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Review:

Antibiotic resistance mechanisms of *Myroides* sp.*

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Abstract: Bacteria of the genus *Myroides* (*Myroides* spp.) are rare opportunistic pathogens. *Myroides* sp. infections have been reported mainly in China. *Myroides* sp. is highly resistant to most available antibiotics, but the resistance mechanisms are not fully elucidated. Current strain identification methods based on biochemical traits are unable to identify strains accurately at the species level. While 16S ribosomal RNA (rRNA) gene sequencing can accurately achieve this, it fails to give information on the status and mechanisms of antibiotic resistance, because the 16S rRNA sequence contains no information on resistance genes, resistance islands or enzymes. We hypothesized that obtaining the whole genome sequence of *Myroides* sp., using next generation sequencing methods, would help to clarify the mechanisms of pathogenesis and antibiotic resistance, and guide antibiotic selection to treat *Myroides* sp. infections. As *Myroides* sp. can survive in hospitals and the environment, there is a risk of nosocomial infections and pandemics. For better management of *Myroides* sp. infections, it is imperative to apply next generation sequencing technologies to clarify the antibiotic resistance mechanisms in these bacteria.

Key words: *Myroides* sp., Antibiotic resistance, Identification methods, 16S ribosomal RNA gene sequencing, Next generation sequencing
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1 Introduction

The genus *Myroides* (*Myroides* spp.) comprises yellow-pigmented, non-motile, Gram-negative, rod-like bacteria (Holmes *et al*., 1977; Cho *et al*., 2011) that release a fruity odor during growth (Holmes *et al*., 1977). The first strain, Stutzer, of the genus *Myroides* was isolated from the stools of patients with intestinal infections (Holmes *et al*., 1977) and was assigned the species name *Flavobacterium odoratum* (Stutzer and Kwaschnina, 1929). For easier clinical recognition, the bacteriological features, pigmentation, biochemical characteristics, and antimicrobial profiles of 10 isolates were examined (Holmes *et al*., 1977). *Myroides* spp. were found to be non-fermentative

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organisms resistant to many antibiotics (Holmes *et al*., 1977). In 1996, after extensive polyphasic taxonomic analysis of 19 strains of *F. odoratum*, the genus *Myroides* was established and included two species, *M. odoratus* and *M. odoratimimus* (Vancanneyt *et al*., 1996). Later, more strains were isolated from forest soil (strain TH-19(T), named *M. xuanwuensis* sp. nov. (Zhang *et al*., 2014)), seawater (strain JS-08(T), named *M. marinus* sp. nov. (Cho *et al*., 2011), strain SM1(T), named *M. pelagicus* sp. nov. (Yoon *et al*., 2006)), deep-sea sediment (strain D25T (Zhang *et al*., 2008)), human saliva (strain MY15T, named *M. phaeus* sp. nov. (Yan *et al*., 2012)), as well as strains from urine, sputum, surgical exudate (Andreoni, 1986), and patients' matter (Table 1). Thus, *Myroides* spp. are widely distributed in nature (Mammeri *et al*., 2002; Ktari *et al*., 2012; Suganthi *et al*., 2013; Ravindran *et al*., 2015).

Myroides sp. is a rare opportunistic pathogen (Schröttner *et al*., 2014). Nevertheless, management of *Myroides* sp. infection is troublesome due to its high resistance to most antibiotics (as summarized in Table 1). For accurate strain identification of *Myroides* sp., current diagnostic methods, such as the Vitek Jr. system (Vitek Systems, bioMerieux) (Spanik *et al*., 1998), are based on bacteriological and biochemical characteristics, and can determine *Myroides* sp. at the species level in most cases. However, they and 16S ribosomal RNA gene sequencing (16S rRNA sequencing), a standardized bacterial strain identification method (Yoon *et al*., 2006; Zhang X.Y. *et al*., 2008; Zhang Z.D. *et al*., 2014) still not widely applied in Chinese hospitals, fail to provide any information on the status and mechanisms of antibiotic resistance in *Myroides* sp. Whole genome sequencing technologies could address these questions, and should be applied to *Myroides* sp. promptly.

2 Antibiotic resistance status of clinical *Myroides* sp. infections

Myroides sp. infections are rare. By searching the PubMed database of English literature using "*Myroides*" or "*Flavobacterium odoratum*" as key words, only a few reports could be found. In immunocompetent people, primary infections by *Myroides* sp. have been rarely reported, such as a case of *M. odoratimimus* cellulitis resulting from a pig bite in an immunocompetent child (Maraki *et al*., 2012). However, secondary infections can frequently arise when human immunity is impaired, such as post catheterization (Holmes *et al*., 1977; Spanik *et al*., 1998), in patients with cancer (Holmes *et al*., 1977; Spanik *et al*., 1998; Song, 2005) or diabetes mellitus (Yang and Wang, 2001), and in neonates (Wang and Su, 1992; Zhang and Zhang, 1996; Zhao, 2000). *Myroides* sp. can cause soft tissue infection (Benedetti *et al*., 2011), cellulitis (Bachmeyer *et al*., 2007), necrotizing fasciitis (Crum-Cianflone *et al*., 2014), ventriculitis (Macfarlane *et al*., 1985), and urinary tract infections (Yağci *et al*., 2000). *M. odoratimimus* even caused an outbreak of urinary tract infection in a hospital (Ktari *et al*., 2012).

By using the same key words to search the China National Knowledge Infrastructure (CNKI) database, we found that most reports of *Myroides* or *F. odoratum* infections contained a single case (Table 1). Two papers reported 23 (Table 2) and 11 strains (Table 3), respectively.

From Tables 1, 2, and 3, we conclude that *Myroides* spp. are resistant to broad antibiotics, and that their extensive antibiotic resistance has resulted in treatment failure and fatalities. We observed a case in July 2009 in which a patient presented with a post-injury urinary tract infection caused by *M. odoratimimus* strain PR63039 (Table 1, our case). Using antibiotic sensitivity testing (AST), the strain was found to be resistant to ampicillin, amoxicillin, clavulanate, amikacin, aztreonam, chloramphenicol, cephalosporin, imipenem, gentamycin, levofloxacin, meropenem, shubatan, sulfamethoxazole, tetracycline, ciprofloxacin, and tazobactam. Even though many antibiotics, such as cefazolin oxime, amikacin, tetracycline, moxifloxacin, ciprofloxacin, and nitrofurantoin, were administered to the patient for 47 d, the infection was not cured.

The reports analyzed in Table 1 reveal that the antibiotic resistance of *Myroides* sp. varies among strains isolated from different sources. For example, a strain from a patient suffering from a hydatid cyst of the liver was sensitive to norfloxacin (An, 1992), but another strain isolated from pulmonary infection patient was reported to be resistant to norfloxacin (Liu and He, 2001). Two strains isolated from patients with cellulitis and a leg amputation, respectively (Hu *et al*., 2013; Crum-Cianflone *et al*., 2014) were resistant to

ciprofloxacin, while another two isolated from trauma and septicemia patients, respectively, were sensitive to ciprofloxacin (Geng *et al*., 2000; Sun and Zhang, 2006).

Why is there so much variation in the antibiotic resistance profiles of *Myroides* sp. strains isolated from different sources? In our opinion, the subtypes and genotypes of *Myroides* sp. might have a great influence on their sensitivity to certain antibiotics. Therefore, it is imperative to obtain accurate information on strain subtype and genotype.

Table 1 Summary of reported infections by *Myroides* sp.

Patient No.	Age (year)/ gender	Underlying diseases or reasons	Site of isolation	Antibiotic resistance status	Treatment strategy	Outcome	Reference
1	87/M	Trauma, old age	Wound	Resistant to all antibiotics except ciprofloxacin. Sensitive to trimethoprim-sulfamethoxazole	iv ciprofloxad	Favorable	Sun and Zhang, 2006
2	34/F	Hydatid cyst of liver	Drainage	Resistant to neomycin, streptomycin, gentamicin, ampicillin, tobramycin. Sensitive to norfloxacin	Norfloxacin	Favorable	An, 1992
3	2/M	Young age	CSF	Resistant to cefazolin, penicillin, chloramphenicol. Sensitive to ampicillin, polymyxin, kanamycin, erythromycin, neomycin	ND	ND	Shi and Zhou, 1993
4	71/M	Chronic bronchitis, old age	Sputum	Resistant to meropenem, imipenem, ampicillin, cefradine, tobramycin, cephalothin, amoxicillin-clavulanic acid, ampicillin-shubatan, ceftriaxone, gentamicin, cefoxitin, ceftazidime, piperacillin. Sensitive to cefepime, levofloxacin	ND	ND	Guo and Liu, 2011
5	30/F	Burn	Blood, central venous catheter, urine	Sensitive to amikcin, norfloxacin	Amikacin	Favorable	Wu, 1998
6	28/F	Injury and surgery	Wound	Resistant to gentamicin, sulfamethoxazole, ciprofloxacin, cefoperazone-sulbactam, tetracycline, tobramycin, cefoperazone, cefepime, imipenem, piperacillin-tazobactam, cefoselis, amikacin, piperacillin, levofloxacin, netilmicin, ceftazidime, cefotaxime, aztreonam, ampicillin-sulbactam. Sensitive to minocycline. Moderately sensitive to meropenem	Debridement, skin transplantation, iv cefperazone-sulbactam and oral minocycline for 3 d, then oral minocycline for another 3 d	Cured	Hu *et al*., 2013
7	76/M	Chronic obstructive pulmonary disease and heart failure, old age	Blood, wound	Resistant to piperacillin, ceftazidime, ceftriaxone, cefepime, aztreonam, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, levofloxacin, tetracycline, trimethoprim-sulfamethoxazole, ampicillin-sulbactam, cefoperazone-sulbactam, piperacillin-tazobactam	Oral minocycline for 9 d	Cured	Huang *et al*., 2014
8	4/M	None	Blood	Resistant to ampicillin, ampicillin-sulbactam, piperacillin, piperacillin tazobactam, aztreonam, cefazolin, cefoxitin, ceftazidime, cefotaxime, azole cefepime, ceftazidime, ceftriaxone, cefepime	Piperacillin and tobramycin for 14 d	Cured	Huang and Lin, 2003
9	58/ND	Diabetes mellitus complicated by heel bursitis	Drainage	Sensitive to cefoperazone and amikacin	Incision and drainage, cefoperazone and amikacin for several days (more than 3 d)	Cured	Yang and Wang, 2001
10	28/F	None	Pus	Resistant to kanamycin, penicillin, erythromycin. Sensitive to ceftriaxone, norfloxacin, trimethoprim-sulfamethoxazole	Abscess incision drainage and norfloxacin and trimethoprim-sulfamethoxazole	Cured	Song *et al*., 1995

To be continued

Table 1

Patient No.	Age (year)/ gender	Underlying diseases or reasons	Site of isolation	Antibiotic resistance status	Treatment strategy	Outcome	Reference
11	24 d/M	Neonate	Blood	Resistant to penicillin, chloramphenicol, carbenicillin, streptomycin, cefazolin. Sensitive to amikacin, erythromycin, ampicillin, benzylpencilline	Ampicillin, and oxacillin for 19 d	Cured	Wang and Su, 1992
12	11 d/M	Preterm birth	Blood, CSF	Resistant to ampicillin, cefazolin, gentamicin, cefoperazone, cefotaxime, cefatrizine, ceftazidime. Sensitive to amikacin, piperacillin, ampicillin, sulbactam-cefoperazone	Antimicrobial treatment for 5 d (the antibiotic was not described)	Failed	Zhang and Zhang, 1996
13	69/F	Lung cancer and surgery, old age	Pleural effusion and sputum	Resistant to tobramycin, gentamicin, ampicillin, erythromycin, clindamycin, tetracycline. Sensitive to amikacin, tobramycin, ceftriaxone	Antimicrobial treatment, but not described in detail	Died	Song, 2005
14	10 months/ F	Child	Blood	Sensitive gentamicin, tobramycin, cephalexin, sulbactam-cefoperazone, ceftriaxone	Cefoperazone, tobramycin for 10 d	Cured	Zhao, 2000
15	60/M	Common bile duct stones	Blood, bile, peritoneal effusion	Resistant to tobramycin. Sensitive to piperacillin, cefoperazone, amikacin, gentamicin, ceftriaxone, cefotaxime	Amikacin and cefoperazone	Cured	Meng *et al*., 1999
16	44/F	None	Blood, bone marrow	Sensitive to norfloxacin, ciprofloxacin, cefazolin, amikacin, ceftazidime	ND	ND	Geng *et al*., 2000
17	67/M	Old age	Sputum (this strain was isolated with *Serratia marcescens*, *Acinetobacter lwoffi*)	Resistant to ampicillin, piperacillin cefazolin, cefuroxime, cefotaxime, ceftazidime, cefotaxime, aztreonam, gentamicin, norfloxacin, trimethoprim-sulfamethoxazole	Antimicrobial treatment, but not described in detail	Cured	Liu and He, 2001
18	45/M	None	Urine	Resistant to ampicillin, amikacin, azithromycin	Application of cefoperazone, cefotaxime, nitrofurantoin, and tobramycin for 3 weeks	Cured	Wuer *et al*., 2000
19	N/A	ND	Blood, sputum, bile, cerebrospinal fluid, urine, all these three isolates were from patients (no further details were available)	All 12 isolates were resistant to erythromycin, penicillin, streptomycin, ampicillin, oxacillin, piperacillin, carbenicillin	N/A	N/A	Li and Zhao, 1995
20	ND	Chronic nephritis	Urine	Two isolates were resistant to meropenem. All three isolates were resistant to ampicillin-sulbactam, piperacillin-tazobactam, cefuroxime, cefotetan, ceftriaxone, aztreonam, gentamicin, ciprofloxacin, levofloxacin, ampicillin, piperacillin, cefazolin, cefuroxime axetil, ceftazidime, cefepime, imipenem, amikacin, tobramycin, levofloxacin, trimethoprim-sulfamethoxazole	One isolate was sensitive to meropenem	ND	Li *et al*., 2010
21	ND	Diabetes mellitus					
22	ND/F	Cervical cancer					
23	61/F	Coma, cerebral hemorrhage	Sputum	N/A	Ceftazidine, chloramphenicol, penicillin G, gentamicin by atomization inhalation, ketoconazole by nasal feeding	Died	Jin and Xiao, 1995

To be continued

Table 1

Patient No.	Age (year)/ gender	Underlying diseases or reasons	Site of isolation	Antibiotic resistance status	Treatment strategy	Outcome	Reference
24	49/M	Chronic alcohol misuse	Blood	Intermediately sensitive to imipenem	Treatment with amoxicillin-clavulanic acid was changed to ciprofloxacin, imipenem-cilastatin used for 10 d, then oral ciprofloxacin for 21 d	Cured	Bachmeyer *et al*., 2007
25	55/F	Liver cirrhosis bilateral lower extremity cellulitis and open wounds	Blood, wound	Resistant to amikacin, gentamicin, tobramycin, aztreonam, ceftriaxone, ciprofloxacin, tetracycline, trimethoprim-sulfamethoxazole, vancomycin. Intermediately sensitive to piperacillin-tazobactam, cefepime, imipenem, and cilastatin	iv vancomycin, piperacillin-tazobactam, and levofloxacin for 18 h, then iv imipenem-cilastatin, daptomycin, clindamycin, then imipenem-cilastatin and doxycycline	Died	Crum-Cianflone *et al*., 2014
26	13/M	Soft tissue infection	Pus	Resistant to piperacillin-tazobactam, aztreonamaminoglycosides. Intermediately susceptible to imipenem. Sensitive to all quinolones tested, cotrimoxazole, chloramphenicol, and amoxicillin-clavulanic acid	Drainage of osteolytic lesions combined with iv ciprofloxacin for 10 d and continued with oral ciprofloxacin for an additional 10 d	Cured	Maraki *et al*., 2012
27	48/F	Cystitis (contaminated)	Urine	Fully resistant to streptomycin, gentamicin, kanamycin, ampicillin, carbenicillin, tetracycline, polymyxin B.	N/A	N/A	Holmes *et al*., 1977
28	34/M	Infected cut finger	Wound				
29	59/F	ND	Urine	Fully resistant or moderately resistant to sulfamethoxazole, trimethoprim-sulfamethoxazole, cephaloridine, erythromycin, chloramphenicol. Moderately sensitive to nalidixic acid			
30	ND/ND	Urinary retention	Urine				
31	ND/ND	Further details are not available	Urine				
32	ND/ND	Varicose ulcer	Wound				
33	76/F	Leg ulcer	Ulcer				
34	67/F	Breast lump	Urine				
35	48/M	Chronic renal insufficiency	Urine				
36	66/M	Urinary tract infection	Urine	Resistant to all β-lactam and non-β-lactam antibiotics tested, including imipenem, vancomycin, ciprofloxacin, chloramphenicol, tigecycline, rifampicin	Imipenem, colistin	Failure	Ktari *et al*., 2012
37	44/M	Bladder colonization	Urine		No treatment	Favorable	
38	44/M		Urine		No treatment	Favorable	
39	47/M		Urine		No treatment	Favorable	
40	77/M	Urinary tract infection	Urine		Ifampicinþ ciprofloxacin	Cured	
41	65/M		Urine		Ifampicinþ ciprofloxacin	Cured	
42	80/M		Urine		Ifampicinþ ciprofloxacin	Cured	
43	59/M	Urinary tract infection	Urine	Resistant to amikacin, gentamicin, imipenem, meropenem, cefazolin, ceftazidime, cefotaxime, cefepime, aztreonam, ampicilllin, piperacillin, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin-tazobactam, colistin, trimethoprim-sulfamethoxazole, chloramphenicol, ciprofloxacin, levofloxacin, moxifloxacin, tetracycline	Levofloxacin was used only temporarily and orally	Failure	Our case

M: male; F: female; N/A: not applicable; ND: not described; CSF: cerebrospinal fluid; iv: intravenous injection

Table 2 Antimicrobial susceptibility testing of 23 strains of *Myroides* sp. using the K-B method

Site of isolation (total samples of positive isolation)	Antibiotic	*R*	*I*	*S*
Sputum (8); Urine (6); Blood (4); CSF (3); Bile (2)	Amikacin	5	10	8
	Cefazolin	11	9	3
	Cefoperazone	9	9	5
	Sulfamethoxazole	4	10	9
	Sulfadiazine	5	7	11
	Ceftazidime	10	6	7
	Erythrocin	3	10	10
	Azithromycin	4	9	10

Translated from Lan and Bao (2009) with permission of the authors. K-B method: Kirby-Bauer disk diffusion method; CSF: cerebrospinal fluid; *R*: resistant; *I*: immediately sensitive; *S*: sensitive

Table 3 Antimicrobial susceptibility testing of 11 strains of *Myroides* sp. isolated from urine using Oxoid culture medium

Patient information	Antibiotic	*R*	*I*	*S*
2–76 years (average 53 years), 9 males, 2 females All patients suffered from urinary retention or urinary tract stones, but none of them had symptoms of urinary tract infection or other discomfort In nine urinarily catheterized patients, the urinary culture when the catheter was in situ was *Myroides* sp. positive, but the urinary testing showed no WBC in these urinary samples, and pus cells were found in only three of them The urinary culture of *Myroides* sp. became negative after removal of urinary catheter in these nine urinarily catheterized patients even though they were not treated	Ampicillin	11	0	0
	Piperacillin	11	0	0
	Cefuroxime	11	0	0
	Cefoperazone-sulbactam	11	0	0
	Ceftazidine	10	1	0
	Cefepime	10	1	0
	Aztreonam	11	0	0
	Imipenem	11	0	0
	Meropenem	11	0	0
	Levofloxacin	8	3	0
	Ciprofloxacin	9	2	0
	Trimethoprim-sulphamethoxazole	0	0	11
	Amikacin	11	0	0

Translated from Chen *et al*. (2009) with permission of *Chin. J. Pract. Med. Tech*. *R*: resistant; *I*: immediately sensitive; *S*: sensitive; WBC: white blood cell

3 Preliminary opinions on the antibiotic resistance mechanisms of *Myroides* sp.

In China, there have been no reports on the antibiotic resistance mechanisms of *Myroides* sp. Although several foreign researchers have investigated this topic, very little information is available. Hummel *et al.* (2007) showed that the β-lactamase gene was responsible for the variable patterns of resistance to β-lactam antibiotics and the decreased susceptibility to carbapenems of different *Myroides* sp. strains. In a study investigating a number of clinical cases involving systemic infections, Mammeri *et al.* (2002) claimed that resistance to β-lactams was due to the production of the chromosome-encoded β-lactamases TUS-1 and MUS-1 in *M. odoratus* and *M. odoratimimus*. The β-lactamases produced by Gram-negative and Gram-positive bacteria play a vital role in resistance against β-lactam antibiotics. However, their study showed that the β-lactamases TUS-1 and MUS-1 could only partly explain the intrinsic resistance of *Flavobacteriaceae* species to β-lactams. Also, a common observation was that these *Escherichia coli* expressed metalloenzymes were much

less resistant to β-lactam than those of primitive origin (Mammeri *et al.*, 2002). Even *Flavobacteriaceae* and *Myroides* sp. belong to the same family, the mechanism of resistance conferred by TUS-1 and MUS-1 in *Flavobacteriaceae* species cannot be assumed to operate in *Myroides* sp. Then, why does *Flavobacteriaceae* serve as a source for a variety of metalloenzymes? As observed for other environmental species, this might be due to the combined biosynthesis of carbapenem derivatives and hydrolyzing β-lactamases (Mammeri *et al.*, 2002).

Suganthi *et al.* (2013) investigated whether the antibiotic sensitivity of plasmid-containing *M. odoratimimus* SKS05-GRD was correlated with the plasmid or was chromosomally-mediated. They revealed that resistance to kanamycin, amikacin, and gentamicin was plasmid-mediated, and that resistance to ampicillin, cefadroxil, cefoperazone, ceftazidine, ceftriaxone, and netillin was chromosomally-mediated. The *Klebsiella pneumoniae* carbapenemase (KPC) family is closely related to resistance to carbapenem in a variety of pathogens and the KPC gene is located in a plasmid. However, *Myroides* sp. WX2856, obtained from an abdominal abscess (Kuai *et al*., 2011), harbored a KPC-2 carbapenemase, but the *KPC-2* gene might not be located on a plasmid as in *K. pneumoniae*. Do these results suggest the possibility of interspecies transmission of the KPC-2 gene? This aspect needs further investigation.

On the other hand, there are several known features of antibiotic resistance mechanisms in bacteria from the same family of *Myroides*, such as in *F. indologenes*, now named *Chryserobacterium indologenes* (Tian and Wang, 2010). First, resistance transfer factors (R-factors) in the cytoplasm determine the bacteria's resistance to antibiotics. R-factor plasmids can carry and transfer a variety of resistance genes among bacteria. In addition, the thick outer membrane and its low permeability, resulting from multidirectional mutations, and the active discharge system of the bacterial cell membrane of *C. indologenes* confer inherent multi-drug resistance. The bacteria also produce a β-lactamase with a broad spectrum of β-lactam hydrolytic activity (Tian and Wang, 2010).

Thus, it is apparent that the antibiotic resistance mechanisms of *Myroides* sp. are unclear and deserve further investigation.

4 Present diagnostic methods do not clarify the antibiotic resistance mechanisms of *Myroides* sp.

In clinical diagnostic laboratories, traditional bacterial identification methods primarily rely on testing biological traits and biochemical characteristics, and include microscopic inspection and metabolic testing of isolated and cultured bacteria. These methods have shown that *Myroides* spp. do not have flagella, release a fruity fragrance, are yellow, oxidase-positive, urea- and indole-negative, and are unable to oxidize sugar (Li and Zhao, 1995; Chen *et al.*, 2009). However, the bacterial strain can be preliminarily identified only as *Myroides* sp., as further strain type designations cannot be determined using these traditional methods. Nearly all cases of *Myroides* sp. infections reported in China have used these traditional identification approaches, and did not describe any *Myroides* sp. subtypes. Using these traditional identification methods, the *M. odoratimimus* strain PR63039 isolated in our case was first identified as *Pseudomonas putida*, then as *M. odoratimimus*, and as the *Acinetobacter calcoaceticus-baumannii* complex, at different time points throughout the 47 d of the patient's hospitalization. It was finally confirmed as *M. odoratimimus* by 16S rRNA sequencing. This case also indicates that these traditional identification methods may not correctly diagnose the strain type.

Recently, other microorganism strain identification technologies have been developed, including VITEK 2 (bioMerieux 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 rRNA sequencing (Table 4). VITEK 2, a routine laboratory method, can help to discriminate among genera but not among 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 (Lee *et al*., 2014; Schröttner *et al*., 2014). According to Schröttner *et al*. (2014), the genus *Myroides* was reliably identified in tests of 22 isolates using VITEK 2. 16S rDNA sequencing further revealed that they shared ≥97% homology, enough for a reliable identification at the species level. Yoon *et al*. (2006) applied 16S rRNA sequencing successfully to clarify the phylogenetic

position of *Myroides* sp. strain SM1T, isolated from seawater in Thailand.

Table 4 Comparison of the present approaches for strain identification of *Myroides* sp.*

Method	Trait
VITEK2	Only suitable to identify bacteria at the genus level, not at the species level
MALDI-TOF MS	Able to distinguish between *M. odoratus* and *M. odoratimimus*
16S rDNA sequencing/16S rRNA sequencing	Able to distinguish microorganisms at the species level
Whole genome sequencing	Able to identify the microorganism and provide the bioinformatics of microorganism

* Yoon *et al*., 2006; Lee *et al*., 2014; Schröttner *et al*., 2014

5 Whole genome sequencing is a feasible way to investigate the antibiotic resistance mechanisms of *Myroides* sp.

In the past decade, many next generation sequencing platforms have been developed, such as 454 invented in 2004 (Margulies *et al*., 2005), Illumina Solexa in 2006 (Bentley, 2006), SOLiD in 2007 (Chi, 2008), Ion Torrent in 2011 (Rothberg *et al*., 2011), and PacBio in 2012 (Koren *et al*., 2012). This has led to a rapid increase in the sequencing of whole genomes of microorganisms, including eukarya, bacteria, archaea, and viruses. These sequences have been deposited in the National Center for Biotechnology Information (NCBI) RefSeq genome collection database (http://www.ncbi.nlm.nih.gov/genome) (Tatusova *et al*., 2015). In 2014, over 10 000 microbial genomes were released (Tatusova *et al*., 2015). Along with the next generation platforms, whole-genome analysis of multi-drug resistance mechanisms has emerged.

For example, *A. baumannii* is a common cause of fatal nosocomial infections because of its extensive antibiotic resistance. Genomic sequencing revealed comprehensive drug-resistance mechanisms, such as a 41.6-kb closely related antibiotic resistance island in the chromosome (Huang *et al*., 2012), the horizontally transmittable carbapenem resistance gene ($bla_{\text{OXA-23}}$) containing a plasmid (in isolate MDR-TJ, with 454 Titanium) among different *A. baumannii* strains (Huang *et al*., 2012; Lee *et al*., 2013), a list of antimicrobial resistance-associated genes (with 454 and SOLiD) (Rolain *et al*., 2013), a diversified resistance gene list (with Illumina Hiseq2000) (Tan *et al*., 2013), and longitudinally evolved antibiotic resistance gene mutations and mutational pathways under pressure from the antibiotic colistin (with 454 Titanium) (Snitkin *et al*., 2013). Since its invention by Rothberg *et al.* (2011), Ion Torrent sequencing technology has been successfully applied to complete genomic sequencing, such as in *Clostridium* sp. BL8 (with Ion Torrent PGM™) (Marathe *et al*., 2014), and clear characterization of drug-resistant genes, such as in *Mycobacterium tuberculosis* (Daum *et al*., 2014). Results can be obtained within five days, comparable to the turnaround time required by current drug sensitivity testing (DST) (Daum *et al*., 2014). PacBio single-molecule real-time technology has frequently been used to perform whole-genome sequencing of many microorganisms, such as *Neisseria gonorrhea* (with the PacBio RSII platform), a Gram-negative β proteobacterium responsible for the sexually transmitted infection gonorrhea (Abrams *et al*., 2015).

Yet, little genomic information about *Myroides* sp. is available. A brief description of the genomes of *M. odoratus* DSM 2801 and CIP 103059 was found in genome database of NCBI (Table 5), but this was not suitable for studying its drug-resistance mechanisms. Recently, the genome sequencing of the urethral catheter isolate *Myroides* sp. A21 was completed (Burghartz *et al*., 2015). The sequence contained 3650 protein-coding sequences (CDSs), 136 RNA-coding genes, and eight copies of the rRNA gene cluster, of which three were resolved as a direct repeat of two rRNA gene clusters. The presence of 106 transfer RNAs (tRNAs) and six noncoding RNAs (ncRNAs) was also predicted. By comparing the genome sequence of *Myroides* sp. A21 with those of *M. odoratimimus* CCUG 10230 and *M. odoratus* DSM 2801, 293 unique CDSs were found in the A21 genome (Burghartz *et al*., 2015). In addition, five genomic islands were predicted by Island Viewer analysis (Burghartz *et al*., 2015). However, as the antibiotic treatment history was not described and the antibiotic resistance status of this strain was not given, the antibiotic resistance mechanisms could not be analyzed from the data.

Table 5 Reported RefSeq genome of *Myroides odoratus* CIP 103059 and DSM 2801

Strain	Name	RefSeq	INSDC	Size (Mb)	Total number of genes	Total number of proteins	rRNA	tRNA	Other genes	GC content (%)	Pseudogenes
CIP 103059	Master WGS	NZ_AGZJ000000 00.1	AGZJ000000 00.1	4.23	3773	3631	10	67	1	35.8	64
DSM 2801		NZ_CM001437.1	CM001437.1	4.3	3838	3695	9	74	1	35.8	59

Cited from GenBank assembly accession: GCA_000243275.1 and GCA_000297875.1. INSDC: International Nucleotide Sequence Database Collaboration

To study these aspects in our *M. odoratimimus* strain PR63039, we extracted its genomic DNA. Agarose gel electrophoresis results from several experiments revealed that it might harbor at least six different types of plasmids which could be related to antibiotic resistance (Fig. 1). However, the exact number of plasmid type needs further confirmation. Many bacterial drug-resistance genes are plasmid- or chromosome-mediated, but we did not know which mechanism was operating in *Myroides* sp. The genome of strain PR63039 was sequenced using an Ion Torrent Personal Genome Machine. We generated 610 contigs and 4221 open reading frames. The total sequence numbers with Gene Ontology (GO) and Clusters of Orthologous Groups (COG) of protein were 2741 and 1026, respectively. However, we could not completely assemble the genome and plasmids, so the multi-drug resistance mechanisms of strain PR63039 still could not be clarified. We are now using PacBio single-molecule real-time technology in the hope of generating a complete genome sequence of both the chromosome and plasmids, to elucidate the mechanisms of resistance and pathogenesis of this strain.

All the infection reports from China indicated that *Myroides* sp. is a serious source of nosocomial infection and has the potential to cause a pandemic. The completion of the *Myroides* sp. genome sequence and detailed bioinformatics analysis are imperative for the understanding of its mechanisms of antibiotic resistance and pathogenesis.

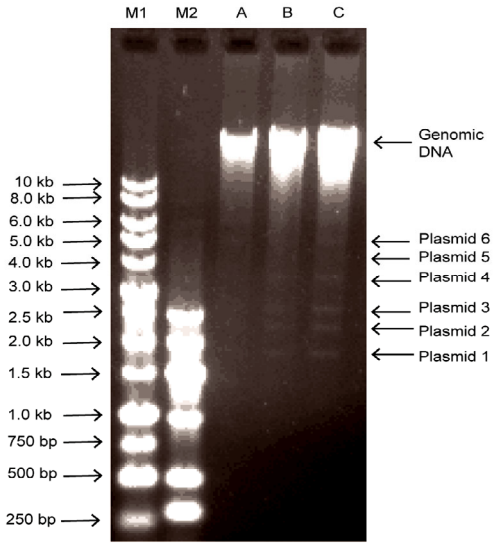

Fig. 1 Agarose gel electrophoresis of genomic DNA of *Myroides odoratimimus* strain PR63039

The genomic DNA of PR63039 was separated by gel electrophoresis using 0.75% (7.5 g/ml) agarose. The genomic DNA was about 23 kb in size. M1: 1 kb DNA marker; M2: DNA marker-G; A: 0.173 μg DNA; B: 0.273 μg DNA; C: 0.328 μg DNA. The genomic DNA was about 23 kb in size. Six different types of plasmids (1–6) were visible

6 Discussion and outlook

Myroides sp. is an opportunistic and extensively antibiotic-resistant pathogen. Infections have not been widely reported, though there have been many cases in China. As the antibiotic resistance mechanisms of *Myroides* sp. are still unclear, and in view of the risk of nosocomial infection and pandemics, novel technologies, such as whole genome sequencing and further bioinformatic analyses, should be applied urgently to *Myroides* sp. An outline of a strategy for whole genome sequencing and bioinformatic analyses is presented in Fig. 2. These analyses will also be helpful in developing appropriate management strategies. Moreover, whole genome sequencing might become a routine diagnosis method for all microbial infections in the near future.

Compliance with ethics guidelines

Shao-hua HU, Shu-xing YUAN, Hai QU, Tao JIANG, Ya-jun ZHOU, Ming-xi WANG, and De-song MING declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

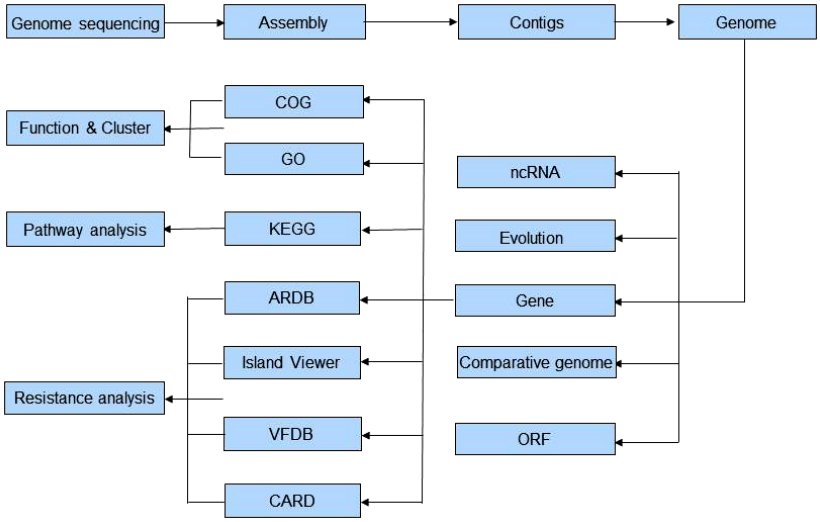

Fig. 2 Procedure of the strategy of whole genome sequencing and bioinformatics analyses

COG: Clusters of Orthologous Group; GO: Gene Ontology; KEGG: kyoto encyclopedia of genes and genome; ARDB: Antibiotic Resistance Genes Database; VFDB: Virulence Factor Database; CARD: Comprehensive Antibiotic Research Database; ncRNA: noncoding RNA; ORF: open reading frame

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中文概要

题　目：芳香黄杆菌的耐药机制

概　要：芳香黄杆菌广泛耐药，一旦感染很难治愈。其耐药机制尚不清楚，需要进一步研究。本文对国内外的芳香黄杆菌病例进行全面总结，分析该细菌的抗生素耐药情况、耐药机制、病人预后、医院内爆发感染和流行的风险，以及目前实验室应用的诊断方法，指出全基因组测序应用于阐明其耐药机制的可行性和迫切性。

关键词：芳香黄杆菌；抗生素耐药；鉴定方法；16S rRNA基因测序；下一代测序