View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by UCL Discovery

JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Case Report

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

Fakhur Uddin[1], Kerry Roulston[2], Timothy D McHugh[2], Taseer Ahmed Khan[1], Muhammad Sohail[1]

[1] *University of Karachi, Karachi, Pakistan*
[2] *UCL Centre for Clinical Microbiology, University College London, London, United Kingdom*

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.

J Infect Dev Ctries 2018; 12(7):597-599. doi:10.3855/jidc.10326

(Received 08 March 2018 – Accepted 03 April 2018)

Copyright © 2018 Uddin *et al*. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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 of source 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 (Biomerieux, Marcy I'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 ±) 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-GGTGTTTGGTCGCATATCGC, R-CCATTCAGCCAGATCGGCATC, IMP-like F-GGAATAGAGTGGCTTAATTC R-CAACCAGTTTTGCCTTACC, and NDM-like F-CACCTCATGTTTGAATTCGCC R-CTCTGTCACATCGAAATCGC. 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 sequencing16s 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 isolatesfrom 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 (µL/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 facilitates.

Acknowledgements

Financial assistance to FU by the Higher Education Commission of Pakistan is greatly appreciated. We are thankful to the researchers & staff of Centre for Clinical Microbiology, University College London, London UK, for their kind support and collaboration.

References

1. Almuzara MN. Vazquez M, Tanaka N, Turco M, Ramirez MS, Lopez EL, Pasteran F, Rapoport M, Procopio A, Vay CA (2010) First case of human infection due to *Pseudomonas fulva*, an environmental bacterium isolated from cerebrospinal fluid. J Clin Microbiol 48: 660–664
2. Liu Y, Liu K, Yu X, Li B, Cao B (2014) Identification and control of a *Pseudomonas* spp (*P. fulva* and *P. putida*) bloodstream infection outbreak in a teaching hospital in Beijing, China. Int J Infect Dis 23: 105–108
3. Warwick S, Duke B, Soleimanian S, Wareham D (2008) A diagnostic algorithm for accurate identification of non-fermentative Gram-negative rods and epidemic strains of *Pseudomonas aeruginosa* from cystic fibrosis patients. Clin Microbiol Infect 14 Suppl 7: 578-579
4. Seok Y, Shin H, Lee Y, Cho I, Na S, Yong D, Jeong SH, Lee K (2010) First report of bloodstream infection caused by *Pseudomonas fulva*. J Clin Microbiol 48: 2656–2674.
5. Cobo F, Jiménez G, Rodríguez-Granger J, and Sampedro A (2016) Posttraumatic skin and soft-tissue infection due to *Pseudomonas fulva*. Case Rep Infect Dis DOI: 10.1155/2016/8716068
6. Clinical and Laboratory Standard Institute (CLSI) (2015) Performance standards for antimicrobial susceptibility testing, 25th informational supplement CLSI document M100-S25 (ISBN 1-56238-989-0)
7. Wolter DJ, Khalaf N, Robledo IE, Vázquez GJ, Santé MI, Aquino EE, Goering RV, Hanson ND (2009) Surveillance of carbapenem-resistant *Pseudomonas aeruginosa* isolates from Puerto Rican medical center hospitals: dissemination of KPC and IMP-18 ß-lactamases. Antimicrob Agents Chemother 53: 1660–1664
8. Kaase M, Nordmann P, Wichelhaus TA, Gatermann SG, Bonnin RA, Poirel L (2011) NDM-2 carbapenemase in *Acinetobacter baumannii* from Egypt J Antimicrob Chemother 66 :1260–1262
9. Dallenne C, Da Costa A, Decré D, Favier C, Arlet G (2010) Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. J Antimicrob Chemother 65: 490–495
10. Chen TL, Lee YT, Kuo SC, Hsueh PR, Chang FY, Siu LK, Ko WC, Fung CP. (2010) Emergence and distribution of plasmids bearing the blaOXA-51-like gene with an 346 upstream ISAba1 in carbapenem-resistant *Acinetobacter baumannii* isolates in Taiwan. Antimicrob Agents Chemother 54: 4575-4581
11. Jiang X, Ni, Y, Jiang Y, Yuan F, Han L, Li M, Liu H, Yang L, Lu Y (2005) Outbreak of infection caused by *Enterobacter cloacae* producing the novel VEB-3 ß-lactamase in China. J Clin Microbiol 43: 826–831.
12. Wu X, Monchy S, Taghavi S, Zhu W, Ramos J, van der Lelie D (2011) Comparative genomics and functional analysis of niche-specific adaptation in *Pseudomonas putida*. FEMS Microbiol Rev 35: 299-323.
13. Peter S, Oberhettinger P, Schuele L, Dinkelacker A, Vogel W, Dörfel D (2017) Genomic characterisation of clinical and environmental *Pseudomonas putida* group strains and determination of their role in the transfer of antimicrobial resistance genes to *Pseudomonas aeruginosa.* BMC Genomics 18: 859.
14. Gilarranz R, Juan C, Castillo-Vera J, Chamizo FJ, Artiles F, Álamo I, Oliver A (2013) First detection in Europe of the metallo-β-lactamase IMP-15 in clinical strains of *Pseudomonas putida* and *Pseudomonas aeruginosa*. Clin Microbiol Infect 19: 424-427.
15. Juan C, Zamorano L, Mena A, Albertí S, Pérez JL, Oliver A (2010) Metallo-beta-lactamase-producing *Pseudomonas putida* as a reservoir of multidrug resistance elements that can be transferred to successful *Pseudomonas aeruginosa* clones. J Antimicrob Chemother 65: 474-478.
16. Sivolodskiĭ EP, Zueva EB, Kunilova ES, Bogumil'chik EA, Domakova TV (2015) The identification of clinical strains *Pseudomonas fulva* using techniques of MALDI-TOF mass spectrometry and common analysis. Klin Lab Diagn 60: 46-49.
17. Sivolodsky EP, Gorelova GV, Bogoslovskaya SP, Zueva EV (2014) Antibiotic susceptibility and identification of clinical *Pseudomonas fulva* isolates. Antibiot Khimioter 59: 33-37
18. Almuzara M, Barberis C, Traglia G, Famiglietti A, Ramirez MS, Vay C (2015) Evaluation of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry for species identification of nonfermenting Gram-negative bacilli. J Microbiol Methods 112: 24-27
19. Rebolledo PA, Vu CCL, Carlson RD, Kraft CS, Anderson EJ, Burd EM (2014) Polymicrobial ventriculitis involving *Pseudomonas fulva*. J Clin Microbiol 52: 2239-2241

Corresponding author

Muhammad Sohail, Ph D.,
Department of Microbiology,
University of Karachi, Main University Road, Karachi-75270, Pakistan.
Phone: (9221) 99261300
Fax: (9221) 99261330
Email msohail@uok.edu.pk

Conflict of interests: No conflict of interests is declared.