

Case Report

Bacteremia in a human caused by an XDR strain of *Pseudomonas fulva*

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Abstract

This is the first report from Pakistan of a case of bacteremia in a human due to *P. fulva*, an opportunistic infection with increased risk of a drug resistant phenotype. *P. fulva* was isolated from blood of a 45 years male admitted in surgical ICU. Isolate was identified by the MALDI-TOF-MS and was extensively drug resistant (XDR) strain. Isolate was found negative for metallo β lactamase (MBL) and extended spectrum β lactamase (ESBL) types by phenotypic and polymerase chain reaction (PCR) assays. It was concluded that *P. fulva* is an emerging opportunistic pathogen.

Key words: Bacteremia; *Pseudomonas fulva*; XDR.

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Introduction

Pseudomonas fulva is an opportunistic pathogen of the Pseudomonad group that rarely causes infection in man. It has mostly been reported from aquatic environments including rice seed, petroleum fields, oil brine and gills of the molluscs [1]. To date, four cases of infections with *P. fulva* have been reported in humans together with an outbreak of 19 patients in China over 3 years in which the source of the bacteremia was identified as contaminated (insulin, isosorbide dinitrate, and potassium magnesium aspartate with 5% glucose) solutions [2]. The case reports regarded isolation of bacterium from sputum of a patient with cystic fibrosis [3], from cerebral drainage of a case of meningitis [1], from the blood of a case of bacteremia [4] and from exudate from a soft tissue wound [5]. Infections with *P. fulva* are characterized by poor clinical outcomes: out of five cases including this case, only 2 patients survived.

Case Report

A 45 years male was admitted to the surgical Intensive Care Unit (ICU) of a tertiary care hospital, in Karachi, Pakistan following a road accident. The case was referred from a public hospital with post-surgical complications, history of head injury and fractured ribs and laceration wounds at various sites of the body together with fever and the need for mechanical ventilation. Total leucocyte count (TLC) was 16.5×10^9

cells/L and septicemia was suspected. Blood was collected for microbiological culture (on the 18th day after surgery) and empirical treatment with Piperacillin/tazobactam and Vancomycin was initiated. The patient's condition did not improve. Blood culture was processed manually and was positive for Gram-negative bacilli on direct Gram-staining from the incubated blood culture bottle after 24 h of incubation showing slight turbidity. Sub-culturing on MacConkey and Blood agar plates revealed the growth of non-hemolytic colonies on blood agar, with lactose negative colonies on MacConkey agar. Using a routine manual method and API 20NF kit (Biomérieux, Marcy l'Etoile France) the growth was identified as *Pseudomonas putida*. Before the complete identification, the patient died. However, the identification characteristics in this case, i.e. motile cells, oxidase positive (weak \pm) colonies and water-soluble yellow pigments were not observed. Variable results for oxidase have previously been reported from negative, weak positive-to-positive for *P. putida* [4]. To confirm the identity, the isolate was submitted in duplicate to MALDI-TOF-MS (Microflex, Bruker Daltonics, Bremen, Germany) at the UCL Centre for Clinical Microbiology and identified as *P. fulva*. Antimicrobial susceptibility to the most common antipseudomonal drugs was determined by the disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI) recommendations. The breaking points for Minimum Inhibitory

Concentrations (MICs) were used as described by Almuzara *et al.* and CLSI, [1,6]. The *P. fulva* isolate was resistant to piperacillin, piperacillin/tazobactam, ceftazidime, aztreonam, cefepime, carbapenems (meropenem and imipenem) amikacin, gentamicin, tobramycin and ciprofloxacin with the zones of inhibition of 7 mm, and was found sensitive to the Colistin by MIC method. The results of disc diffusion were confirmed by determination of MICs using Etest strip (BioMérieux, Lyon, France and M.I.C. Evaluator, Oxoid, Basingstoke, UK). The data (Table 1) revealed the strain of *P. fulva* as extensively drug resistant (XDR). Modified Hodge test (MHT) and Rapidec Carba NP (Biomerieux, Marcy-l'Etoile, France) were used to identify carbapenemases and results were found negative. Isolate was subjected to Ethylenediaminetetraacetic acid (EDTA) double disc synergy test with Impipenem and Meropenem and Rosco kit test for *Klebsiella pneumoniae* carbapenemase (KPC) / metallo β lactamase (MBL) for *P. aeruginosa* and *Acinetobacter* species (ROSCO's Diagnostic, Tassstrup, Denmark). It appeared positive for MBL using these phenotypic methods, however, production of MBLs could not be confirmed by the genotypic assay using primers VIM-like F-GGTGTTTGGTTCGCATATCGC, R-CCATTACGCCAGATCGGCATC, IMP-like F-GGAATAGAGTGGCTTAATTC R-CAACCAGTTTTGCCTTACC, and NDM-like F-CACCTCATGTTTGAATTCGCC R-CTCTGTCCACATCGAAATCGC. Polymerase chain reaction (PCR) conditions were maintained as previously described [7-9]. The isolate was also found negative for extended spectrum β lactamase (ESBLs), PER and VEB types) by PCR method [10,11]. This suggests that the resistance mechanism was other than these β -lactamases.

Discussion

The members of *Pseudomonas putida* group are ubiquitously present in variety of environmental niches [12]. They are rarely involved in infections in human but few reports of bacteremia and infections of soft tissues have been emerged [13]. Studies state higher rate (46%) of VIM positive strains of this group from human specimens [1]. Furthermore, these species have been described as reservoirs for the dissemination of the antibiotic resistance genes to the more pathogenic species such as *P. aeruginosa* [13-15]. *P. fulva* is the less characterized member of this group which has scarcely been demonstrated as human pathogen. It is mainly due to its misidentification as *Pseudomonas putida* (99%) by the Vitek 2 system [1,2] resulting in its under reporting. In all the cases, *P. fulva* was identified either by 16S rRNA typing or MALDI-TOF-MS.

The MALDI-TOF-MS has been validated by different researchers as an alternate assay for the accurate and reliable tool to distinguish *P. fulva*, from *P. putida* group (*P. putida*, *P. fulva*, *P. monteilii*, and *P. mosselii*) in comparison to sequencing 16s rRNA, gyrB, or rpoD genes. It identifies *P. fulva* with 100% sensitivity and specificity among *P. putida* group [16-18]. Hence, cautionary measures should be taken to identify an isolate when unusual biochemical results of *Pseudomonas* spp. are observed. Correct identification of the isolates from samples may increase the number of cases caused by this species.

History of the patient revealed that source of *P. fulva* may be traced back to hospital environment or contaminated intravenous solutions as described earlier [2]. Susceptibility pattern of our strain was slightly different to the previously isolated stains [1,2,19]. Almuzara *et al.* [1] reported susceptibility of *P. fulva* to Cefepime, Amikacin, Ciprofloxacin, and Colistin. However, in this case, the isolate was susceptible to Colistin only. The presence of MBL was also detected

Table 1. Antibiotic susceptibility pattern (MIC) of *P. fulva*.

Antibiotic	MICs (μ L/mL)	Interpretation
Piperacillin	128	Resistant
Piperacillin-tazobactam	128/4	Resistant
Ceftazidime	> 32	Resistant
Cefepime	> 32	Resistant
Aztreonam	> 32	Resistant
Imipenem	> 32	Resistant
Meropenem	> 32	Resistant
Tobramycin	32	Resistant
Gentamicin	32	Resistant
Amikacin	128	Resistant
Ciprofloxacin	8	Resistant
Colistin	0.5	Resistant

by Rebolledo *et al.* [19] using phenotypic tests, EDTA double disc synergy test and ROSCO test, however, genotypic results did not affirm this finding in our case. Contrarily, the presence of *bla*_{VIM}, MBL type was reported in previously isolated *P. fulva* strains [1,19].

Conclusion

The increasing isolation of *P. fulva* foreshadows the increased risk of this organism causing severe infections in critically ill patients. Due to capability of acquiring resistance determinants such as carbapenemase (VIM), it appears as an emerging threat to public health care facilities.

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