

Case Study

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Urosepsis due to Multi Drug Resistant *Myroides odoratimimus*: A Case Report

Manish Ranjan¹, Santosh Karade^{1,2*}, Praveen Rahi³, S.P. Singh¹ and Sourav Sen¹

¹Department of Microbiology, Armed Forces Medical College, Pune - 411040, India

²Department of Pathology, Command Hospital (SC), Pune - 411040, India

³National Centre for Microbial Resource, National Centre for Cell Science, Pune - 411021, India

*Corresponding author

ABSTRACT

Keywords

Myroides, Multi drug resistance, Septicaemia, Urinary tract infection.

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Members of the genus *Myroides* namely, *M. odoratus* and *M. odoratimimus* are ubiquitous in nature. Over past decade, these Gram negative bacteria are increasingly being implicated in various life threatening infections in health care setting. Here we report a case of urosepsis due to multi-drug resistant *M. odoratimimus* in a 41 year old male with anaplastic astrocytoma. The case highlights the importance of surveillance of multidrug resistant organisms in intensive care units of hospital.

Case Report

A 41 year old male; a known case of frontal astrocytoma was brought to this centre with history of headache, altered sensorium and an episode of seizure. He was diagnosed as a case of anaplastic astrocytoma six months back and underwent surgical resection followed by chemotherapy and radiotherapy. Clinical evaluation revealed bedridden but responsive patient with Glasgow coma scale of 6/15. Radiological investigations confirmed recurrence of lesion in right frontal lobe. He was admitted to the intensive care unit (ICU) and underwent emergent surgical resection of the tumor.

The patient had a stormy post-operative course, requiring ventilatory as well as

ionotropic support. Histopathological examination of the resected tumor specimen confirmed a grade III anaplastic astrocytoma. On third post-operative day, he developed continuous, moderate to high grade fever with features of hemodynamic instability. Total leucocytes count increased from 11500/cumm on first post-operative day to 16,200/cumm. Peripheral blood smear examination indicated neutrophilia with left shift and toxic granulation. Urine examination showed presence of traces of albumin, reducing substances and numerous pus cells. Blood, urine and surgical drain samples were sent to microbiology laboratory for culture and antibiotic sensitivity testing.

Urine culture on CLED agar showed non-lactose fermenting, 2-3 mm dome shaped pale yellow colonies with smooth edge and entire margins (Figure 1a). Gram stained smears from these colonies revealed Gram negative bacilli. The organisms were non-motile and negative for catalase enzyme activity. It was positive for cytochrome oxidase activity. The colonies failed to grow on MacConkey agar, when incubated at 42 °C. This non-fermenter isolate was identified as *Myroides* sp. by Vitek 2 compact SL (BioMerieux), with good identification score of 94%.

Further identification of the isolate was done using MALDI Biotyper 3.1 (Bruker Daltonics), equipped with autoflex speed and Biotyper database (5597 strains). Mass spectrum was acquired in a linear positive ion extraction mode at a laser frequency of 200 Hz within a mass range from 2,000 to 20,000 Da (Figure 2). The results of MALDI Biotyper showed the highest (2.47) score value with *Myroides odoratimimus* LMG 12839, confirming species level identification.

Antibiotic sensitivity testing was performed both by modified Kirby Bauer disk diffusion method and Vitek 2 compact SL (BioMerieux) and interpreted as per CLSI 2016 guidelines (CLSI 2016). Except for minocycline, the isolate was resistant to all available antibiotics recommended for the panel of *Pseudomonas* sp. The MIC value of tested antibiotics is shown in table 1. Patient, who was initially managed with inj cefoperazone/sulbactam and amikacin was changed to inj meropenem, inj amikacin and tab minocycline. *M. odoratimimus* was also isolated from blood culture by fifth postoperative day. Isolation of similar colonies from both urine and blood cultures confirmed the diagnosis of urosepsis in this case. Our patient continued to remain febrile, developed uraemia, multi organ dysfunction

and eventually succumbed to his illness on seventh post-operative day.

Furthermore, we explored for the presence of plasmid families responsible for resistance. The plasmid DNA was extracted using HiPur A™ Plasmid DNA Miniprep Purification kit as per the manufacturer's instructions and preserved at - 40 °C. We investigated the presence of major plasmid families circulating in the family *Enterobacteriaceae* as described previously (Carattoli *et al.*, 2005, Carattoli 2009). The primers used for the study were FIA, FIB, FIC, HI1, HI2, I1-Iy, L/M, N, P, W, T, A/C,K, B/O, X, Y, Frep B, and FIIA. As both intergenic and intragenic transfer of plasmids are known to confer resistance (Pucci *et al.*, 1988), we used 18 sets of primers divided into 5 multiplex PCRs and 3 simplex PCRs to detect plasmid families (Carattoli *et al.*, 2005). Electrophoresis of the amplified products was performed on 1% agarose gel and visualized under UV light.

Of the 18 sets of primers tested, we detected presence of a single Frep B plasmid family of amplicon size 270bp in the given isolate (Fig. 3). Frep B plasmid has been shown to confer resistance to broad range of beta lactam antibiotics including carbapenems (Huang, Yu *et al.*, 2016). Additionally a commercially available rapid test for detection of carbapenemases production, RAPIDEC® CARBA NP (BioMérieux, France) was performed. Development of orange – yellow colour in this test indicated carbapenemases production in the given isolate of *M. odoratimimus* (Poirel and Nordmann 2015).

Results and Discussion

The genus *Myroides* consists of non-motile, oxidase positive, Gram negative bacteria; *M. odoratus* and *M. odoratimimus* (Garrity *et al.*, 2004). *Myroides* was taxonomically separated from genus *Flavobacterium* based on

genotypic and phenotypic data (Vancanneyt *et al.*, 1996). Although *Myroides spp.* are ubiquitous, they are frequently encountered in wet environments, sea water, soil and sewage treatment plants. Over past decade, these Gram negative bacteria are increasingly being implicated in various life threatening infections in health care setting (Elantamilan *et al.*, 2015, Endicott-Yazdani *et al.*, 2015). These infections include skin and soft tissue infections, urinary tract infection, bacteraemia

and sepsis (Benedetti *et al.*, 2011, Beharrysingh 2017). They have now been increasingly reported as a cause of life-threatening infections such as, sepsis, urinary tract infection, endocarditis and soft tissue infections primarily in immuno-compromised individuals (Prateek *et al.*, 2015, Beharrysingh 2017). We reported a case of urosepsis due to *M. odoratimimus* in a 41 year old male.

Table.1 Minimum inhibitory concentration (MIC, µg/ml) of *Myroides odoratimimus* for various antimicrobial agents determined by using the VITEK 2 system

S. No	Antibiotic tested	MIC	Result
1	Piperacillin/Tazobactam	>= 128	R
2	Ceftazidime	>=64	R
3	Cefoperazone/Sulbactam	>=64	R
4	Cefepime	>=64	R
5	Aztreonam	>=64	R
6	Doripenem	>=8	R
7	Imipenem	>=16	R
8	Meropenem	>=16	R
9	Amikacin	>=64	R
10	Gentamicin	>=16	R
11	Ciprofloxacin	>=4	R
12	Levofloxacin	>=8	R
13	Minocycline	<= 1	S
14	Tigecycline	>=8	R
15	Colistin	>=16	R
16	Trimethoprim/Sulfamethoxazole	>=320	R

Abbreviations:
R - Resistant, S - Susceptible

Fig.1a and 1b 1 (a) shows 2-3 mm dome shaped pale yellow colonies of *Myroides odoratimimus* on nutrient agar. 1 (b) RAPIDEC® CARBA NP test showing positive result for carbapenemase production indicated by orange yellow colour change in the well e



Fig.2 Matrix assisted laser desorption and ionisation, time of flight mass spectrometry (MALDI-TOF MS) spectrum of whole-cell protein (2-20KDa) urinary isolate of *Myroides odoratimimus*

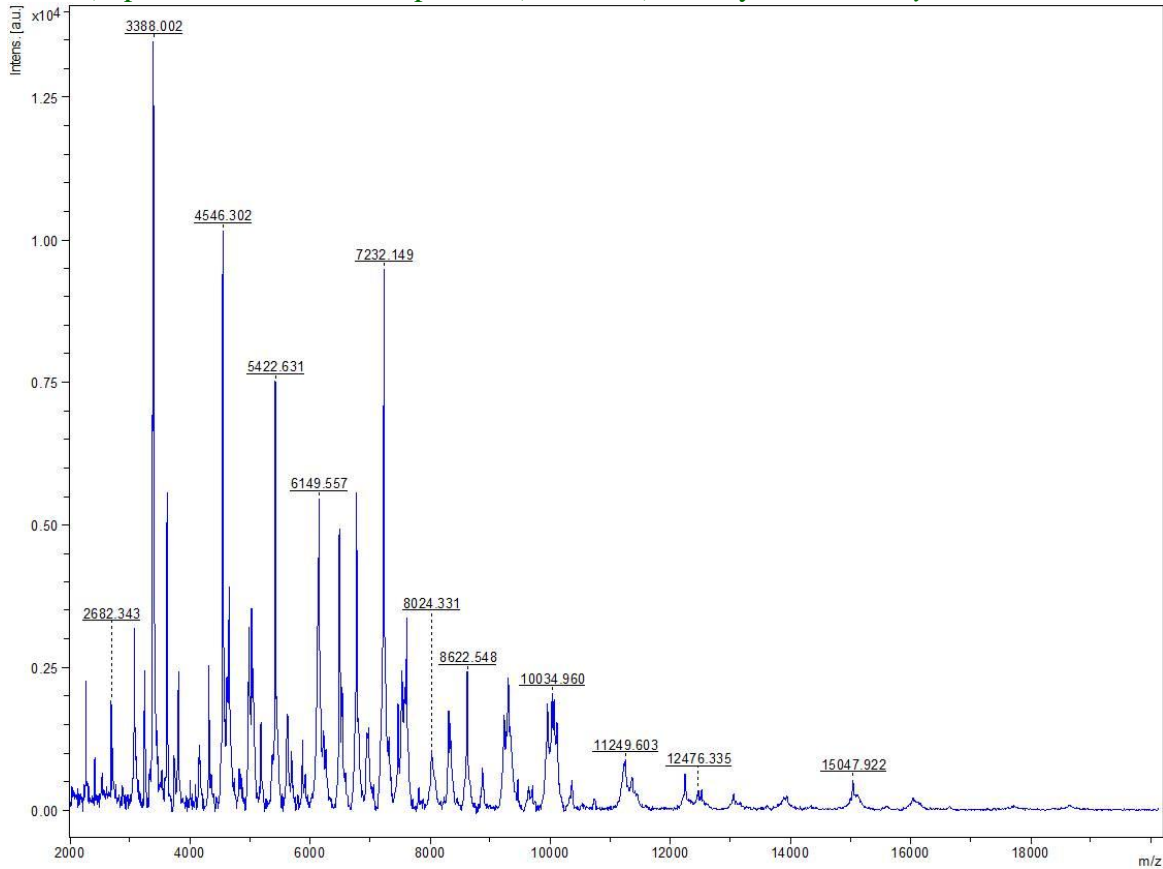
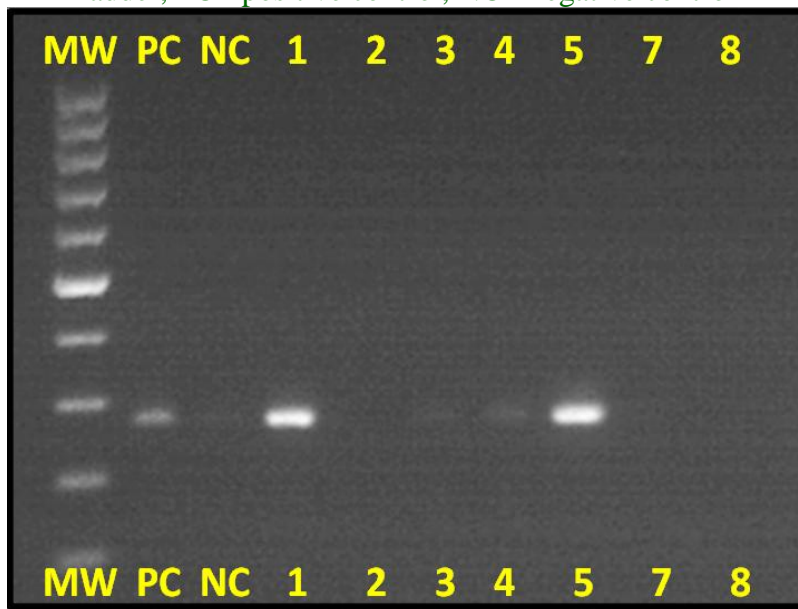


Fig.3 Agarose Gel Electrophoresis of the amplified products of extracted plasmid shows presence of 270 bp FrepB plasmid in lane 1 and 5. Abbreviations: MW - 100bp molecular weight ladder, PC - positive control, NC - negative control



Resistance to multiple antibiotics in *Myroides spp.*, especially intrinsic resistance to beta-lactam antibiotics has been reported previously (Mammeri *et al.*, 2002, Hu *et al.*, 2016). The present isolate was resistant to all available antibiotics except minocycline. Immuno-compromised status due to ongoing cycles of chemotherapy and radiotherapy, recent surgical intervention, repeated hospital admissions with exposure to multiple antibiotics and prolonged urinary catheterization were important risk factors for acquiring multi-drug resistant *Myroides* infection in our patient. Although *Myroides* is commonly found in soil and water, the mode of acquisition in this case could not be ascertained. There was no history of animal bite, injuries or exposure to contaminated water bodies, which is often implicated as source of infection (Maraki, Sarchianaki *et al.*, 2012). *Myroides* infections are rare in Indian settings and presently only few case reports are published till date (Deepa *et al.*, 2014, Elantamilan *et al.*, 2015, Prateek *et al.*, 2015). Recovery of organism from urine and blood with similar antibiotic sensitivity pattern rules out the possibility of environmental contamination. Also, scrutiny of the recent and past fomite-surveillance data did not reveal recovery of *Myroides sp* from ICU. The species level confirmation was done by MALDI-TOF which is an accepted tool for identification (Schrottner *et al.*, 2014, Almuzara *et al.*, 2015). However, in spite of extensive antibiotic therapy, the patient succumbed to septicemia and surgical complication. To conclude, this case highlights the role of *M. odoratimimus* as an emerging multi drug resistant pathogen in health care settings warranting periodic hospital environmental surveillance to ascertain the source of infection. Use of newer diagnostic modalities is assisting microbiologists not only in rapid diagnosis of exotic infection but also in recommending appropriate antibiotic therapy.

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