 43–49.