

The identification of polymicrobial bacteraemia should alert to the presence of an uncontrolled focus, generally intra-abdominal.<sup>6</sup> In our patient, we suspect that the most likely mechanism was the deep extension of an SSTI due to her history of pressure ulcer with adjacent pyomyositis and arthritis. This case illustrates the importance of adequate management of chronic wounds and pressure sores, as they can be a focus of polymicrobial bacteraemia.

From the microbiology point of view, anaerobic bacteria and enterobacteria are usually isolated<sup>7</sup> In our case it was a polymicrobial infection with predominance of *Clostridium* spp. and *Bacteroides* spp. Given the severity of anaerobic bacteraemia, antibiotic sensitivity tests must be performed to confirm the sensitivity of these species to metronidazole, piperacillin/tazobactam and carbapenems, as sensitivity can vary between different species and strains.<sup>8</sup>

Contrast-enhanced CT is the most sensitive test for diagnosing EO, showing the “pumice stone sign” in more than 90% of cases. Other typical radiographic findings are emphysema in the surrounding soft tissues and the absence of destruction of the cortical bone.<sup>1</sup>

Treatment is combined with parenteral broad-spectrum antibiotic therapy for at least four weeks and surgical control of the focus.<sup>1,4,9</sup>

It is important to define the optimal duration of antibiotic therapy and the need for surgical intervention in all cases, as multiple or complex surgical interventions can increase mortality rates.<sup>9</sup>

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## First characterization of a *Klebsiella pneumoniae* clinical isolate producing VEB-1 and OXA-436 in Spain



## Primera caracterización de un aislado clínico de *Klebsiella pneumoniae* productor de VEB-1 y OXA-436 en España

Sir,

Extended-spectrum  $\beta$ -lactamases (ESBLs)-producing Gram-negative bacilli are a major cause of resistance to third-generation cephalosporins. The genes encoding these beta-lactamases are often carried in plasmids, which have allowed them to spread horizontally worldwide, and are currently endemic in community and hospital-acquired Enterobacterales. These enzymes hydrolyse third-generation cephalosporins and aztreonam, but are not active against cephamycins, and they are inhibited by clavulanic acid and by tazobactam. Currently, CTX-M, SHV and TEM family of ESBLs are the most common in most geographical areas worldwide and specifically in Spain. However, there are other less frequent families such as PER, GES or VEB.<sup>1</sup>

VEB (*Vietnamese extended-spectrum  $\beta$ -lactamase*) is a family of ESBLs, that has so far localised in class 1 integrons.<sup>2</sup> It was first detected in Southeast Asia and subsequently in African and Amer-

ican countries.<sup>3,4</sup> This *bla* gene has been found in Enterobacterales, *Achromobacter xylosoxidans*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. In Europe it has been found in both *Klebsiella pneumoniae* and in non-fermentative Gram-negative bacteria since 2001.<sup>5–10</sup> Recently, a clinical case of VEB-producing *K. pneumoniae* was detected in our hospital of Seville and, to date, this is the first description of VEB-1-producing *K. pneumoniae* in Spain.

In addition, the incidence of clinical infections caused by carbapenemase-producing organisms has increased in the last years.<sup>11</sup> A rare plasmid-mediated variant of OXA-48 called OXA-436 has been described.<sup>12</sup> The enzyme has been shown to be a class D carbapenemase similar to OXA-48 in terms of substrate specificity. It has a higher activity at higher temperatures, resembling a human infection scenario, whereas OXA-48 has activity at lower temperatures, indicating an environmental scenario. However, no significant difference is shown in antimicrobial susceptibility profiles *in vivo*.<sup>13</sup> It was initially discovered in an *Enterobacter asburiae* isolate from a patient admitted to a hospital in the capital of Denmark and subsequently detected in other enterobacteria (*Citrobacter freundii*, *K. pneumoniae*, *Escherichia coli*) in different Danish hospitals.<sup>11,12,14</sup> OXA-436 has also been identified in a strain of *Shewanella putrefaciens* from a Pakistani hospital.<sup>15</sup>

A 57-year-old woman presented to the emergency department with a one-week history of fever, chills, cough, and asthenia. She had no past medical history. She had no previous contact with health-care or rural areas, and no history of travel in the 6 months prior to admission.

Clinical examination, including gynecological examination, was unremarkable. Laboratory data revealed an increase in acute phase reactants (CRP = 237 mg/l, PCT = 3.70 ng/ml), with non-pathological urine sediment. In the computed tomography of the neck, chest and abdomen with contrast and in the cholangio-MRI no data of interest were found. Colonoscopy showed no pathological lesions.

An empirical antibiotherapy with ceftriaxone was started. Blood cultures yielded two different Gram-negative isolates: *E. coli* and *K. pneumoniae*, both identified by MALDI-TOF (Bruker).

Due to the epidemiology of our hospital,  $\beta$ -LACTA and  $\beta$ -CARBA tests (Bio-Rad) were carried out for a rapid detection of resistance. The  $\beta$ -LACTA test was positive in both isolates. The *K. pneumoniae* isolate was identified as an OXA-48 producer based on positive results in the  $\beta$ -CARBA test and NG-Test CARBA 5 (NG biotech). The  $\beta$ -CARBA test for *E. coli* was negative. Susceptibility was determined using the MicroScan Walk-Away (Beckman Coulter®) NMDR panel.

The *K. pneumoniae* isolate was susceptible to ciprofloxacin, levofloxacin, amikacin, cotrimoxazole, colistin and tigecycline, and to newer antibiotics such as ceftazidime/avibactam, meropenem/vaborbactam and cefiderocol. It was resistant to third-generation cephalosporins, amoxycillin/clavulanic acid, piperacillin/tazobactam, ceftolozane/tazobactam, ertapenem, gentamicin and tobramycin. It was susceptible with increased exposure to meropenem and imipenem, according to EUCAST breakpoints.

The patient did well clinically and was discharged after completing treatment with 12 days of ceftazidime/avibactam.

In this case, only the *K. pneumoniae* isolate was sent to the reference center for sequencing, since only carbapenemase-producing isolates were sent. Initial bacterial typing of OXA-48-producing *K. pneumoniae* was carried out by pulsed field gel electrophoresis with *Xba*I, and the isolate was found to be different (>6 bands of difference) to previous local isolates. Whole genome sequencing was performed by the Illumina MiSeq, using Nextera Flex, library preparation and genome assembly was performed using CLC Genomics Workbench software. Annotations of resistance determinants and MLST typing were done by using the tools from the Center for Genomic Epidemiology (CGE; <https://cge.cbs.dtu.dk/services/>). The isolate was assigned to clone ST37 and carried the resistance determinants *bla*<sub>OXA-436</sub>, *bla*<sub>OXA-10</sub> and *bla*<sub>VEB-1</sub>. The coverage and homology of *bla*<sub>OXA-436</sub> was 100% by Resfinder and CARD. It is located in a 15176 bp contig with a depth coverage of x30.5. It is identical to a *bla*<sub>OXA-436</sub> sequence deposited in Genbank KY863418 of plasmidic nature, which correlates to a Danish strain of *E. asburiae* AMA 497.

Therefore, resistance to cephalosporins was due to the production of VEB-1 and resistance to carbapenems to the production of OXA-436 and/or OXA-10.

In Europe, the first VEB-1-producer was identified in ceftazidime-resistant *P. aeruginosa* isolates in Bulgaria (2001–2005).<sup>5</sup> Subsequently, in this same species, in the United Kingdom, three more cases of VEB-1-producing *P. aeruginosa* were detected in 2003–2007: the first case was a patient transferred from a hospital in India; the second case was a patient transferred from the previous hospital; and the third case was a patient repatriated to the United Kingdom from Thailand.<sup>6</sup> Subsequently, VEB-1-producing *A. baumannii* isolates were identified in France and in Belgium in 2003. Epidemiological investigations showed that frequent patient transfers between French hospitals and

long-term care facilities in Belgium could be responsible for the spread of strains between countries. The genetic environment of *bla*<sub>VEB-1</sub> revealed an integron identical that found in most *P. aeruginosa* isolates from Thailand.<sup>7,8</sup>

A few years later, cases of VEB-producing *Enterobacteriaceae* infections began to be detected in Europe. In Greece there was an outbreak of *K. pneumoniae* producing KPC-2 and VEB-25 (which differs from VEB-1 in only one mutation) occurred in 2019.<sup>9</sup>

In Spain, a non-conjugative VEB-4-producing isolate (this variant differs from VEB-1 in two amino acid substitutions)<sup>10</sup> was identified in a survey of a ESBL-producing *Proteus mirabilis* collection in Barcelona in 2000–2005, although epidemiological data were not included in the report. Our case is the second described in our country and the first in *K. pneumoniae*. Most importantly, the patient had not travelled outside Spain, which could indicate that this type of enzyme has already been introduced into our country and is circulating in the community. This highlights the need for continuous monitoring of the determinants associated with third-generation cephalosporin-resistant isolates to detect changes in prevalence. Currently, there is a useful method consisting of a set of six multiplex PCRs and one simplex PCR available for rapid screening of  $\beta$ -lactamases, including VEB. This PCR method is a fast, low-cost and reliable tool for the screening of frequently encountered  $\beta$ -lactamases. It will assist in monitoring their emergence and their spread, and it could be used in epidemiological surveys.<sup>16</sup>

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## A case of bacteremia by *Streptococcus pseudopneumoniae*



### Un caso de bacteriemia por *Streptococcus pseudopneumoniae*

#### Case report

A 70-year-old man with a history of polycystic kidney disease, presented to the Urology outpatient clinic with symptoms of chronic urinary obstruction. He was diagnosed with benign prostate hyperplasia and he was treated with photovaporization. Few hours after the procedure he presented with septic shock. Treatment with antibiotics (meropenem, linezolid and daptomycin) and vasoactive drugs was initiated with significant improvement after 72 h. *Streptococcus pneumoniae* was tentatively identified in one of two blood cultures. Urine antigen of *S. pneumoniae* (BinaxNOW, Abbott) and HIV serology were negative. There were no signs of lung consolidation in the chest radiography.

The presumptive identification of *S. pneumoniae* in the microbiology laboratory was performed by optochin disc diffusion test incubated in CO<sub>2</sub> and in ambient air (susceptible in both atmospheres), and mass spectrometry (MALDI-TOF, Bruker). Susceptibility testing was performed with disc diffusion according to EUCAST (v13.1) ([www.eucast.org](http://www.eucast.org)). The isolate was susceptible to cefotaxime, clindamycin and vancomycin, and susceptible with increased exposure to penicillin and levofloxacin. Treatment was deescalated to ceftriaxone with good clinical progress.

As part of a surveillance programme at the Madrid Autonomous Community, all isolates of *S. pneumoniae* are analyzed at the Regional Public Health Laboratory for characterization. These results showed that this particular isolate was non typable, and soluble in bile. The capsular polysaccharide biosynthesis A (*cpsA*) and autolysin (*lytA*) genes were not detected, while the amplification of the pneumolysin gene (*ply*) was positive. Given this variety in our findings, whole genome sequencing (WGS) was performed with MinION (Oxford Nanopore) leading to identification of *Streptococcus pseudopneumoniae*.

*S. pseudopneumoniae* is a bacterial species closely related to *S. pneumoniae*, first identified in 2004 as part of the *Streptococcus mitis* group.<sup>1</sup> It is considered an opportunistic pathogen associated with septicemia and meningitis,<sup>2,3</sup> particularly in patients with haematologic disease.

Phenotypic characteristics can provide some clues for its identification, but these may not be definitive, as they can overlap with other streptococcal species. *S. pseudopneumoniae* colonies have a similar appearance to *S. pneumoniae* in blood agar plates with alpha-hemolytic activity, but unlike the latter, *S. pseudopneumoniae* lacks capsule, is often resistant to optochin (inhibition zones <14 mm) when incubated with CO<sub>2</sub> but susceptible to optochin (inhibition zones >14 mm) when incubated in ambient atmosphere, and not soluble in bile.<sup>1,4</sup> However, there are exceptions and a variable proportion of *S. pneumoniae* strains do not have a specific agglutination in the Quellung reaction due to lack of capsule (non-typeable), can be optochin resistant and bile insoluble.<sup>5,6</sup> Additionally, a proportion of *S. pseudopneumoniae* has also been reported as optochin susceptible when incubated in CO<sub>2</sub> (16.4%), or bile soluble (36.1%)<sup>6</sup> like in our case, although this does not represent the majority of the isolates. Moreover, commercial tests (such as antigen detection or DNA hybridization probes), might not easily discriminate between both species.<sup>1,7</sup>

The *lytA* and the *cpsA* genes are harbored by *S. pneumoniae* (although the latter may be absent in non-typeable strains), however none of these genes are present in *S. pseudopneumoniae*, hence being useful for discrimination between both species.<sup>6,8</sup> Nevertheless, there are some rare *S. pneumoniae* strains that show some molecular peculiarities of the *lytA* gene that confer an atypical bile insoluble phenotype.<sup>9</sup>

In contrast to the *lytA* and the *cpsA* genes absent in *S. pseudopneumoniae*, some isolates can carry the *ply* gene.<sup>6,10</sup>

Our isolate was non-typeable, optochin susceptible when incubated both in CO<sub>2</sub> and in ambient air, and bile soluble. The PCR was positive for *ply*, while negative for *lytA*.

Resistance rates are often higher in *S. pseudopneumoniae*, particularly for penicillin (60.7%) and erythromycin (42.6%),<sup>6</sup> therefore the correct identification could affect empiric treatment.

In our patient, who presented with an invasive disease (bacteremia), the urinary tract seemed the most likely focus, although urine culture was sterile. Nevertheless, routine culture media used for urine are not suitable for *Streptococcus* spp. and culture in enriched media was not performed. To our knowledge, no cases of urinary tract infection caused by *S. pseudopneumoniae* have been described previously.

In conclusion, it is important to note that relying solely on phenotypic characteristics for the identification of *S. pseudopneumoniae*