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Genomic Insights of A Multi-Drug Resistant *Comamonas kerstersii* Isolate

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Abstract: A multi-drug resistant *C. kerstersii* 121606 was isolated from a patient with a urinary tract infection, and the antimicrobial susceptibility testing (AST) and whole genome sequencing were performed, then comparative genomic analyses were conducted with seven representative *Comamonas* species strains and *Acidovorax* species by average nucleotide identity (ANI) analysis using OrthoANI and single nucleotide polymorphism (SNP) analysis by snpTree web server. Finally, genomic sequence annotation was conducted with RAST server, as well as functional annotation of orthologous clusters by OrthoVenn, prediction of antibiotic resistance genes (ARGs) by CARD, CRISPRs by CRISPR recognition tool, and prophages by PHAST. These data revealed that *C. kerstersii* 121606 was a multi-drug resistant bacterium with similar genetic components to other seven *Comamonas* species and *Acidovorax* species. The presence of ARGs in its genome helped to explain its multi-drug resistance mechanisms. These findings provide valuable insights for the study of novel antibiotics to manage multi-drug resistant *C. kerstersii* infections.

Keywords: *Comamonas kerstersii*; multi-drug resistance; comparative genomic analysis; antibiotic resistance gene; CRISPR; prophage

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一株多药耐药 *Comamonas kerstersii* 细菌的基因组分析

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摘要: 从1名尿路感染患者中分离出了1株多药耐药的 *Comamonas kerstersii* (*C. kerstersii*) 菌株 121606, 对其进行了抗微生物药敏试验 (AST) 和全基因组测序; 然后将其与7个具有代表性的 *Comamonas* 菌株和 *Acidovorax* 菌种进行基因组比较分析, 包括使用 OrthoANI 分析平均核苷酸同一性 (ANI), 以及通过 snpTree 网络服务器进行单核苷酸多态性 (SNP) 分析。最后, 使用 RAST 服务器进行基因组序列, 使用 OrthoVenn 软件对同源簇进行功能注释, 通过 CARD 数据库对抗生素耐药基因 (ARGs) 进行预测, 并利用 CRISPR 识别工具预测 CRISPR, 以及利用 PHAST 软件预测前噬菌体。结果表明: *C. kerstersii* 121606 是一种多药耐药细菌, 其遗传成分与其他7个 *Comamonas* 和 *Acidovorax* 菌种相似; 其基因组中存在的 ARGs 有助于解释其多药耐药机制。这些发现为研究新型抗生素来控制多药耐药 *C. kerstersii* 感染提供了有价值的见解。

关键词: *Comamonas kerstersii*; 多药耐药性; 比较基因组分析; 抗生素耐药性基因; CRISPR; 前噬菌体

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Comamonas kerstersii (*C. kerstersii*) is a gram-negative, aerobic, motile rod bacterium^[1] that mainly caused the gastrointestinal tract infections^[2-4]. It is rarely resistant to antibiotics^[2, 5]. However, we isolated a multi-drug resistant strain, 121606, from the urine of an 82-year-old male patient with a urinary tract infection secondary to prostatic hyperplasia. This strain was resistant to 18 out of 21 antibiotics, including β -lactams, aminoglycosides, quinolones, sulfonamide and colistin, indicating that it was a multi-drug resistant strain. The property of multi-drug resistance of *C. kerstersii* 121606 has not been reported. As the infections of *C. kerstersii* are rapidly increasing, it is imperative to understand the genomic features and the antibiotic resistance mechanisms of *C. kerstersii* strain 121606 for better management of the increasing *C. kerstersii* infections. To achieve these purposes, in this paper, we performed genomic sequencing for strain 121606, conducted comparative genomic analyses, and correlated the antibiotic resistance genes (ARGs) in the genome with its multi-drug phenotype.

1 Materials and Methods

1.1 Bacterium Identification and Antimicrobial Susceptibility Testing

Bacterium identification and antimicrobial susceptibility testing (AST) of *C. kerstersii* 121606 were performed by BD PhoenixTM automated identification and antibiotic susceptibility testing system with the NMIC/ID-4 panel (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), according to CLSI categories.

1.2 DNA Extraction and 16S rRNA Gene Sequencing

DNA extraction and 16S rRNA gene sequencing of *C. kerstersii* 121606 were performed as described previously^[6]. It was deposited into GenBank under genus *Acidovorax* (1 460 bp, GenBank No. KY014106).

1.3 Genome Sequencing, Assembly, Annotation and Analysis of General Components in *C. kerstersii* 121606 Genome

Sequencing of the genomic DNA of *C. kerstersii* 121606 was completed with Illumina HiSeqTM 2000 (Illumina, San Diego, CA, USA) with 500 bp library preparations. The clean data were obtained (476 Mb). Genome assembly was carried out by SOAP *de novo* (version 2.04), and the genomic size was 3.34 Mb (196 contigs, GenBank assembly accession No. GCA_002002445.1, ASM200244v1).

The genomic sequences were annotated with RAST server^[6]. The identification of rRNA genes, genomic GC content, insertion sequence (IS) elements was completed with RNAmmer 1.2 server^[7].

1.4 Comparative Genomic Analyses of *C. kerstersii* Strains

To understand the origin, genomic compositions of *C. kerstersii* 121606, five representative *Comamonas* species and two *Acidovorax* species were comparatively analysed (GenBank accession numbers: *C. kerstersii* 8943, CP020121.1; *C. kerstersii* J29, NZ_LFYYP00000000.1; *C. kerstersii* UBA11446, DOQQ00000000.1; *C. testosterone* TK102, CP006704.1; *C. aquatica* CJG, CP016603.1; *Acidovorax* sp. RAC01, NZ_CP016447.1; *Acidovorax* sp. 1608163, NZ_CP033069.1). Both *C. kerstersii* 8943 and *C. kerstersii* J29 were isolated from the same source—dialysis effluent of patients with peritonitis caused by continuous ambulatory peritoneal dialysis (CAPD) by the same team from Zhejiang Province, China^[8].

Average nucleotide identity (ANI) analysis was operated by OrthoANI^[9]. The single nucleotide polymorphism (SNP) differences between each *C. kerstersii* genomes were calculated using the snpTree web server for the assembled genomes with minimum coverage and minimum distance be-

tween SNPs^[10]. Functional annotation of orthologous clusters among the *C. kerstersii* strains was conducted by OrthoVenn^[11]. The prediction of CRISPRs (clustered regularly interspaced short palindromic repeats), prophages in our *C. kerstersii* 121606 were carried out with CRISPR recognition tool (CRT, version CRT1.1)^[12], PFAST^[13] respectively.

1.5 Analysis of Antibiotic Resistance Mechanisms of *C. kerstersii* 121606 at Genomic Level

The ARGs in *C. kerstersii* 121606 was predicted through CARD database, then filtered with more stringent cut-off parameters (identity>50%, query coverage>50%, subject coverage>50%, match length>100 amino acids, identical amino acids>100).

2 Experimental Results and Analysis

2.1 Identification and Antibiotic Susceptibilities Profiles of *C. kerstersii* 121606

With BD PhoenixTM automated identification and AST system, *C. kerstersii* 121606 was initially misidentified as *Sphingobacterium multivorum* (92% confidence). Moreover, because its 16S rRNA gene sequence displayed high identity with *Acidovorax* sp. 98-63833 (GenBank No. AY258065.1), it was again misidentified as *Acidovorax* sp. and deposited as *Acidovorax* sp. in GenBank after BlastN analysis.

The AST (table 1) displayed that *C. kerstersii* 121606 was resistant to 18 out of 21 tested antibiotics, including aminoglycosides (amikacin, gentamicin), nearly all β -lactams {penicillins (ampicillin,

Tab. 1 Antibiotic resistance profiles of *C. kerstersii* 121606

表 1 *C. kerstersii* 121606 的耐药性谱

Antibiotics classification	Name of antibiotics	121606
Aminoglycosides	Amikacin	R (>32)
	Gentamicin	R (>8)
Carbapenems	Imipenem	R (>8)
	Meropenem	IS (4)
Cephalosporins	Cefazolin	R (>16)
	Ceftazidime	R (>16)
	Cefotaxime	R (>32)
	Cefepime	R (>16)
Monobactams	Aztreonam	R (>16)
	Ampicillin	R (>16)
Penicillins	Piperacillin	R (>64)
	Amoxicillin-clavulanate	R (>16/8)
	Ampicillin-sulbactam	R (>16/8)
	Piperacillin-tazobactam	IS (64/4)
Chloramphenicols	Chloramphenicol	R (>16)
Polypeptides	Colistin	R (>2)
	Ciprofloxacin	R (>2)
Quinolones	Levofloxacin	R (>8)
	Moxifloxacin	R (>4)
Sulfonamides	Trimethoprim-sulfamethoxazole	R (>2/38)
Tetracyclines	Tetracycline	S (4)

Notes: S represents sensitive; IS represents intermediately sensitive; R represents resistant.

注: S 表示敏感; IS 代表中度敏感; R 代表抗性。

piperacillin, amoxicillin-clavulanic acid, ampicillin-sulbactam, piperacillin-tazobactam), cephalosporins (cefazolin, ceftazidime, cefotaxime, cefepime), carbapenems (imipenem), monobactams (aztreonam)}, chloramphenicols (chloramphenicol), polypeptides (colistin), quinolones (ciprofloxacin, levofloxacin, moxifloxacin), sulfonamides (trimethoprim/sulfamethoxazole), intermediately suscepti-

ble to meropenem, piperacillin-tazobactam, and sensitive to tetracycline. These data showed that *C. kerstersii* 121606 possessed a multi-drug resistant phenotype.

2.2 General Genomic Components of *C. kerstersii* 121606

The assembled genome was composed by 196 contigs and 53 scaffolds, the repeat accounted for 31.2%. The genome size was 3 419 381 bp, and GC content was 59.68%. It had 3 291 coding sequences (CDS), 3 128 insertion sequences (ISs), 17 tRNAs, 3 rRNAs.

2.3 Subsystem Features of *C. kerstersii* 121606 and Orthologues in Four Representative *Comamonas* Species and Two *Acidovorax* Species

The subsystem features of *C. kerstersii* 121606 generated by RAST server revealed that it contained 27 subsystem categories (A-A' in figure 1). For example, it contained 80 genes related to virulence, disease and defense, 6 related to phage, prophage (table 2). Further subsystem category analysis revealed that *C. kerstersii* 121606 had two phages, which was consistent to the number predicted by PHAST, as described below.

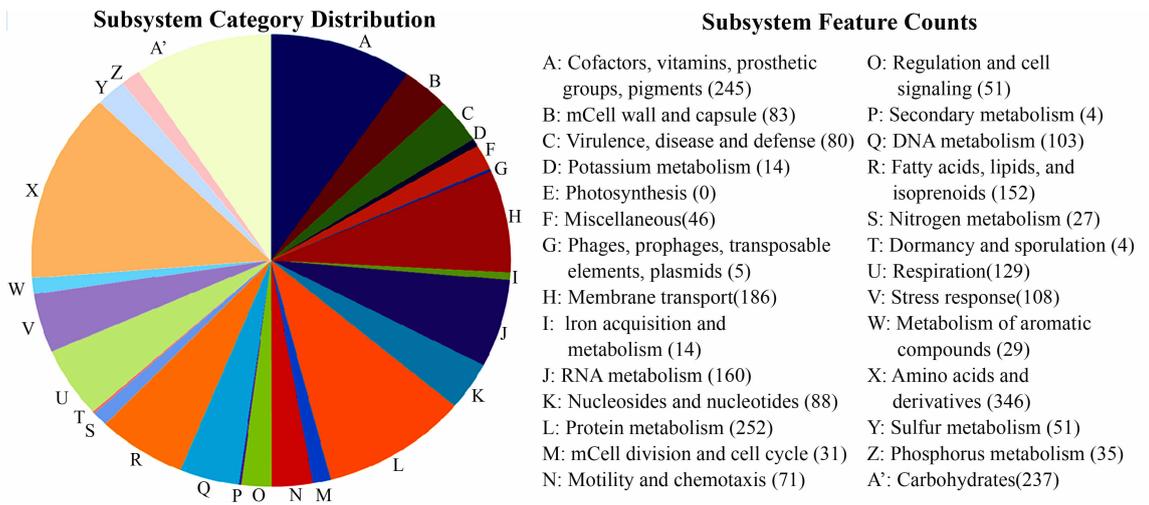


Fig. 1 Subsystem categories distribution of genome of *C. kerstersii* 121606 analyzed by RAST server

图 1 应用 RAST 服务器分析 *C. kerstersii*121606 基因组的子系统分类分布

Notes: In the 27 subsystem categories of A-A', the values in parentheses represent the gene count of that subsystem category.

注: 在 A~A' 的 27 个子系统类别中, 括号内的数值表示该子系统类别的基因计数。

Tab. 2 Subsystem features of *C. kerstersii* strain 121606

表 2 *C. kerstersii* 菌株 121606 的子系统特征

Subsystem features	Function classification	Function description	Subsystem feature counts
Virulence, disease and defense	Resistance to antibiotics and toxic compounds	Multiple antibiotic resistance MAR locus	1
		Copper homeostasis	16
		Bile hydrolysis	3
		Cobalt-zinc-cadmium resistance	9
		Resistance to fluoroquinolones	4
		Arsenic resistance	4
		Copper homeostasis: copper tolerance	4
		β -lactamase	2
		Multidrug resistance efflux pumps	18

Continue table

续表

Subsystem features	Function classification	Function description	Subsystem feature counts
Virulence, disease and defense	Bacteriocins, ribosomally synthesized antibacterial peptides	Tolerance to colicin E2	1
		Colicin V and bacteriocin production cluster	9
	Invasion and intracellular resistance	Involved in DNA transcription	2
		Involved in protein synthesis	7
Phages, prophages, transposable elements and plasmids	Phages, prophages	Phage replication	2
		Phage packaging machinery	2
		Phage tail fiber proteins	1
		Phage capsid proteins	1
		Phage lysis modules	0

2.4 ANI Analysis and Phylogenetic Tree of *C. kerstersii* 121606 With Five Representative *Comamonas* Species and Two *Acidovorax* Species

To reconfirm this *C. kerstersii* isolate 121606, we calculated the ANI values with other five representative *Comamonas* species and two closely related *Acidovorax* species. *C. kerstersii* 121606 showed high identities with *C. kerstersii* 8943, J29, UBA11446 (99.39%, 99.40%, 99.08%, respectively), and its identities with *C. testosterone* TK102, *C. aquatica* CJG, *Acidovorax* sp. 1608163, and *Acidovorax* sp. RAC01, were low (76.71%, 81.34%, 74.95%, 75.07%, respectively). According to the microbial taxonomy for species delineation ((95±0.5)% cut-off for ANI)^[14], these data suggested that *C. kerstersii* 121606 really belonged to the species *C. kerstersii*, the identification as *S. multivorum* by the BD PhoenixTM automated identification method or as *Acidovorax* sp. by 16S rRNA gene sequence was not correct.

The phylogenetic tree based on SNPs showed that *C. kerstersii* 121606, 8943, J29 and UBA11446 grouped together and other bacteria constituted a monophyletic cluster (figure 2). In figure 2, the tree was built using snpTree server with default parameters; the maximum likelihood tree was based on SNPs alignment; tree was shown in the rectangular cladoGram format in which branches were drawn with reference to branch lengths. The tree topology evaluation was based on percentage of concordance. Their distance to *C. testosterone* TK102, *C. aquatica* CJG, *Acidovorax* sp. 1608163 and *Acidovorax* sp. RAC01 was the same as the ANI-based phylogenetic tree (not shown).

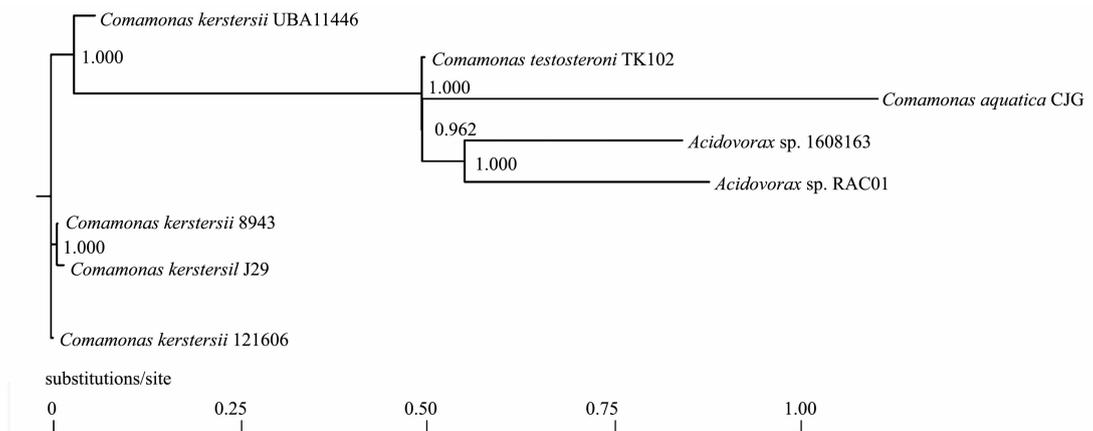


Fig. 2 SNP phylogenetic tree of *C. kerstersii* strains and *Acidovorax* species

图 2 *C. kerstersii* 菌株及 *Acidovorax* 菌种的 SNP 系统发育树

The orthologues of *C. kerstersii* 121606 were compared with other three *C. kerstersii* genomes (strain 8943, J29 and UBA11446). It showed that strain 121606 had 2 955 proteins, 2 943 clusters,

11 singletons. 2 095 clusters were shared by these four *C. kerstersii* strains. Further, the gene family clusters of these five *Comamonas* species and two *Acidovorax* species were presented in figure 3. In agreement with above statements, *C. kerstersii* strain 121606, 8943, J29 and UBA11446 exhibited a closely related similarity in single- or multiple-copy orthologs.

2.5 Prediction of CRISPR in *C. kerstersii* 121606

CRISPRs are vital constituents of RNA-based viral defense systems possessed by bacteria and archaea^[15]. CRISPR loci are composed by leader sequence, repeats, spacers and cas gene cassette^[15]. *C. kerstersii* 121606 was predicted to contain 10 nearly identical 29-nucleotide CRISPR repeats (GGTTGCCCGCACACGCGGGGATAGGCC) (Range: 2657125-2657702 in genome), which separated by 32 nucleotides spacers in average length. The constituent characteristics of these CRISPRs were same as those originally found in *Escherichia coli* genome in 1987^[16].

2.6 Prediction of Prophages in *C. kerstersii* 121606

The *C. kerstersii* 121606 genome harbored an incomplete (a) and an intact prophage region (b) (figure 4). The intact prophage region b was predicted to extend from 1 799 206 bp to 1 836 855 bp (37 650 bp in length) and it carried 35 CDSs. Significantly, one integrase used as the marker of mobile

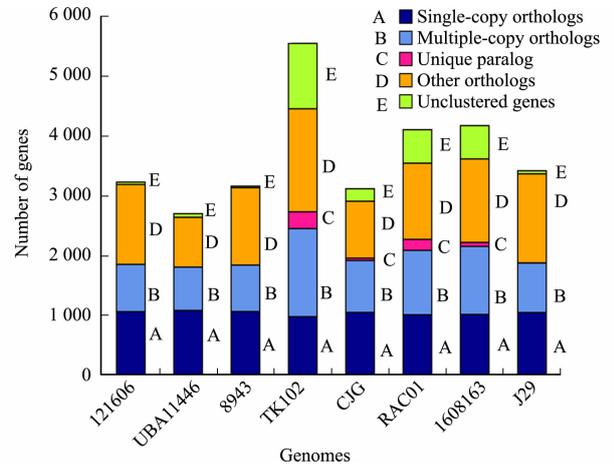


Fig. 3 Orthologous distribution of *C. kerstersii* strains and *Acidovorax* species

图3 *C. kerstersii* 菌株及 *Acidovorax* 菌种的同源分布

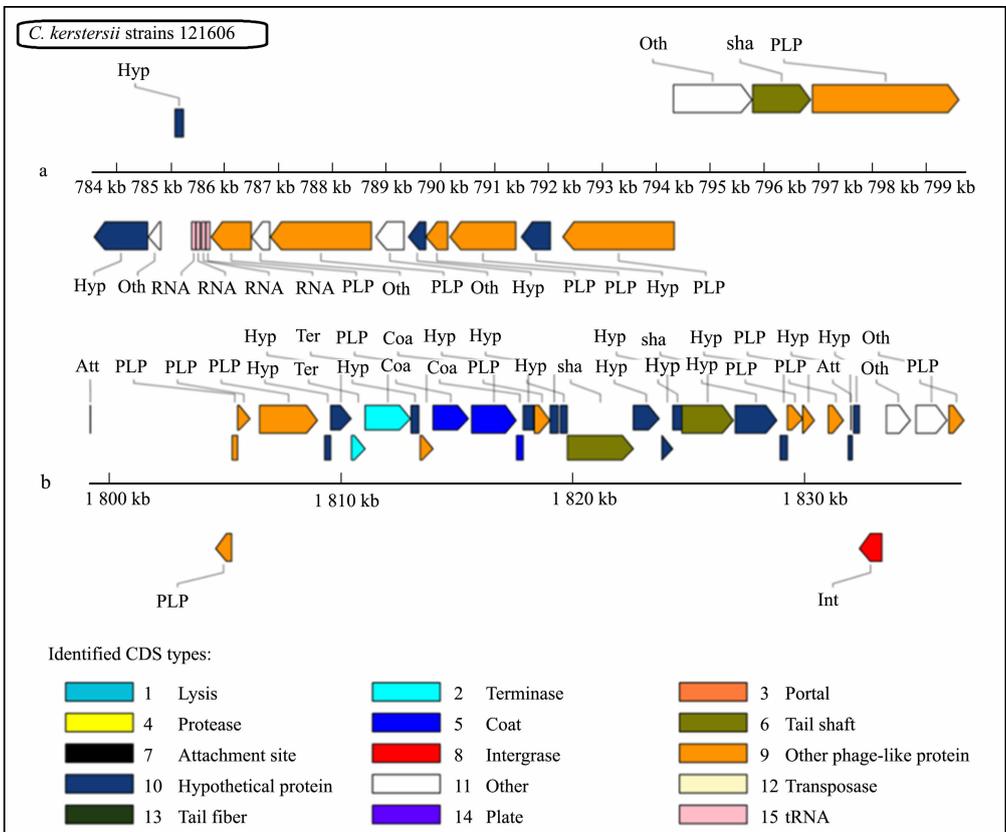


Fig. 4 Predicted prophage regions and elements in genomes of *C. kerstersii* 121606

图4 *C. kerstersii* 121606 基因组中预测的前噬菌体区域和组成原件

DNA elements^[17] was identified in b, and the *att* sites used to determine the extent of the prophage^[18]

were also found in b, meaning that the intact prophage region b is disseminatable? This assumption requires laboratory verification.

2.7 Resistome of *C. kerstersii* 121606

C. kerstersii 121606 (table 3) harbored a number of ARGs contributing to the resistance to β -lactams (*bla*_{KPC-2}, *bla*_{SPM-1}), fluoroquinolone (*AANT(4')*-*Ib*, *apmA*, *mfd*, *opmH*, *oqxB*), polymyxin (*basR*), elfamycin (*basS*, *lpxC*), fosfomycin (*pvrR*), aminocoumarin, elfamycin, fluoroquinolone, isoniazid, lipopeptide antibiotics, and rifamycin[*tet(35)*], or encoding efflux pump complex subunits or regulators (*acrB*, *acrD*, *acrF*; *mexA*, *mexB*, *mexQ*, *oprM*, *mexR*; *smeB*, *smeD*, *smeE*; *adeJ*; *mdtF*). The presence of these collective ARGs was consistent with the AST profiles of *C. kerstersii* 121606.

Tab. 3 Predicted antibiotic resistance genes in *C. kerstersii* 121606 genome

表 3 *C. kerstersii* 121606 基因组中抗生素抗性基因的预测

Antibiotic resistance genes	Functions	Antibiotic resistance genes	Functions	
<i>bla</i> _{KPC-2}	<i>Klebsiella pneumoniae</i> carbapenemase-2	<i>ANT(4')</i> - <i>Ib</i>		
<i>bla</i> _{SPM-1}	Sao Paulometallo- β -lactamases-1	<i>apmA</i>	Determinant of fluoroquinolone resistance	
<i>acrB</i>		<i>mfd</i>		
<i>acrD</i>		<i>opmH</i>		
<i>acrF</i>		<i>oqxB</i>		
<i>mexA</i>		<i>basR</i>	Determinant of polymyxin resistance	
<i>mexB</i>		<i>basS</i>	Determinant of elfamycin resistance	
<i>mexQ</i>	Efflux pump complex or subunit conferring antibiotic resistance	<i>lpxC</i>	Determinant of elfamycin resistance, protein(s) conferring antibiotic resistance via molecular bypass	
<i>oprM</i>				
<i>mexR</i>			<i>pvrR</i>	Determinant of fosfomycin resistance
<i>smeB</i>				
<i>smeD</i>				
<i>smeE</i>				Determinant of resistance to aminocoumarin, elfamycin,
<i>adeJ</i>			<i>tet(35)</i>	fluoroquinolone, isoniazid, lipopeptide antibiotics, rifamycin
<i>mdtF</i>				

Notes: +/− indicates presence or absence of the resistance gene in the genome predicted by CARD; bold indicates discussed.

注: +/− 分别表示 CARD 预测的基因组中抗性基因的存在或不存在;粗体表示已讨论。

3 Discussion

3.1 Underestimated *C. kerstersii* Infections in Clinical Diagnosis

As mentioned above, during our laboratory diagnosis, *C. kerstersii* 121606 was initially misidentified as *S. multivorum*. Later, it was misidentified as *Acidovorax* sp. even by the gold standard diagnosis method, 16S rRNA gene sequencing. In fact, we had the same misidentification experience with other two *C. kerstersii*, 202149 and 12322-1 (unpublished). Their identities as *C. kerstersii* were not determined until their genomic sequences were obtained for ANI analysis. There are increasing reports of *C. kerstersii* misidentification due to unreliable phenotypic identification tests prior to the application of MALDI-TOF^[4,19-20]. As a result, actual *C. kerstersii* infections might be seriously underestimated.

3.2 Origin of *C. kerstersii* 121606 Revealed by Comparative Genomic Analyses

The phylogenetic trees based on both ANI values and SNPs confirmed that *C. kerstersii* 121606 is a strain of the species *kerstersii* and is closely related to *C. kerstersii* 8943 and *C. kerstersii* J29. Additionally, the orthologous analysis revealed that strains *C. kerstersii* 121606, 8943, and J29 contained

highly similar single- or multiple-copy orthologs. It is known that *C. kerstersii* 8943 and *C. kerstersii* J29 were isolated from the same source in Zhejiang Province, China^[8], which is not far from Quanzhou City, the isolation site of *C. kerstersii* 121606. Therefore, *C. kerstersii* 121606 might have the same ancestor as *C. kerstersii* 8943 and J29.

3.3 Multi-Drug Phenotype of *C. kerstersii* 121606 Explained by Presence of Number of Antibiotic Resistance Genes in Genome

According to table 1, *C. kerstersii* 121606 exhibited the multi-drug phenotype of *C. kerstersii*, which had not been previously reported. For instance, it was noted that nine of the antibiotics to which *C. kerstersii* 121606 was resistant (amikacin, gentamicin, imipenem, cefepime, cefotaxime, ciprofloxacin, ampicillin-sulbactam, piperacillin-tazobactam, trimethoprim-sulfamethoxazole) were sensitive in 14 clinical *C. kerstersii* strains^[21]. Additionally, 11 of the antibiotics to which *C. kerstersii* 121606 was resistant (ampicillin, ampicillin-sulbactam, cefotaxime, ceftazidime, cefepime, imipenem, gentamicin, amikacin, ciprofloxacin, colistin, trimethoprim-sulfamethoxazole) were susceptible in four clinical *C. kerstersii* isolates^[5]. The AST profiles of strain 121606 (table 1) could be explained by the corresponding ARGs in its genome (table 3).

β -lactams are the primary antibiotics used to treat bacterial infections. The rapid increase in bacterial resistance to β -lactams posed a serious health threat. The mechanisms of β -lactam resistance include inactivation by β -lactamases, efflux pumps, reduced permeability, and altered transpeptidases^[22]. *C. kerstersii* 121606 possessed at least the former two mechanisms.

Firstly, the presence of the β -lactamase encoding genes *bla*_{SPM-1} and *bla*_{KPC-2} in *C. kerstersii* 121606 explained its resistance to the tested β -lactams, including penicillins (ampicillin, piperacillin, amoxicillin-clavulanic acid, piperacillin-tazobactam), cephalosporins (ceftazidime, cefotaxime, cefepime), monobactams (aztreonam), and carbapenems (imipenem) (table 1).

SPM-1 (Sao Paulo metallo- β -lactamases) is a Class B metallo- β -lactamase, first identified in *Pseudomonas aeruginosa* strain 48-1997A isolated in Sao Paulo, Brazil^[23]. It shows hydrolytic activity against penicillins (benzylpenicillin, ampicillin, carbenicillin, azlocillin, piperacillin), with the highest activity for benzylpenicillin, ampicillin, and piperacillin, and preferential hydrolyzing activity against cephalosporins (cephalothin, cefuroxime, cefotaxime, cefoxitin, cefepime), carbapenems (imipenem, meropenem), the serine β -lactamase inhibitor tazobactam, but not against the monobactam aztreonam or the serine β -lactamase inhibitor clavulanic acid. The latter two are competitive inhibitors of SPM-1^[23]. The presence of *bla*_{SPM-1} was consistent with the resistance of *C. kerstersii* 121606 to ampicillin, piperacillin, cefotaxime, cefepime, and imipenem.

KPC-2 is a homolog of various *Klebsiella pneumoniae* carbapenemases (KPCs), the most typical example of a class A carbapenemase, which also confers resistance to most β -lactams. KPC-1 was first found in a *K. pneumoniae* isolate in 1996 in the United State, and showed hydrolytic activity against penicillin (benzylpenicillin, ampicillin, cloxacillin, piperacillin), cephalosporins (cefotaxime), carbapenem (imipenem and meropenem), and monobactams (aztreonam)^[24]. The KPC-1 transforming expressing bacteria showed resistance to penicillins (ampicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam), cephalosporins (ceftazidime, cefoxitin, cefpodoxime, cefotaxime, ceftriaxone), carbapenems (imipenem, meropenem), and monobactams (aztreonam)^[24]. Initially identified KPC-2 also has hydrolytic activity against penicillins and cephalosporins but not cephamycins and ceftazidime. Recently emerged KPC-2 variants evolved by single and double amino acid substitution among clinical isolates showed increased hydrolytic activity against ceftazidime and consequently increased resistance to ceftazidime, while maintaining the resistance capacity to penicillins and carbapenems^[25]. The presence of *bla*_{KPC-2} was closely related to the resistance of *C. kerstersii* 121606 to ampicillin, amoxicillin-

clavulanic acid, piperacillin-tazobactam, ceftazidime, cefotaxime, imipenem, and aztreonam.

Secondly, for the efflux pumps, the *C. kerstersii* 121606 genome possessed several type of multi-drug efflux systems, which would further enhance resistance to β -lactams and other antibiotics listed in table 1.

The genes *acrD*, *acrB*, and *acrF* encode three types of RND (Resistance Nodulation and cell Division) efflux pumps that confer resistance to monobactams (aztreonam), cephalosporins (cefazolin), and aminoglycosides (amikacin, gentamicin) and quinolones (ciprofloxacin, levofloxacin, moxifloxacin). For example, the AcrD (encoded by *acrD*) containing AcrAD-TolC efflux pump confers resistance to aztreonam, cefazolin in *Salmonella typhimurium*^[26], and aminoglycosides (amikacin, gentamicin, neomycin, kanamycin, and tobramycin) in *E. coli*^[27]. AcrAB-TolC, a major and clinically important efflux pump in Gram-negative bacteria, and another type of RND efflux pumps, AcrEF-TolC, contribute to resistance to fluoroquinolones, which are often used as therapeutic alternatives in infections^[28].

For the component and structure of RND efflux pumps in Gram-negative bacteria, for example, the AcrAB-TolC multidrug efflux pump complex is formed by assembling the homotrimeric inner membrane protein (IMP) AcrB, which recognizes, binds and translocates substrates, and transduces the energy (proton motive force, PMF) for the drug/proton antiport process, the hexameric membrane fusion protein (MFP) AcrA, which forms a membrane fused tubular structure inside the periplasmic space, and the homotrimeric outer membrane factor (OMF) TolC^[29], constituting a main means of intrinsic resistance against cytotoxic substances^[30].

The TolC encoding gene *tolC*, and the AcrEF efflux pump encoding genes *acrE* and *acrF* were predicted in *C. kerstersii* 121606, but not *acrA*. *acrE* was predicted to encode a 399 amino acid-AcrE protein in *C. kerstersii* 121606 through CARD database, but it was filtered out and not listed in table 3 because its identity with the reference gene was below 50%. However, AcrE and AcrD/AcrF are homologues of AcrA and AcrB, and AcrE can substitute AcrA to form a functional complex with AcrD by associating with the same residues identified in AcrB binding^[31]. So, we supposed that *C. kerstersii* 121606 possessed functional AcrEF, AcrED, or AcrEB-TolC multidrug efflux pumps, resulting in resistance to monobactams (aztreonam), cephalosporins (cefazolin), aminoglycosides (amikacin, gentamicin) and quinolones (ciprofloxacin, levofloxacin, moxifloxacin) (table 1).

In addition, *C. kerstersii* 121606 may have MexAB-OprM, which is found in *P. aeruginosa*, as it harbored their encoding genes *mexA*, *mexB*, *oprM* and one of the repressor gene, *mexR*. Overexpression of MexAB-OprM induced by *mexR* mutations can export various antibiotics, including penicillins (penicillin G cloxacillin, nafcillin, amoxicillin, piperacillin, carbenicillin, sulbenicillin), cepheims (cefamandole, cefuroxime, cefoperazone, cefotaxime, ceftizoxime, ceftriaxone, ceftazidime, cefpirome, cefepime, ceftazopran, cefoselis, ceftioxin), oxacephems (moxalactam, flomoxef), monobactams (aztreonam), quinolones (nalidixic acid, piromidic acid, pipemidic acid, cinoxacin, norfloxacin, ofloxacin, enoxacin, ciprofloxacin, tosufloxacin, sparfloxacin), macrolides (erythromycin, oleandomycin, spiramycin), lincosamides (lincomycin), chloramphenicols (chloramphenicol), and novobiocin (novobiocin)^[32]. If MexAB-OprM really conferred the resistance of *C. kerstersii* 121606 to penicillins (amoxicillin, piperacillin), cephalosporins (cefotaxime, ceftazidime, cefepime), monobactams (aztreonam), quinolones (ciprofloxacin), chloramphenicol (chloramphenicol)] (table 1), sequencing of the full-length *mexR* gene in *C. kerstersii* 121606 should be performed to verify whether it is a loss-of-function mutant^[33], leading to overexpression of MexAB-OprM and contributing to the resistance. In fact, *C. kerstersii* 121606 may express a series of Mex efflux pumps as it contained the genes *mexA-N*, *mexP-R*, *mexV-Y*, *mexA-N*, *mexP-R*, *mexV-Y*, *oprA*, *oprJ*, *oprM*, *oprN*,

oprZ^[34], most of which were not listed in table 3 due to the stringent filtering parameters mentioned above.

C. kerstersii 121606 might also possess another two homologues of the mexAB-*oprM* efflux pumps, SmeABC and SmeDE (a SmeB homologue) F (a SmeC homologue) multidrug efflux pump, as their encoding genes *smeA* (encoding the inner-membrane fusion lipoprotein), *smeB* (encoding the RND transporter), *smeC* (encoding the outer-membrane efflux lipoprotein), *smeD*, *smeE*, *smeF* (encoding the outer membrane protein), and the regulation genes *smeR* (encoding the two-component system sensory kinase which positively regulates expression of the *smeA*, *smeB*, *smeC* genes), *smeS* (encoding the two-component system response regulator) were predicted through CARD database, and *smeS* and *smeR* were located upstream of the *smeABC* genes and the *smeDEF* genes as reported^[35]. We should mention that, *smeA*, *smeC*, *smeF*, *smeR*, and *smeS* were not put into table 3 because their identities with the reference genes in CARD database were below 50%. The SmeABC efflux pump confers the resistance to aminoglycosides (amikacin, gentamicin, kanamycin, streptomycin, tobramycin), penicillins (penicillin, carbenicillin, ampicillin), (cefsulodin, cefotaxime, cefoperazone, cefepime, cefpirome), quinolones (nalidixic acid, ciprofloxacin, norfloxacin, trovafloxacin)^[35]. The efflux pump SmeDEF has broad substrate specificity and contributes to the resistance to a variety of antimicrobials, including quinolones, macrolides, chloramphenicol, and novobiocin^[34], trimethoprim-sulfamethoxazole in clinical isolates of *Stenotrophomonas maltophilia*^[36]. Aside from conferring the resistance to β -lactams (listed in table 3), the probable expression of AcrEF, AcrED, AcrEB-TolC, MexAB-OprM, *smeABC*, SmeDEF multidrug efflux pump also contributed to the resistance to aminoglycosides (amikacin, gentamicin), quinolones (ciprofloxacin, levofloxacin, moxifloxacin), chloramphenicol (chloramphenicol), sulfonamides (trimethoprim-sulfamethoxazole).

Overall, these ARGs contained in *C. kerstersii* 121606 genome explained its multi-drug resistance.

4 Conclusion

Comparative genomic analyses revealed that our clinical *C. kerstersii* isolate 121606 belonged to the species *C. kerstersii* and shared similar genetic components with five other *Comamonas* species and the closely related *Acidovorax* species. The ARGs in the genome were consistent with its multi-drug resistance phenotype. All the data needs to be verified by laboratory experiments and could help to find new drug to combat infections caused by multi-drug resistant *C. kerstersii*.

Ethics Statement: This article does not contain any studies with humans or animals performed by any of the authors. This work was approved by the Ethics Committee of Quanzhou First Hospital.

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