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(CASE REPORT)

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Pseudomonas aeruginosa producing beta metalloenzyme (VIM, NDM): A case report

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Abstract

Pseudomonas aeruginosa is an opportunistic bacterium with a high rate of acquisition of new resistance. The production of metallo-ß-enzyme is a resistance mechanism that is still relevant today. We report the case of a diabetic patient presenting with a trauma to the lower limb; the patient underwent immediate surgery, a discharge of pus was marked in the evolution; the bacteriological profile was in favour of a strain of *Pseudomonas aeruginosa* producing type VIM metallo carbapenemase, with a positive EDTA synergy test and a positive carba test. Despite repeat surgery, the introduction of antibiotic therapy, treatment and control of the glycaemic cycle, the evolution was unfavourable, threatening the vitality of the limb.

Keywords: *Pseudomonas aeruginosa*; Multi-resistant bacteria; Beta metalloenzyme; EDTA synergy test; Rapid carbapenemase detection test

1. Introduction

Multi-resistant *Pseudomonas aeruginosa* is one of the multi-resistant bacteria (MRB) with an acquired mechanism resistance. MRB can be defined as bacteria resistant to numerous antibiotics or, more scientifically, as bacteria resistant to at least three major classes of antibiotics. The appearance of new terms such as pan- or Toto-resistant bacteria or bacteria with very broad resistance made these definitions even more difficult. [1] The production of acquired metallo-β-lactamases (MBLs) is a mechanism that confers on *Pseudomonas aeruginosa* the ability to hydrolyse a wide range of β-lactam antibiotics, including carbapenems, which play an essential role in the management of serious hospital infections and often used as antibiotics of last resort. The emergence of Gram-negative pathogens is making resistance to carbapenems a worrying development. The paucity of antibacterial drugs available to target these pathogens compounded this concern [2, 3].

The aim of this case report is to provide an update on the resistance mechanism of a *Pseudomonas aeruginosa* producing a beta metalloenzyme, and the importance of environmental sampling in controlling the risk of infection and reducing the emergence of new antibiotic resistance mechanisms.

2. Case report

We report the case of a 58-year-old patient with type 2 diabetes who had been on oral antidiabetic drugs for 6 years and who was poorly monitored. He admitted to the trauma department of ibn Tofail Hospital following a road traffic accident that caused trauma to his left lower limb (a floating knee fracture associated with an open leg fracture and a sub trochanteric femur fracture). A 2nd generation Hoffman fixator inserted as an emergency.

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The post-operative course marked by sepsis of the material, with pus coming from the surgical scar.

The patient treated with a combination of antibiotics (ciprofloxacin, ceftazidime, metronidazole and vancomycin), analgesics, anticoagulants, blood sugar control and daily care.

The evolution marked by a loss of skin substance on the posterior aspect of the leg, with exposure of the bone (Figure 1).

Pus samples (deep swabs) taken and sent to the microbiology laboratory.

Direct examination revealed a very strong cellular reaction consisting of altered neutrophils with the presence of Gram-negative bacilli.

Cultures were positive, polymorphic and very abundant (Figure 2 (A)).

Three types of colony were isolated and studied; three antibiograms were performed and a biochemical study was carried out using an oxidase test and the API 20 E AND 20NE gallery.

Interpretative reading of the antibiograms and identification by biochemical methods led to the isolation of the following bacterial strains:

- *Proteus mirabilis*, whose resistance mechanism is a low-level cephalosporinase,
- *Klepsiella pneumoniae*, whose resistance mechanism is an extended-spectrum beta-lactamase,
- *Pseudomonas aeruginosa*, which produces a class B carbapenemase (figure 2(B-C)), which immediately showed resistance to all beta lactam antibiotics, including carbapenems, with the exception of aztreonam; the presence of a beta metalloenzyme was detected using the EDTA synergy test, which showed inhibited growth of the imipenem disc in EDTA.

A qualitative immunochromatographic test used to detect two types of carbapenemase, the VIM variant and NDM (figure 2(D)).

The antbioresistance of this strain affected all antibiotic families with the exception of colistin.

Figure 1 Loss of substance with exposed bone

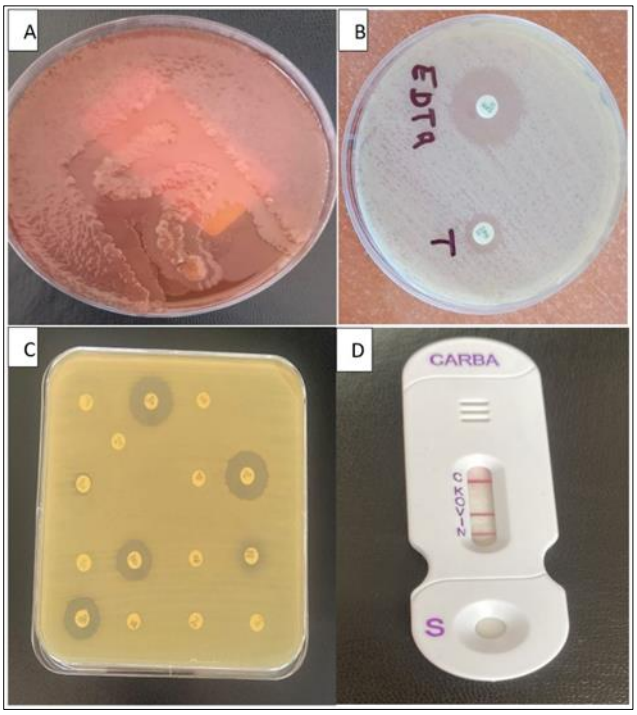

Figure 2 A/ culture on Mac Conkey positive polymorphic and very abundant B/EDTA synergy test with inhibition of imipenem disc C/Antibiogram of a *Pseudomonas aeruginosa* strain producing a metallo Beta enzyme D/rapid carbapenemase detection test positive with 2 types of variants VIM and NDM

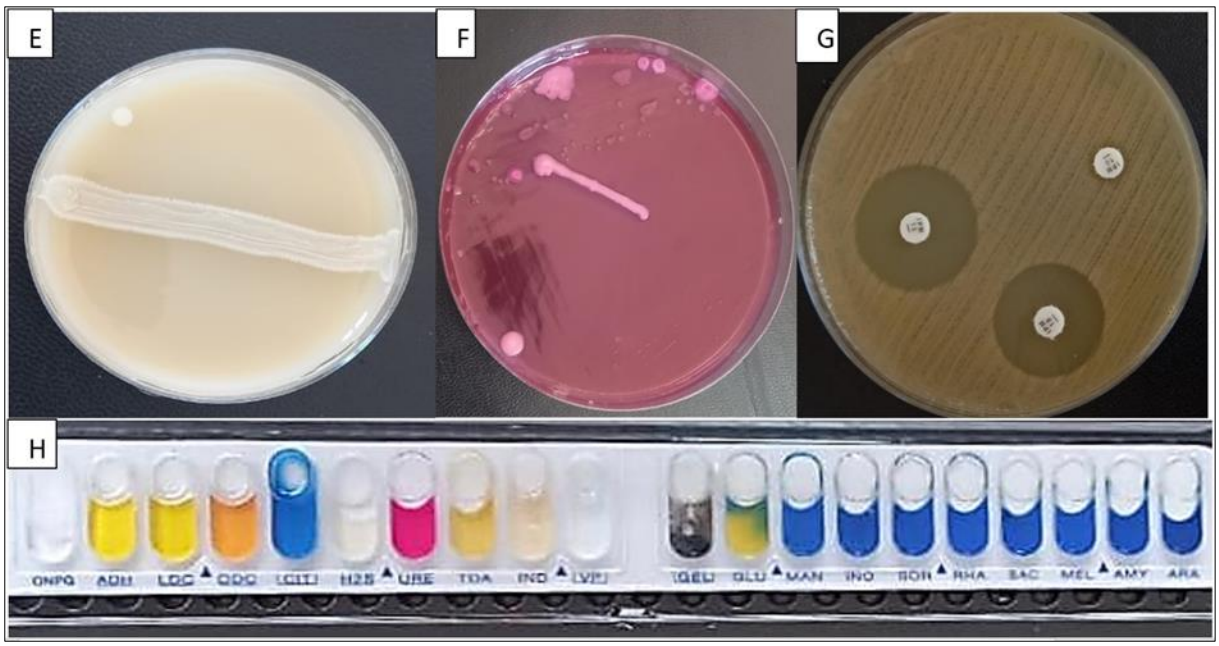

Figure 3 E/Negative DNase test F/Positive polymorphic culture from the patient's telephone G/EDTA synergy test for Pseudomonas on the telephone H/API gallery for identification of *Pseudomonas aeruginosa* on the floor

A control pus sample requested and environmental samples sent; seven sites studied bed rails, bedside table, gurney, treatment trolley, floor, door handle and patient's telephone. Three sites showed positive results.

- Gram-positive coccis clusters were isolated from the bedside table, with a negative DNase test (Figure 3(E)) identifying an opportunistic white *Staphylococcus*.
- A *Pseudomonas* was isolated from the floor, with a negative EDTA synergy test (Figure 3(H)).

- From the patient's telephone: three colonies were isolated and identified using the same methods described above (figure 3(F)): a *Klebsiella oxytoca* with a low-level penicillinase resistance mechanism, an *Enterobacter cloacae* with a low-level cephalosporinase resistance mechanism, and a *Pseudomonas aeruginosa* producing a metallo-beta enzyme (figure 3(G)).

The control pus sample showed: a *Klebsiella* made sensitive after probabilistic antibiotic treatment; in addition, the *Proteus mirabilis* isolated in the first sample still showed the same resistance profile and, at the end, the resistance of the multi-resistant strain of *Pseudomonas aeruginosa* producing metallo-beta enzyme.

After studying and comparing the different bacteriological profiles detected in the pus and environmental samples, for this patient we could prescribe only two antibiotics: fosfomycin and aztreonam, taking into account the antibiotic resistance of each bacterial strain isolated, in association with hospital hygiene measures and the patient's technical and geographical isolation.

The course was unfavourable, with persistence of the infection, the discharge of pus from the wound and the onset of necrosis of the leg. A repeat operation was required to assess the vitality of the limb.

3. Discussion

Pseudomonas aeruginosa remains a significant pathogen in hospitals today [4].

In our case, infection with *Pseudomonas aeruginosa* producing Ambler class B carbapenemase or MBL, a very common enzyme in this pathogen which is highly contagious and acquires multiple antibiotic resistance [2].

Mainly certain environmental bacteria produce Metallo-β-enzymes (MßL). These enzymes contain a zinc ion at their active site, which is essential for their activity, hence the inhibitory effect of divalent cation chelating agents such as EDTA [5].

The EDTA synergy test is the only phenotypic test for diagnosing MBL. It consists of looking for a synergy image between a carbapenem disc and a disc containing EDTA, which we can add it directly to the carbapenem disc. The test is positive when we observe an increase in diameter greater than or equal to 5 mm between the zones of inhibition around the carbapenem disc and the combined disc [2].

Over the last decade, wide varieties of carbapenemase reported worldwide. However, the most common belong to class B metallo-β-lactamases (NDM, VIM, IMP), Ambler class A carbapenemases (KPC) and class D oxacillinases (OXA-48 and OXA-48-like) [6].

The carba test used in our study is a qualitative immunochromatographic multiplex rapid diagnostic test for the detection and differentiation of the five main carbapenemase families (KPC; OXA 48; VIM; IPM; NDM).

Pseudomonas aeruginosa isolates reported to contain a wide variety of carbapenemases worldwide. For example, in Latin America these include KPC, GES, IMP, VIM, NDM and SPM [7].

The spread of metallo-β-enzymes represents a major challenge both for the few therapeutic options available to patients and for control policies of hospital infection, highlighting the lack of preparedness of healthcare structures in the face of this emergency.

The main risk factors for acquiring resistance in *Pseudomonas aeruginosa* linked to the host, the way the patient is treated, or the hospital environment. Screening samples are therefore essential for assessing the emergence of this bacterium in hospitals [8].

This work shows that mobile phones play a role in the transmission of nosocomial and community-acquired infections. It would be difficult to ban the use of mobile phones, but we could easily prevent the spread of bacterial infections by making phone users aware of the importance of hand washing and the use of hydro alcoholic solutions [9].

Hospital hygiene training remains essential, necessary and urgent for the prevention of nosocomial infections. We must design it as a coherent model capable of dealing with the clinical, bacteriological and epidemiological aspects of these infections, and for evaluating the effectiveness of continuing training programmes on the rates of nosocomial infections.

The role of the bacteriology laboratory is central to the prevention and control of nosocomial infections, detecting multi-resistant bacteria, studying their profile and participating, in collaboration with the clinician, in the choice of treatment in order to limit the uncontrolled use of antibiotics, which increase the emergence of highly pathogenic strains or even acquire new resistance. With the introduction of nosocomiovigilance measures, monitoring of antibiotic resistance and environmental surveillance can be effective [10].

4. Conclusion

This clinical case shows the presence of a multi-resistant strain of *Pseudomonas aeruginosa* producing a beta metallo enzyme resistant to all possible treatments, as well as the lack of marketing in Morocco of certain antibiotics such as aztreonam, complicating the task for the clinician.

These multi-resistant bacteria present a major epidemiological risk. Efforts must therefore be stepped up to halt their emergence, starting with strict control of preventive measures and maintenance of the hospital environment, combating the random and uncontrolled use of antibiotics and screening and treating patients with risk factors for developing hospital- acquired infections in order to limit this scourge.

Compliance with ethical standards

Disclosure of conflict of interest

No disclosure of conflict of interest

Statement of informed consent

Informed consent obtained from all individual participants and included in the study by signing the Free and Informed Consent Form

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