

Draft Genome Sequence of *Enterobacter hormaechei* ST93, a Clinical Tigecycline Resistant Strain Harboring blaNDM-1

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Abstract

Objective: The emergence of Carbapenem-resistant *E. cloacae* complex has been a serious challenge to manage in the clinic because of multi-drug resistance. Tigecycline is regarded as one of the last-resort for Carbapenem-resistant *E. cloacae* complex infections. Here, we report the draft genome sequence of a tigecycline resistant NDM-1-producing *E. hormaechei* strain ETR1 that was isolated from a male in China.

Methods: Whole-genomic DNA of *E. hormaechei* strain ETR1 was extracted and was sequenced using an Illumina-Hiseq™ X Ten platform. The generated sequence reads were assembled using CLC Genomics Workbench. The draft genome was annotated using Rapid Annotation using Subsystem Technology. Bioinformatics analysis was further performed.

Results: The 5,141,975 bp genome contains various antimicrobial resistance genes conferring resistance to beta-lactam, fosfomycin, fluoroquinolone and tetracycline. Notably, the strain was also resistant to carbapenem and tigecycline. In addition, virulence factor encoding genes were also identified.

Conclusion: The genome sequence will provide valuable information to understand antibiotic resistance mechanisms and pathogenic mechanisms in this strain. Close surveillance is urgently needed to monitor the spread of tigecycline resistant NDM-1-producing isolates.

Keywords: *Enterobacter cloacae* complex; NDM-1; Whole-genome sequencing

Introduction

Enterobacter cloacae complex is an opportunistic pathogen that can cause a variety of infections, especially in immune-compromised individuals. Moreover, it is able to carry a number of antibiotic resistance genes, which make it a difficult-to-treat pathogen [1]. There is a growing recognition that *E. cloacae* complex is now the third major *Enterobacteriaceae* species associated with healthcare-associated infections after *Escherichia coli* and *Klebsiella pneumoniae* [2]. It is worrying that (New Delhi metallo- β -lactamase) NDM-producing *E. cloacae* complex has reported during recent years, limiting the therapeutic options [1]. Tigecycline is regarded as one of the last-resort for CRE (carbapenem-resistant *Enterobacteriaceae*, CRE) infections. However, the emergence of tigecycline resistant CRE strains has been a serious challenge to public health.

Methods

In addition, few studies reported tigecycline resistant *E. hormaechei* strains. In the present study, the whole genome sequence of a clinical tigecycline resistant *E. hormaechei* sequence type 93 strain producing

NDM-1 carbapenemases was identified to understand antibiotic resistance and pathogenic mechanisms.

Strain *E. hormaechei* ETR1 was recovered from an ascites sample of a male patient who was admitted to the hospital for gall-stone in Hangzhou, Zhejiang Province, China, in 2016. The strain was grown overnight at 37°C in MH agar (Oxoid, United Kingdom). Strain *E. hormaechei* ETR1 was preliminarily identified by the VITEK 2 system (Sysmex-bioMérieux, Marcy l'Etoile, France) and further confirmed by 16S rRNA gene sequencing analysis. Antimicrobial susceptibility testing was performed by agar dilution or E-test method. All susceptibility tests were repeated at least three times and MICs were interpreted according to the CLSI guidelines, except tigecycline and polymyxin B, for which European Committee on Antimicrobial Susceptibility Testing breakpoints were used [3]. Whole-genomic DNA of this strain was extracted using a QIAamp DNA MiniKit (Qiagen, Valencia, CA, USA) following the manufacturer's recommendations. The genome was sequenced on an Illumina-Hiseq™ X Ten (Illumina Inc, San Diego, U.S.A) using a paired-end 2×100 bp protocol. Sequence reads were assembled using CLC Genomics Workbench v.10.0 software (QIAGEN, Hilden, Germany). The contigs were initially annotated using Rapid Annotation using Subsystem Technology (RAST) and were then manually checked. Resistance and virulence genes, the sequence type (ST) and plasmid Inc type of the strain were analysed using ResFinder 3.0, Virulence Finder 1.5, MLST 1.8 server and PlasmidFinder 1.3, which are available at the Center for Genomic

Epidemiology [4]. In addition, the ISfinder, CRISPRFinder and PFAST databases were used to characterize insertion elements (ISs), clustered regularly interspaced short palindromic repeats (CRISPRs) and prophage sequences, respectively, of strain *E. hormaechei* ETR1.

Discussion

Strain ETR1 was resistant to the majority of the antimicrobial agents tested, including ampicillin/sulbactam, aztreonam, ceftazidime, cefepime, ceftriaxone, ertapenem, cefotetan, imipenem, piperacillin/tazobactam, ciprofloxacin, levofloxacin, minocycline, tetracycline and tigecycline, with exception of polymyxin E and classified MDR bacteria

according to the recently proposed international classification scheme [5].

The draft genome consists of 5,141,975 bp with an N50 value of 155,713 bp with a 54.7% GC content. Using RAST analysis, 4671 ORFs and 80 RNAs gene were predicted, of which 3836 (82.1%) could be functionally annotated (Figure 1). The distribution of subsystems in this strain was described by RAST server based annotation of the whole genome. Proteins responsible for carbohydrates (651 ORFs), derivatives and amino acids (466 ORFs), and cofactors, vitamins, prosthetic groups and pigments (261 ORFs) were ample among the subsystem categories.

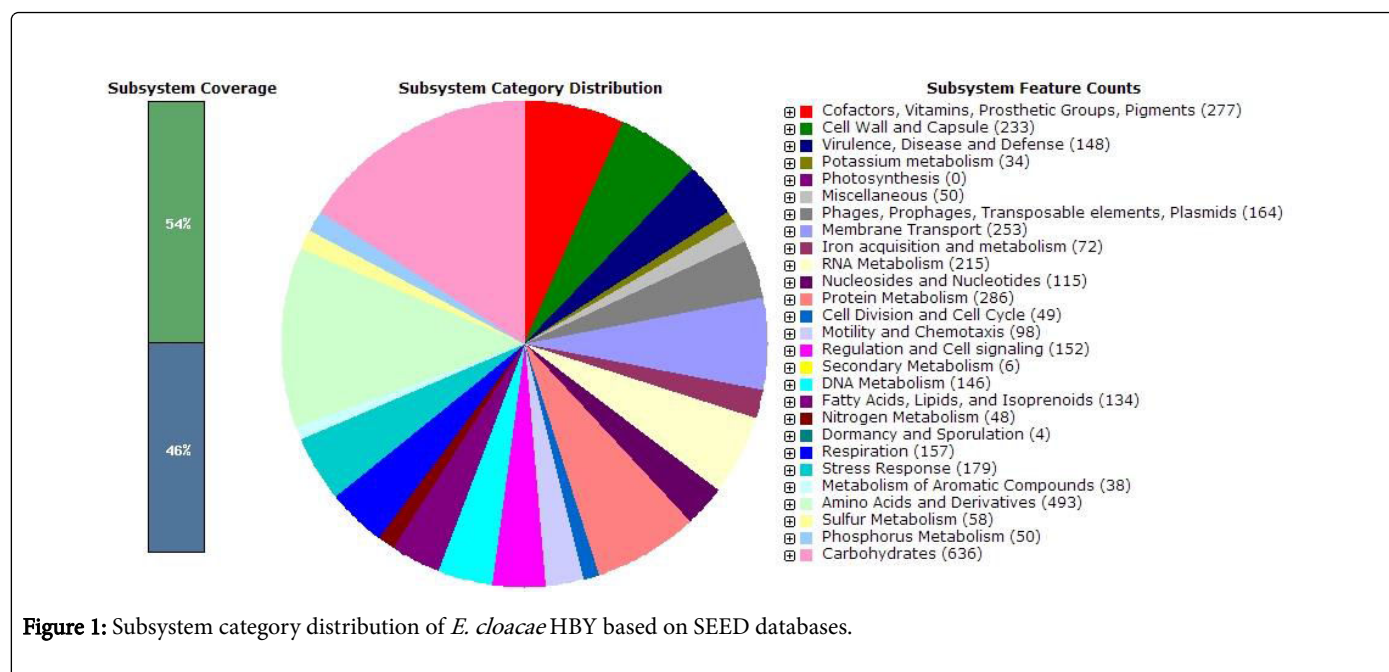


Figure 1: Subsystem category distribution of *E. cloacae* HBY based on SEED databases.

Resistance genes to beta-lactam (*blaACT-7*, *blaNDM-1* and *blaSHV-12*), fosfomycin (*fosA*), fluoroquinolone *oqxB* and *oqxA* and tetracycline (*tet34*) were identified. Furthermore, southern blot showed that the *blaNDM-1* gene was located in the IncX3 plasmid (ca. 50k). Tigecycline resistance in strain *E. hormaechei* ETR1 may be mediated by efflux pump system because RND and MFS family multidrug transport proteins were identified in this genome. MLST analysis showed that the strain belonged to ST93. Plasmid Inc types of IncQ1 and IncX3 were also identified. In addition, the genome contains several IS elements, the majority belonging to the *IS3*, *IS21*, *IS66* and *IS630* families. Similarly, there are five complete and three incomplete prophage sequences, and two CRISPR sequences that can be predicted in the genome. In addition, the type 1 fimbriae genes *fimABCDEF*GHI, encoding virulence determinants, were identified by the RAST annotation. Furthermore, type IV fimbrial genes *pilABC* were also identified. Other virulence genes for hyperadherence (*vidE*), virulence protein (*MsgA*) and several putative virulence factors were also identified in this genome. The identified virulence determinants might have contributed to the infection or colonization of this strain.

Conclusion

In conclusion, whole genome sequencing and the results of the bioinformatics analysis of this strain provide a basis for understanding

the resistance and pathogenic mechanisms. Furthermore, this information will be useful for raising awareness of the emergence of a tigecycline resistant *E. hormaechei* strain from China producing blaNDM-1.

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