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Fatal multidrug-resistant *Acinetobacter baumannii* pneumonia in Maputo, Mozambique: a case report.

Acinetobacter baumannii is one of the six most important multidrug-resistant (MDR) microorganisms isolated in hospitalised patients worldwide, having an extraordinary capacity to spread to different areas[1].In the last three decades *A. baumannii* has acquired resistance to antibiotics including carbapenems and even polymyxins, representing a challenge for achieving effective antibacterial treatment[1,2]. In the global priority list of antibiotic-resistant bacteria of the World Health Organization, *A. baumannii* is considered the most critical pathogen[3]. Knowledge of the epidemiology and antibacterial susceptibility profile of *A. baumannii* is still incomplete in many parts of the world including Africa. Here, we report a fatal pulmonary infection by MDR *A. baumannii* in Maputo, Mozambique.

In 2014, a woman in her 20's, with HIV infection on antiretroviral treatment for the preceding 12 months, was admitted to the Maputo Central Hospital with cough, dyspnoea and seizures of acute presentation. Physical examination revealed: a Glasgow score of 15/15, temperature 38.2°C, blood pressure 180/120mmHg, heart rate 100 bpm, and respiratory rate 24 rpm. Thick and thin smear tests for malaria were negative. Laboratory analyses during hospitalization showed anaemia (haematocrit 24.9% and haemoglobin 8.3g/dL), leukopenia (white blood cell count 2.9 x 10^9/L), elevated transaminases (AST 157 IU and ALT 726IU), and kidney failure (maximum creatinine and urea levels

were 363 µM/L and 29.4 µM/L respectively); the estimated glomerular filtration (Cockcroft-Gault Equation) was 18.8 mL/min. Chest X-ray was performed and only a cardiomegaly was reported. The nadir CD4 count was 192cells/mL. Sputum Gram stain and blood culture were not performed. The patient received penicillin, cotrimoxazole, and oxygen but died on day 14 of hospitalisation. Pre-mortem clinical diagnoses were: HIV/AIDS, Kaposi's sarcoma, dilated cardiomyopathy, kidney failure, and pulmonary hypertension. The patient was not intubated or in mechanical ventilation.

The case was included in the CaDMIA project, a validation study of a minimally invasive autopsy (MIA) protocol against the complete diagnostic autopsy (CDA)[4,5]. A universal screening for several key pathogens was conducted, and microbiological analysis were performed according to the histopathological findings[4,5]. The autopsy revealed a severe pyogenic pneumonia (Figure1). Serum samples tested positive for antibodies against HIV with a viral load of 182 copies/mL. Lung samples resulted negative for tuberculosis, *Cryptococcus*, *Toxoplasma gondii*, *Pneumocystis jirovecii* and respiratory viruses by PCR testing. *A. baumannii* was isolated from brain, lung and liver samples. Gram negative bacilli were visible in the Gram stain of histological lung samples (Fig1), brain, and liver. *A*. *baumannii* was also identified by 16S rRNA PCR in plasma, brain, lung, liver and cerebrospinal fluid samples. The cause of death was assigned to fatal pneumonia caused by a MDR *A. baumannii* infection, following a previously described algorithm [6]. Clinical diagnosis of Kaposi's sarcoma and dilated cardiomyopathy were not confirmed at autopsy.

Antibiotic susceptibility tests were performed and interpreted according to the EUCAST guidelines (version 7.0,2017; http://www.eucast.org) which consider *A. baumannii* intrinsically resistant to penicillins and cephalosporins. In addition, the strain was resistant to the following antibiotics: ciprofloxacin, levofloxacin, trimethoprim-sulphamethoxazole, and gentamicin; showing intermediate resistance to meropenem (4 µg/ml) and susceptibility to amikacin, tobramycin, imipenem and colistin. The MIC of tigecycline was 1µg/ml. Multi-Locus Sequence Typing following the Pasteur scheme (https://pubmlst.org/abaumannii/) identified all the *A. baumannii* isolates as belonging to international clone II and sequence type 2(ST2).

Few data are available in the literature regarding *A. baumannii* in Africa. A recent report analysed 65 strains from 5 different countries and found a high prevalence of MDR strains[7]. International clone II/ST2 isolates belong to one of the major clonal lineages associated with the spread of MDR *A. baumannii* worldwide, but in Africa they have only been reported in Algeria and Kenya[8,9]. Two recent studies[10,11] (one of them conducted at the Maputo Central Hospital) reported non-MDR *A. baumannii* in Mozambique, whereas, to our knowledge, this is the first report of a MDR *A. baumannii* strain in this country. The patient had several known risk factors for acquiring *A. baumannii* infection such as severe immunosuppression and having been hospitalised for two weeks. However, the final cause of death was only identified after a complete diagnostic autopsy and in depth microbiological studies were carried out. Diagnostic autopsies are rarely performed in sub-Saharan Africa due to, among others, the lack of resources and trained pathologists. We show that a

standardised minimally invasive sampling procedure can provide accurate identification of a pathogen causing death. This method may improve the capacity of the current surveillance methods to detect bacterial infections and associated antimicrobial resistance. Our report highlights the utility of post-mortem investigations for accurate determination of cause of death and the need for microbiological surveillance to tackle the growing problem of nosocomial MDR infections in low-income countries.

References

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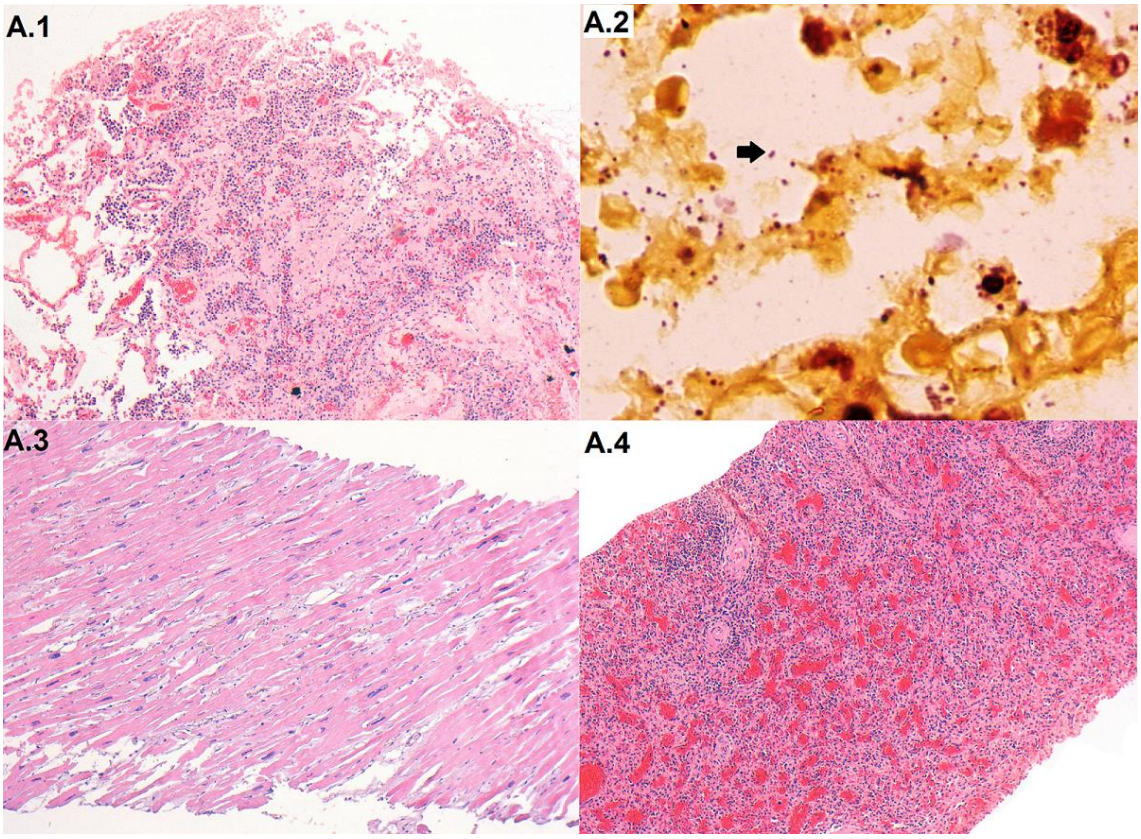

Figure1. Fatal MDR *A. baumannii* infection: microbiological and pathological results of post-mortem samples.

Histological images of relevant findings. (A.1) Lung with pyogenic pneumonia (hematoxylin and eosin, 100×); (A.2) Gram negative bacteria (*A. baumannii*) pneumonia (arrow) infecting the lung (gram stain, 1000×); (A.3) Heart with hypertrophy (hematoxylin and eosin, 100×) (A.4) Spleen with lymphocytic depletion and congestion (hematoxylin and eosin, 100×)