ORIGINAL ARTICLE CODEN: AAJMBG

Footstep of a new superbug *Myroides spp.*: A study based on a series of cases from a tertiary care hospital of Eastern India

Sayan Pal[1], Prabir Kumar Ghosh[2], Raveendra D. Totad[3], Raja Ray[4] and Hirak Jyoti Raj[4*]

[1]Department of Microbiology, Bankura Sammilani Medical College, PO: Kenduadihi, Dist: Bankura-722102, West Bengal, India, [2]Department of Microbiology, Prafulla Chandra Sen Government Medical College and Hospital, Arambagh, Hooghly-712601, West Bengal, India, [3]Department of Microbiology, Al Ameen Medical College and Hospital, Athani Road, Vijaypur-586108, Karnataka, India and [4]Department of Microbiology, IPGME & R, SSKM Hospital, 244 AJC Bose Road, Kolkata-700020, West Bengal, India

Received: 02nd September 2022; ***Accepted:*** 10th December 2022; ***Published:*** 01st January 2023

Abstract: *Background:* Infections caused by *Myroides spp.* have been found very rarely in recent scientific literature. These Gram-negative bacilli however are increasingly being implicated in various life threatening infections, of late, in some of the critical care settings. *Objective: T*o detect *Myroides spp* infections with strong degree of suspicion and to review the surveillance of multi drug resistant organism spread and containment in critical care settings of hospital. *Methodology:* Suspected isolates were found in the course of routine laboratory activities of blood and urine culture from patients admitted in intensive care unit, from amongst the non - fermenter Gram negative bacteria set as the inclusion criterion, and subjected to identification and antimicrobial susceptibly testing in automated system following standard microbiological methodology by Vitek-2 automated identification and susceptibility testing system. Interpretation of the results was done following the guidelines of Clinical and laboratory standards Institute. History and other relevant investigation results, as required, of the patients were retrieved from hospital record section. *Result:* Out of the 620 non-fermenter gram negative bacilli included in the study, *Myroides spp.* isolates were found five in number from blood and urine samples of the five patients. Tigecycline and minocycline were found to be most effective drug against the organism followed by colistin and imipenem. *Conclusion:* A robust Antimicrobial stewardship policy and implementation tool with efficient infection control measures are backbone to limit emergence and spread of such microbes in unforeseen days.

Keywords: Myroides spp, Uncommon, Pathogen, Drug Resistant.

Introduction

Myroides spp. is a rare pathogen ubiquitously distributed in the environment. Of late, it is being reported from a variety of clinical settings. Clinicians should remain alert of this pathogen as a possible etiologic agent for invasive infection, especially in the settings of immune-compromised state or when there is treatment refractoriness.

Myroides spp are oxidase-positive aerobic, non-motile, gram negative, rod, which typically emit a sweet odour during growth and produce yellowish pigmant. They have been identified as causative agents in Urinary Tract Infection (UTI), Pneumonia, meningitis, fasciitis, ventriculitis in mostly immuno-compromised patients. However, few cases have been reported in immuno-competent hosts also [1-2].

Myroides spp. grow in MacConkey's agar. Colonies are yellow and produce a fruity odor. The organisms are positive for catalase, urease, gelatinase, oxidase and negative for indole production. *Myroides spp.* are frequently found in environmental sources like soil and water. They behave as low grade opportunistic pathogens, affecting immuno-compromised hosts such as those with liver cirrhosis, diabetes mellitus, on long-term corticosteroid treatment.

The genus has been reported as an etiologic agent most commonly in cases of cellulites and necrotizing fasciitis and urinary tract infections. Two nosocomial outbreaks have been reported among urologic patients, all of whom (except one) underwent endo-urologic operations and were hospitalized for a prolonged period. All of these patients also had urinary stones or urinary neoplasms. There have also been (very few) reported from cases of surgical site infection, endocarditis, ventriculitis.

Prosthetic joint infection and necrotizing pancreatitis. It should also be noted that the invasive potential of the species has been demonstrated in several reported cases of bacteremia [3-4]. Antibiotic treatment of *Myroides* infection can be quite difficult. The production of chromosome-encoded metallo-beta-lactamases has also been documented both in *M. odoratus* (TUS-1) and in *M. odoratimimus* (MUS-1). Many strains have thus been recognized as resistant to β-lactams, carbapenems and aztreonam. They exhibit variable susceptibility to aminoglycoside, fluoroquinolone, co-trimoxazole [5-6]. The pathogenicity and resistance mechanisms are not clearly understood at this time [7].

Flavobacterium meningosepticum, the most important species of the genus *Flavobacterium* is now a member of the genus *Elizabethkingia*, whereas *Flavobacterium indologenes* is now in the genus *Chryseobacterium*. *Flavobacterium odoratum*, an uncommon clinical isolate, has been placed in the genus *Myroides* and divided into two species, *M. odoratus and M. odoratimimus* [8-9]. The comparison of biochemical characters of *Myroides spp*. and its congeners has been depicted in Table 1 [38].

Table-1: Comparison of *Myroides spp.* with the Morphological and Biochemical congeners [38]

Biochemical characteristics	***Myroides odoratus & Myroides odoratimimus***	***Sphingobacterium spp.***	***Elizabethkingia spp.***	***Chryseobacterium spp.***
Oxidse	+	+	+	+
Pigment	Yellow	Pale yellow	Pale yellow	
Motility	Nonmotile	Non motile	Non motile	Non motile
Growth on Mac Conkey Agar	+	+_	+_	V
OF Glucose	_	+	+	+
OF Mannitol	_	+	+	_
Indole	_	_	+	+
Nitrate reduction	_	_	-	V
Gelatinase	+	_	+	+
Esculin	_	+	+	+
ONPG	NA	+	+	+
DNAase	+	+	+	V
Urea hydrolysis	+	+	V	+
Penicillin	'S' in 19% of isolates	R	R	R
Polymyxin	R	R	R	R
V= Variable				

These bacterial species were originally isolated in feces from patients suffering from typhoid fever and acute gastroenteritis. Review of the literature demonstrates isolation of *Myroides* spp. from urine, blood, respiratory secretions and wound samples from a variety of infections ranging from simple urinary tract infection to more severe conditions such as necrotizing fasciitis

[10-13]. The pathogenicity of *Myroides* spp. in human hosts continue to be under documented, however this bacterium's opportunistic nature lends credence to critical infections in immuno compromised patients [14-15].

Myroides spp. are acknowledged as having broad, intrinsic antimicrobial resistance profiles and have the propensity to develop biofilms [16-17]. Myroides are broadly resistant to β-lactams, including carbapenems, with variable susceptibility to aminoglycosides, quinolones, and sulfamethoxazole [18]. Cutaneous as well as systemic infections have been successfully treated with a quinolone or trimethoprim-sulfamethoxazole based on the results of in vitro susceptibility testing [18].

Though not part of the human microbiota, *Myroides spp*. are commonly found in the environment and infections are typically attributed to contact with contaminated water. Nosocomial infections are usually believed to originate from a hospital water source. The salient features of *Myroidesspp* and its morphological and biochemical congeners that belonged to erstwhile genus *Flavobacterium* has been given in Table 1.

Recently, advanced technologies have been developed for microorganism strain identification, including VITEK 2 (bio Merieux VITEK-2, France), matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS), 16S rDNA sequencing, and another more frequently used nomenclature, 16S r-RNA sequencing . VITEK 2, a routine laboratory method, can detect *Myroides* isolates upto genus level, but unable to discriminate between species. MALDI-TOF MS and 16S rDNA sequencing/16S rRNA sequencing can be used to identify species and are more frequently used for research purposes.

Material and Methods

All clinical samples were taken from patients admitted in our hospital between July 2021 and November 2021.

Objective:

i) To detect isolates of *Myroides spp.* from the clinical samples under study out of the non-fermenter gram negative bacilli (NFGNB).
ii) To understand antimicrobial susceptibility pattern of the organism
iii) To understand the association of co-morbidities and other clinical conditions with *Myrodes spp.* infection, if any.

Inclusion and Exclusion criteria: Isolated NFGNBs only from the Intensive care units (ICU) were included in the study and further processed to identify the organism of interest. All other organisms were not included in the present study. As the study began after obtaining the growth in the bacteriology laboratory, requirement of consent from the patients was waived by the institutional ethics committee (IEC).

During the study period, in a total, 620 isolates of NFGNBs were included in the study. To describe the basic microbial methodology, first, the samples were put up in conventional culture in suitable media according to types of clinical samples. Mostly, cystiene lysine electrolyte deficient (CLED) agar, MacConkey's agar and 5% Sheep blood agar (SBA) and automated Bact T/Alert blood culture bottles were used for inoculation. The inoculated media were put on aerobic culture at 37°C for 24 hours.

They were then observed for bacterial growth. The bacterial growth is then subjected to Gram staining following the observation of colony characters. Identification of NFGNBs were done by conventional biochemical test for identification (Table 2) and with the help of automated identification system namely Vitek-2 compact as well when required. Cultute techniques and conventional biochemical testing followed the methodology of standard text book [19-20].

The suspected *Myroides spp.* on blood agar appeared to be 2-3 mm diameter, round, convex with yellowish tinge and revealed a fruity fragrance. On MacConkey's agar they appeared as non-fermenter pale colonies. Gram staining revealed gram negative bacilli which were non-motile. Those organisms were indole negative, oxidase and catalase positive. Suspected isolates were put up on Vitek-2 compact system for final identification and antimicrobial susceptibility testing (AST).

Table-2: Biochemical comparison of isolated Non-fermenters in the present study						
Biochemical test	***P. aeruginosa***	***A. baumannii***	***B. cepacia complex***	***B. pseudomallei***	***Stenotropho monas maltophilia***	***Myroides spp.***
Oxidse	+	–	+	+	–	+
Pigment	Green, greenish yellow, brown red, dark brown	–	Grey, yellow	Cream or tan	Grey, slight yellow, lavender	
Motility	Motile	Non-motile	Motile	Motile	Motile	Motile
Growth on Mac Conkey Agar	+	+	+	+	+	+
NO_3 reduction	–	V	V	+	V	–
Esculin hydrolysis	–	–	V	–	+	–
ONPG	–	+	V	V	+/-	NA
DNAase	–	–	–	–	+	+
Polymyxin B	S	S	R	R	S	R

**P. aeruginosa- Pseudomonas aeruginosa; A. baumannii- Acinetobacter baumannii; B. cepacia complex- Burkholderia cepacia complex; B. pseudomallei –Burkholderia pseudomallei;* £V- Variable

All isolates were first identified as *Myroides* spp. using the Vitek-2 GN ID cards (BioMérieux). However, speciation could not be done in our present study. AST was performed by VITEK N281 card (BioMerieux, France) having a panel of antimicrobials on a single card to which susceptibility results could be tested. As Clinical and Laboratory Standards Institute (CLSI) did not recommend any direct guideline for *Myroides*, as of yet, we utilized breakpoint criteria suggested by CLSI for non-enterobacteria non-fastidious, glucose-non-fermenting, gram-negative bacilli for the purpose of interpretation of resistance and susceptibility [22].

This panel has antibiotics which are intended to be used against non-fermenter bacteria. The following antimicrobial agents were tested: ticarcillin-clavulenic acid, piperacillin/ tazobactam, ceftazidime, cefoperazone-sulbactum, cefepime, aztreonam, imipenem, meropenem, amikacin, gentamicin, clevofloxacin, minocycline, colistin, and co-trimoxazole. Hospital records of all patients with positive *Myroides spp.* growth in culture, were reviewed retrospectively. The collected data included the demographic characteristics of the patients, co-morbidities, and the presence of an indwelling urinary catheter. The antimicrobial treatment and the clinical outcomes were reviewed in a case to case basis.

Results

We got five isolates of *Myroides spp.* as detected by Vitek-2 compact system out of total 620 NFGNB isolates included in the study. Other bacteria which were identified and processed but not registered for the results of antibiogram as they were organisms other than *Myroides spp.,* comprised of *Acinetobacter baumannii, Pseudomonas aeruginosa, Burkholderia cepacia complex, Burkholderia pseudomallei* and *Stenotrophomonas maltophila.* The antibiogram of five *Myroides spp* isolates was depicted in Table-3.

All the isolates were resistant to ticarcillin-clavulenic acid, piperacillin-tazobactam, cefepime, ceftazidime, cefoperazone–sulbatam, meropenem, amikacin, gentamicin, levofloxacin and aztreonam. Two out of five isolates were found susceptible to minocycline and tigecycline each. Only one isolate was susceptible to co-trimoxazole and colistin each.

Table-3: Antibiogram of *Myroides spp* isolates		
Antimicrobials	**Number of Resistant isolates (Total n = 05)**	**Number of Susceptible isolates (Total n = 05)**
Piperacillin-tazobactam	05	00
Ticarcillin-clavulanic acid	05	00
Ceftazidime	05	00
Cefoperazone-sulbactam	05	00
Cefepime	05	00
Imipenem	04	01
Meropenem	05	00
Aztreonam	05	00
Amikacin	05	00
Gentamicin	05	00
Levofloxacin	05	00
Minocycline	02	03
Tigecycline	02	03
Co-trimoxazole	04	01
Colistin	04	01
R= Resistant; S= Susceptible		

Discussion

The two most commonly encountered *Myroides spp*. occurring in human infection are *Myroides odoratus* and *Myroides odoratimimus* [6, 8, 18]. Common clinical scenerios are bacteremia, isolated outbreaks of urinary tract infections, cellulitis followed by incidental exposure to contaminated water and/ or trauma [19, 21-22]. The classical epidemiology of *Myroides spp.* involves infection of typically immune-compromised hosts. The common co-morbidities associated with *Myroides spp.* Infection are immune-compromised state, terminal illness and debilitating conditions like end stage kidney disease, cirrhosis of liver, chronic obstructive lung disease, malignancies [23-25]. Out of the five reports of *Myroides spp*. infections, three were nosocominal UTIs and two isolated from blood stream infection (BSI).

Here, we report a case of an 80-year-old cancer chemotherapy recipient male patient, retired corporate employee, admitted with recurrent episodes of hematuria along with fever with chills and rigor to urology outpatient department (OPD). The patient was then admitted for further work up. The clinical history of the patient revealed that the patient had been suffering from diabetes mellitus for the last 25 years. A midstream clean catch urine sample was collected and cultured on CLED and 5% sheep blood agar media by semi-quantitative culture technique, which yielded a NFGNB in quantitatively significant amount i.e. colony count > 10^5 CFU/mL (Fig. 1).

Fig-1: Growth of *Myroides spp* in CLED medium from urine sample

The isolate was identified as *Myroides spp.* by the automated ID & AST system. The patient was treated with oral minocycline according to the antibiotic susceptibility testing (AST) report. However, the blood culture was sterile after seven days of aerobic incubation in Bac-T/Alert automated blood culture system. The patient came round after two weeks without any complication.

A 78-year-old male patient was admitted to general medicine ward of the hospital with chief complaints of vomiting and disorientation for last three days. On investigation of biochemical tests, creatinine was 9.8 mg/dL, potassium 5.1 mEq/L with typical EKG changes. He was diagnosed to have renal failure and immediately shifted to critical care unit facility. He was immediately put on hemodialysis to fight renal failure along with hyperkalemia. On the next day, the patient developed high fever with chills rigor. Full microbiological investigations were done.

His catheterized urine sample, following culture in MacConkey's agar and 5% SBA, showed growth round, smooth, convex, yellow pigmented colonies. Identification of the organism was completed by Vitek-2 Compact system, and the pathogen was diagnosed to be *Myroides spp*. having susceptibility only to tigecycline among all the antimicrobials tested. His blood cultute was put up on Bac-T Alert 3D which gave positive signal after 24 hours. Inoculation from liquid culture suspension following culture in 5% SBA and MacConkey's agar showed non-fermenter colonies on overnight aerobic incubation at 37°C. It was diagnosed as *Myroides spp* with the same resistance pattern when put up on Vitek-2 Compact system. However the patient succumbed to cardiac arrest on the same evening.

Fig-2: Patient in ICU

A 49 year old male (Fig. 2) with multiple co-morbidities presented to hospital with high grade fever with chills and generalized body ache. He was suffering from uncontrolled diabetes mellitus for last 16 years, further complicated by alcoholic liver diseases. He was diagnosed with oral squamous cell carcinoma, and on regular radiotherapy along with cisplatin. The patient's white blood cell count was initially 45,000/L and serum hemoglobin was 8.9 gm%, which was quite consistent with chronic anaemia. All other laboratory parameters including kidney and hepatic function tests were within normal clinical range.

He was empirically put on ciprofloxacin, imipenem and metronidazole following admission. On the third day of his stay in the hospital, he was transferred to CCU for altered mentation. His electrolyte analysis showed hyponatremia. He was initially suspected to develop diabetic ketoacidosis and managed on that line. Blood samples were sent for malarial parasites, scrub typhus and dengue NS1 antigen ELISA, all of which found to be non-reactive / negative as reported by dept of Microbiology. Blood cultures were taken at the same time with blood collected from two peripheral veins and put in automated blood culture bottles (BacT- Alert).

Both the cultures were found to be positive for gram negative non-fermenter rods. Identification and AST were put up in Vitek-2 compact system which identified the organism as *Myroides spp*. Based on microbiology reports, the patient was started on intravenous tigecycline. The patient unfortunately succumbed due to dyselectrolytemia and cardiac dysrrhythmia, only after 24 hours of starting of the therapy.

We encountered a case of urinary tract infection by *Myroides spp* in a child. That two years old male patient, presented with fever, chills and rigor for last three days to pediatrics OPD. The child was admitted for further work up. Complete hemogram revealed, haemoglobin 8.5gm%, RBC 2.9 and total leukocyte count (TLC) was 18,000/mL. Urineon aerobic culture yielded non-hemolytic, colonies on blood agar and non lactose fermenting colonies on CLED agar with a sweet odour. Meanwhile as the patient's clinical condition deteriorated, the clinicians sent blood for culture as well microbiology laboratory in BacT/Alert 3D bottle.

The automated culture system developed positive signal of growth just after 48hours. On direct smear from the bottle's suspension, Gram stain showed numbers of WBC and Gram negative bacilli which on subculture in solid media showed non-hemolytic pale colonies on 5% SBA and non lactose fermenting (NLF) colonies on MacConkey's agar. Mycological culture revealed nothing even after two weeks, hence discarded as negative. For quick and correct diagnosis of the organism, culture identification and AST

for both urine as well as blood culture were put up in VITEK 2 Compact System which identified the organism to be same for both, that is *Myroides spp.* The bacteria showed moderate sensitivity to the antibiotics tested. Based on the laboratory reports, the patient was put on antibiotics imipenem and minocycline. The patient came round and got discharged after two weeks of hospital stay.

Fig-3: Patient in ICU

An 80 yrs old female patient (Fig. 3) was admitted in CCU, with a diagnosis of cerebrovascular accident (CVA). The patient was first admitted in a private nursing home, received basic life support and sent to our institution after two days of the incident. Physical examination and assessment was done at emergency department which revealed that the patient was febrile, unconscious, with heart rate of 96/min, BP 100/60 mm Hg. On evaluation, the patient was found to be suffering from uncontrolled diabetes mellitus, on oral hypoglycemic drugs for last 10 years; having dys-electrolytemia and acute renal failure.

The blood parameters of the patient were as follows: Hb- 9.8 gm %, RBC 3.5 m, WBC 16000/mL ,random blood sugar -408 mg/dL, Na^+:131 mEq/L,K^+: 3.9 mEq/L. On admission, herother biochemical parameters were blood urea nitrogen (BUN) 55 mg/dL, creatinie - 1.9 mg/dl, serum total protein 3.6mg/dl and otherwise unremarkable liver function test. Her hemoglobin was 9.8gm/dL, platelet count 1,60,000/µl, TLC-16000/µl with 78% neutrophils. She was seronegative for HIV, HBsAg and anti HCV in serological tests. The patient was started empirically on imipenem and amikacin. Catheter aspirated urine sample was sent to department of Microbiology. It was cloudy, with a reddish tinge.

The urine routine microscopy showed 5-7 pus cells/high power field and bacilli with red blood cells on direct wet mount examination. Urine sample was inoculated onto blood agar and MacConkey agar and incubated aerobically overnight at 37°C. On next morning, culture media showed growth of a non-fermenter organism with fruity odour in significant number (> 10^5CFU/mL). The isolated organism was catalase –ve, oxidase +ve, non-fermenter, gram negative bacillus.

Further evaluation of the isolate using VITEK-2 Compact (bioMerieux) identified it as a *Myroides spp*. The organism was susceptible only to minocycline, and resistant to ceftazidime, cefoperazone-sulbactam, ticarcillin-clavulenic acid, cefepime, aztreonam, imipenam, meropenam, amikacin, gentamicin, levofloxacin, tigecycline ,colistin, cotrimoxazole. The antibiotic sensitivity report was sent to CCU at the earliest and the clinicians were suggested to prescribe minocycline, unless contraindicated. Unfortunately, the patient died of multi organ dysfunction after 24 hrs of arrival of the report.

Myroides spp. grows well in presence of high concentration of glucose. In the present study diabetes mellitus was present as a co-morbidity in four out of five cases (80%).Association between diabetes mellitus and UTI has been reported by Solanki *et al* ,where 54% cases of *Myroides spp* mediated UTI had diabetes mellitus as co-morbid condition [26]. Eight (73%) out of these eleven patients were long standing diabetics. Verma *et al* found 100% patients with diabetes in similar UTI settings [27].

Myroides spp has strong adherence property especially at low temperature,which make them avid biofilm producers. This property has been exploited in causation of UTI with Foley's catheter in situ. In our study, three out of five (60%) cases were catheterized. Their ubiquitous presence and ability of auto aggregation, co-aggregation leading to biofilm

formation may explain infection of debilitated or immune-suppressed hosts with indwelling urinary catheters [28-29]. Chen *et al* found 82% of their study subjected with catheter in situ developing UTI with *Myroides spp* [30].

As a whole, there are limited published cases of *Myroides spp*. available in the literature. One study presented infection with *Myroides odoratamimus* following pig bite which caused osteolytic lesion on the right tibia [1]. Another study described *Myroides* bacteremia in a diabetic person [27]. *Myroides spp*. can be regarded as a multi drug resistant (MDR) environmental organism and can have multiple mechanisms simultaneously [7,14-15,18]. Intrinsic resistance to β-lactamases is due to the presence of two metallo-β-lactamases, MUS-1 and TUS-1, which share a 73% of amino acid identity [31].

Furthermore, a resistance island was found on the chromosome of the bacterium [9-33]. This region has different types of resistance genes, including tetX (conferring tetracycline resistance), cat (chloramphenicol resistance) and bla-OXA-347 and bla-OXA-209 (conferring β-lactam resistance) [33]. Moreover, it has been recently found that *Myroides odoratimimus* not only have common virulence factors, like *bauE* gene to acquire iron competing with the host and adherence factors (*DnaK, Hsp60*), but also can survive intracellularly (*katA, clpP, EF-Tu, and sodB*), even in human stomach (*ureA, ureB, ureG*), can disseminate easily and is able to destroy human tissues [9, 33].

Biofilm formation is one of the important virulence factor for many pathogens including *Myroides spp*; in fact, it has become obvious that sessile bacterial cells in the biofilms express properties which are different from the properties of planktonic cells, for example, the ability to escape host defense, but also the higher resistance to antibacterial agents [7, 14]. The production of a strong biofilm is a serious problem because it increases pathogenicity in device-related infections and it is often associated with therapeutic failure, as well as persistence of infections [14-16].

The isolates of *Myroides spp* in the present study were resistant to most of the tested antimicrobials. Minocycline and tigecycline were only two drugs against which three isolates showed susceptibility. *Agarwal M et al* have noticed 100% susceptibility of *Myroidesspp* towards minocycline. Though they did not comment on tigecycline. Licker *et al* also reported 100% susceptibility against minocycline while resistance to all other antibiotics [15].

The problem with tigecycline is that it cannot be relied upon in UTI as it is not excreted through kidney, hence, not concentrated in urinary bladder. In case of bacteremia, the option of tigecycline comes far later because of the fact that tigecycline being highly lipophilic remains concentrated in tissues and only a small fraction of the dose is available in blood stream. So, even with good susceptibility profile tigecycline could not become a reliable drug in UTI and bacteremia caused by *Myroides spp.* Though *Licker M. et al* claimed in their study that two out of four *Myroides odoratimimus* isolates from UTI were successfully treated with tigecycline [15].

All the isolates in this study were resistant to anti-pseudomonal β-lactam drugs. This finding is similar to other studies done on *Myroides spp* antimicrobial resistance pattern. Though some workers found meropenem to be better alternative for *Myroides spp* than imipenem, our finding is just the opposite. Moxifloxacin has been suggested among fluoroquinolone by some workers. But as VITEK-2 compact system did not have standardized option for it, we could not test the drug. But, all the isolates were resistant to levofloxacin in present study. Susceptibility towards cotrimoxazole was found in one isolate. Whereas, Chen et al. reported 100% sensitivity against co-trimoxazole [30].

Among the cases from India Ahamed I. from Bengaluru reported an UTI case from where *Myroides odoratimimus* was isolated, in 74 years old male diabetic patient who ultimately died of multiorgan failure [11]. Prateek S. from Uttarakhand reported a case of pericardial effusion, from where *Myroides odoratus* was isolated, in a patient with chronic kidney disease on maintenance hemodialysis [35]. The isolate was pan

resistant. The patient finally succumbed to death due to cardiac arrest. *Mahapatra A. et al* reported a case of liver abscess in a 43 year old chronic alcoholic and ganja addict male from Odisha. *Myroides spp* was isolated from empyema pus of that patient. Surprisingly it was susceptible to wide range of antimicrobials including amoxiclav, piperacillin-tazobactam, carbapenems, and ciprofloxacin, but resistant to cephalosporins [36].

The patient was successfully treated with intravenous piperacillin/tazobactam and ciprofloxacin. However, a number of *Myroides spp.* mediated UTI cases have been reported by *Agarwal M et al.* in his article. He reported 16 cases of UTI. All the patients were catheterized [37]. So, urinary tract catheterization or instrumentation imposes a risk for invasion by *Myroides spp.* In our study, we have tried to show that various immune-compromised and debilitated conditions associated with risks from where *Myroides spp* has been isolated (Table 4). It must be noted that till date no case or case series or article has been reported from eastern part of India on *Myroides* infection. This original work may in that sense should be considered and claimed as eye opener index one.

Table-4: Comorbidity profile of patients (n=05)

Comorbidity	Present in patients (%)
Diabetes mellitus	04 (80%)
Malignancy	03 (60%)
Urinary Catheterization	03 (60%)
Stay in intensive care unit of hospital	04 (80%)

However, the present study has its share of limitations. Speciation of the isolates could not be done as Vitek-2 Compact could detect up till genus level of this pathogen. Current strain identification methods based on biochemical traits are unable to identify strains accurately at the species level.16S ribosomal RNA (rRNA) gene sequencing can accurately achieve this [34].

Schröttner P. demonstrated in their pioneering that VITEK-2, though reliably identified the genus *Myroides*, but could not differentiate between *M. odoratimimus* and *M. odoratus*. In contrast to this, both MALDI-TOF MS and 16S rDNA sequencing efficiently distinguished between these two species [9, 34]. Speciation of the genus may be important for epidemiological and statistical purpose, but, the susceptibility *vs* resistance pattern has no established difference between these two species.

Conclusion

In conclusion, it may be said that the clinicians should be cautious and suspicious about the atypical pathogens, in particular, in immune-compromised population. Urine culture should be considered at an earlier stage in these kinds of patients due to the presence of less virulent organisms that may be harbouring important resistance mechanisms.

A well-designed antimicrobial stewardship associated with efficient infection control practices are essential to limit the spread of these new emerging pathogens.

Acknowledgement:

Staff, resident doctors and faculty of Department of Microbiology, Medicine and Critical care unit.

Financial Support and sponsorship: Nil

Conflicts of interest: There are no conflicts of interest.

References

1. Maraki S, Sarchianaki E, Barbagadakis S. *Myroidesodoratimimus* soft tissue infection in an immunocompetent child following a pig bite: Case report and literature review. *Braz J Infect Dis*. 2012; 16:390-392.
2. Bachmeyer C, Entressengle H, Khosrotehrani K. Cellulitis due to Myroidesodoratimimus in a patient with alcoholic cirrhosis. *Clin Exp Dermatol.* 2008; 33(1):97-98.
3. Spanik S, Trupl J, Krcmery V. Nosocomial catheter-associated Flavobacterium odoratum bacteraemia in cancer patients. *J Med Microbiol.* 1998; 47(2):183.
4. Beharrysingh R. *Myroidesbacteremia*: a case report and concise review. *ID Cases.* 2017; 8:34-36.

5. Bartoletti R, Cai T, Wagenlehner FM, Naber K, Bjerklund-Johansen TE. Treatment of urinary tract infections and antibiotic stewardship. *EurUrol Suppl.* 2016; 15:81-87.
6. Holmes B, Snell JJ. Lapage SP. Flavobacterium odoratum: a species resistant to a wide range of antimicrobial agents. *J Clin Pathol.* 1979; 32:73-77.
7. Hu SH, Yuan SX, Qu H, Jiang T, Zhou YJ, Wang MX, Ming DS. Antibiotic resistance mechanisms of Myroides sp. *J Zhejiang Univ Sci B*. 2016;17(3):188-99.
8. Vancanneyt M, Segers P, Torck U. Reclassification of Flavobacterium odoratum (Stutzer 1929) strains to a new genus, Myroides, as Myroidesodoratuscomb. Nov. And Myroidesodoratimimusspnov. *Int J SystBacteriol.* 1996; 46:926-932.
9. Hu S, Cao L, Wu Y. Comparative genomic analysis of Myroides odoratimimus isolates. *Microbiologyopen.* 2019;8(2); 1-14.
10. Crum-Cianflone NF, Matson RW and Ballon-Landa G. Fatal case of necrotizing fasciitis due to *Myroides odoratus*. *Infection.* 2014; 42:931-935.
11. Ahamed I, Annapandian VM, Muralidhara KD. *Myroidesodoratimimus* urinary tract infection. *Saudi J Kidney Dis Transpl* [serial online] 2018 [cited 2022 Apr 7]; 29:1220-2.
12. LaVergne S, Gaufin T, Richman D. *Myroides injenensis* Bacteremia and Severe Cellulitis. *Open Forum Infect Dis.* 2019; 6(7):ofz282.
13. Jover Sáenz A, Pérez Villar F, Barcenilla Gaite F. Severe sepsis caused by infected prosthesis joint due to Myroides odoratimimus. *Medicina Clinica (English edition)*. 2016; 147:276-277.
14. Lorenzin G, Piccinelli G, Carlassara L. *Myroidesodoratimimus* urinary tract infection in an immunocompromised patient: an emerging multidrug-resistant micro-organism. *Antimicrob Resist Infect Control.* 2018; 7:96.
15. Licker M, Sorescu T, Rus M. Extensively drug-resistant *Myroidesodoratimimus* - a case series of urinary tract infections in immunocompromised patients. *Infect Drug Resist.* 2018; 11:743-749.
16. Niveditha S, Pramodhini S, Umadevi S, Kumar S, Stephen S. The isolation and biofilm formation of uropathogens in the patients with catheter associated urinary tract infections (CAUTI). *J Clin Diagn Res.* 2012; 6:1478-1482.
17. Stepanović S, Vuković D, Hola V, Di Bonaventura DS, Cirković I, Ruzicka F. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. *APMIS.* 2007; 115:891-899.
18. Gunzer F, Rudolph WW, Bunk B, Schober I, Peters S, Muller T, Oberheitmann B, Schröttner T. Whole-genome sequencing of a large collection of Myroidesodoratimimus and Myroidesodoratus isolated and antimicrobial susceptibility studies. *Emerg Microbes Infect.* 2018; 7:61.
19. Betty A. Forbes, Daniel F. Sahm, Alice S. Weissfeld; In: Infection of the urinary tract. Bailey & Scott"s Diagnostic Microbiology. 12th edition: USA; Mosby Elsevier; 2007; 842-855.
20. Betty A. Forbes, Daniel F. Sahm, Alice S. Weissfeld; In: Bloodstream infection. Bailey & Scott"s Diagnostic Microbiology. 12th edition: USA. *Mosby Elsevier.* 2007; 778-797.
21. Pompilio A, Galardi G, Gherardi G. Infection of recurrent calcaneal ulcer caused by a biofilm-producer Myroidesodoratimimus strain. *Folia Microbiol (Praha)* 2018; 63(2):203-207.
22. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; thirty first informational supplement.(M100-S31). *Cls document. Pensylvania, Wayne,pa,USA.* 2021.
23. Bachmeyer C, Entressengle H, Khosrotehrani K, Goldman G, Delisle F, Arlet G, Grateaum G. Cellulitis due to Myroides odoratimimus in a patient with alcoholic cirrhosis. *Clin Experim Dermatol.* 2007; 33:97-98.
24. Prateek S, Gupta P, Mittal G, Singh AK. Fatal case of pericardial effusion due to Myroidesodoratus: a rare case report. *J Clin Diagn Res.* 2015; 9:DD01-2.
25. Ranjan M, Karade S, Rahi P, Singh SP, Sen S. Urosepsis due to Multi Drug Resistant *Myroidesodoratimimus*: A Case Report. *Int J Curr Microbiol App Sci.* 2017; 6:1930-1935.
26. Solanki R, Dawar R, Aggarwal DK, Rani H, Imdadi F, Jasuja S. Nonfermenting gram-negative bacilli and urinary tract infection -sorting the mystery of infections caused. *J Med Microb Diagn.* 2015; 4:210.
27. Verma Y, Hemachander SS, Shah K. Urinary tract infection caused by Myroides spp. In diabetic patients: To be or not to be. *Indian J Case Reports.* 2018; 4:1-3.
28. Bendetti P, Rassu M, Pavan G et al. Septic shock, pneumonia, and soft tissue infection due to *Myroidesodoratimimus*: report of a case and review of *Myroides* infections. *Infection*, 2011; 39:161-165.
29. Pompilio A, Galardi G, Gherardi G, Verginelli F, Geminiani C, Pilloni AP et al. Infection of recurrent calcaneal ulcer caused by a biofilm-producer *Myroidesodoratimimus* strain. *Folia Microbiol.* 2018; 63:203-207.
30. Chen XC, Liang L, Lin W. Analysis of elven *Myroides sp.* isolates from urine culture. *Chin. J. Pract. Med. Tech.* 2009; 16:691-692.
31. Mammeri H, Bellais S, Nordmann P. Chromosome-encoded beta-lactamases TUS-1 and MUS-1 from *Myroidesodoratus* and *Myroidesodoratimimus* (formerly *Flavobacterium odoratum*), new members of the lineage of molecular subclass B1 metalloenzymes. *Antimicrob Agents Chemother.* 2002; 46(11):3561-3567.
32. Yağci A, Cerikçioğlu N, Kaufmann ME et al. Molecular typing of *Myroidesodoratimimus (Flavobacterium odoratum)* urinary tract infections in a Turkish hospital. *Eur J Clin Microbiol Infect Dis*. 2000; 19:731-732.
33. De Song M, Chen Q, Chen X. Analysis of resistance genes in pan-resistant Myroides odoratimimus clinical strain PR 63039 using whole genome sequencing. *Microb Path.* 2017; 112:164-170.
34. Schröttner P, Rudolph WW, Eing BR, Bertram S, Gunzer F. Comparison of VITEK2, MALDI-TOF MS, and 16S rDNA sequencing for identification of

Myroidesodoratus and Myroidesodoratimimus. *Diagn Microbiol Infect Dis.* 2014; 79(2):155-159.
35. Prateek S, Gupta P, Mittal G, Singh AK. Fatal Case of Pericardial Effusion Due to MyroidesOdoratus: A Rare Case Report. *J Clin Diagn Res.* 2015; 9(11):DD01-2.
36. Mahapatra A, Mohapatra PR, Choudhury SD. Nerbadyswari Empyema caused by *Myroidesodoratimimus* in a patient with liver abscess. *Lung India.* 2019; 36(5):459-460.
37. Mohit Agrawal, Ved Prakash Mamoria,Sonali Mittal, Ayushi Sharma. Myroides: an emerging pathogen causing urinary tract infections in hospitalized patients. *International Journal of Contemporary Medical Research*, 2019; 6(11):K11-K14.
38. Washington C. Winn Jr., Stephen D. Allen, William M. Janda, Elmer W. Koneman, Gray SW. Procop, Paul C. Schreckenberger et al. The Non fermentative Gram-Negative bacilli: Koneman's Color Atlas and Textbook of Diagnostic Microbiology; 7th edition; USA; *Lippincott Williams & Wilkins – A Wolters Kluwer Company,* 2019; 316-432.

Cite this article as: Pal S, Ghosh PK, Totad RD, Ray R and Raj HJ. Footstep of a new superbug Myroides spp.: A study based on a series of cases from a tertiary care hospital of Eastern India. *Al Ameen J Med Sci* 2023; 16(1): 46-56.

*All correspondences to: Dr. Hirak Jyoti Raj, Associate Professor, Department of Microbiology, IPGME & R, SSKM Hospital, 244 AJC Bose Road, Kolkata-700020, West Bengal, India. E-mail: drhjrmicro@gmail.com