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# Genomic Analysis of Antibiotic Related Genes of *Morganellamorganii* MS-27 iq

**Moamin M.Khdir*, and Sahar S. Al-Nakkar**

Department of Biology, College of Science, University of Mosul, Mosul, Iraq.

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**Abstract**

The strain of *Morganellamorganii* MS-27 iq which was isolated from patient had urinary tract infection, in Mosul City (Iraq), has a genome size 3.91 Mb with GC content of 51.46%. This study investigate the antibiotic resistance of *M. morganii* MS-27 iq. Isolate appeared resistance to ampicillin, meropenem, imipenem, ceftriaxone, cefotaxime, cefixime, ciprofloxacin, and levofloxacin whereas sensetive to gentamycin and doxycycline. Genomic analysis of this strain showed, it had 8 genes related to antibiotic resistance mechanisms. 3 genes were related to antibiotic efflux pumps *CRP*, *rsmA*, and*qacG*. 3 genes were related to antibioticinactivation $bla_{DHA\text{-}1}$, *cat A2*, *fosA8*. 2 genes were related to target alternation resistance mechanisms*Arn T* and*gyrB*.

**Key words:** *M.morganii*, genome sequencing, Urinary tract infections

The limited range of therapeutic options available for treating *M. morganii* infections is due to its innate resistance to many antibiotics such as β-lactam and colistin. Resistance genes from the same or different species can also be horizontally exchanged by *M. morganii* (Shi *et al*., 2012). Drug resistance in clinical isolates has expanded quickly due to the ability of lateral gene transfer to confer acquired antimicrobial resistance in bacteria (Poirel *et al.,* 2019). Mobile genetic elements including integrons and plasmids have been found in multidrug-resistant (MDR) *M. morganii* cases that have been described in the literature recently (Van Hoek *et al.,* 2011). Even with advised treatment regimens, clinical failure has resulted from mobile genetic elements mobilizing numerous resistance genes into pathogens (Poirel *et al.,* 2019). Because of its aggressiveness and rising antibiotics resistance, it has been acknowledged as a pathogen with growing importance (Van Hoek *et al.,* 2011). The purpose of this study was to highlight the relevance of *Morganella-morganii*as an uropathogen and analysis whole genome sequencing to detect mechanisms of antibiotic resistance, at the first time in Mosul, Iraq.

**Corresponding author* : Email: moaminalasmar20@gmail.com

## Materials and Methods

**Culture and Isolation**: Urine sample was collected from patient and cultured on MacConkey and blood agar, then incubated at 37° C for 18-24hrs.The bacterial strain identified according to its morphology of colonies on MacConkey and blood agar, Gram staining, oxidase and catalase testing.VITEK-2 SYSTEM (Bio-Merieux, France) was then used to identify the bacterial isolate. The identification of bacterial isolates was verified using molecular techniques based on 16S rRNA. The extraction of DNA was done by using Genomic DNA Extraction Kit (Geneaid Biotech, Taiwan). By using a nano-drop spectrophotometer (Cambridge CB4, England), the concentration and purity of the extracted DNA were determined, at wave length 260-280 nm.

**Antibiotic Susceptibility Testing**: The Kirby-Bauer disc-diffusion method was used for carrying out the test on Muller-Hinton Agar, as per CLSI's guidance.

**Genetic analysis**: The genome sequence of *M.morganii*MS-27iq has been deposited at

DDBJ/ENA/GenBank under the accession number JAUPWO000000000. The raw reads were *de novo* assembled to contigs using SPAdes 3.5 bioinformatics tool applying settings of k-mer length of 21,33,55,77. QUAST software was used to generate assembly statistics. The assembled genome was annotated using the RAST server. The SEED tool was used for predicting functional genes in subsystem categories.

**Detection of the antibiotic resistance genes in the Genome of M.morganiiMS-27iq**: The antibiotic and the *qac* resistance genes in the *M.morganii*MS-27iqgenome were detected using the Comprehensive Antibiotic Resistance Database (CARD) program version 3.2.6.

**General Features of M. morganii strain MS-27iq Genome**: The genome was annotated using the NCBI Prokaryotic Genomes Annotation Pipeline (Table I).

## Results

Bacterial identification, and antibiogram Study: The strainisolate was isolated from patient had urinary tract infections in Mosul city, Iraq. FromVITEK 2 analysis, it was identified as *M. morganii (M. morganii* strain MS-27iq, Accession OR064283). Table II displays the *M. morganii strain MS-27iq* resistance profile to the antibiotics being investigated.

The *M. morganii*MS-27iqhas a genome size 3.91Mb with GC content of 51.46%. About total genes were identified in the genomes, 97% of them were the protein-coding sequences(CDSs), 1.9%encoded RNAs, and 1.1%represented pseudogenes.

Functional annotation was carried out using the Rapid Annotations using Subsystems Technology (RAST) server, and 3,810 coding sequences with 59. Among the annotated subsystem features, 379 genes were identified as amino acids and derivatives, 314 genes encode cofactors or vitamins, and 270 genes were associated with protein metabolism. A total of 83 genes were found to be associated with virulence, disease, and defense.

Antimicrobial Resistance Genes were detected with the *M.morganii*MS-27iq strain. The results showed that this strain was harboured with 8 Antimicrobial Resistance Genes: $bla_{\text{DHA-1}}$, *arn T,fosA8*, *gyrB*, *CRP*, *rsmA*, *cat* A2 and *qacG*.

## Discussion

It has been demonstrated that *M. morganii* possesses inherent resistance to oxacillin, ampicillin, amoxicillin, the majority of 1st and 2nd generation of cephalosporins, macrolides, lincosamides, glycopeptides, fosfomycin, fusidic acid, and colistin. Additionally, the acquired resistance of *M. morganii* may also be caused by many factors such as mutations in specific genes, furthermore by conjugative plasmids, prophages, transposons and integrons (Shi *et al*., 2012;Liu *et al*., 2016). Several antibiotic resistance genes were identified in *M. Morganii*MS-27iq, which they were : $bla_{\text{DHA-1}}$, *arn T*, *fosA8*, *gyrB*, *CRP*, *rsmA*, *cat A2* and *qacG*.

*CRP*, *rsmA*, and *qacG*efflux pumps, which may allow resistance to the majority of commonly used antibiotic classes, including penam, cephalosporins, aminoglycosides, macrolides, fluoroquinolones, and colistin. The action of efflux pumps can contribute to the construction of other resistance mechanisms because they allow bacteria to live for a longer amount

**Table I.** General genome features of *M.morganii*MS-27iqgenerated using QUAST software and RAST server.

| Feature | Value | Feature | Value |
|---|---|---|---|
| Genome total length (bp) | 3,908,586 | Genes (RNA) | 72 |
| Number of contigs | 135 | Number of tRNA genes | 62 |
| Largest contig (bp) | 499,281 | Number of rRNA genes | 6 |
| Smallest contig (bp) | 1000 | ncRNAs genes | 4 |
| GC content (%) | 51.46 | Pseudo Gens | 44 |
| Genes (total) | 3811 | N50 | 351,644 |
| protein-coding sequences (CDSs) | 3695 | | |

**Table II.** Resistance Profile of *M. morganii MS-27iq* to the Antibiotics

| No. | Antibiotics | Abbreviation | Conc. | Results |
|---|---|---|---|---|
| 1 | Ampicillin | AMP | 30 | R |
| 2 | Meropenem | MEM | 10 | R |
| 3 | Imipenem | IPM | 10 | R |
| 4 | Ceftriaxone | CRO | 10 | R |
| 5 | Cefotaxime | CTX | 30 | R |
| 6 | Cefixime | CFM | 5 | R |
| 7 | Gentamycin | CN | 10 | S |
| 8 | Doxycycline | DO | 10 | S |
| 9 | Ciprofloxacin | CIP | 5 | R |
| 10 | Levofloxacin | LEV | 5 | R |

R=Resistant, S=Sensitive

of time, increasing the likelihood of spontaneous mutations that lead to the development of high-level resistance to particular antimicrobials (Ebbensgaard *et al.*, 2020).

The *rsmA* efflux pump gene responsible for drug resistance, controls bacterial communication and quorum sensing mechanisms with development of biofilm. Additionally, it controls the synthesis of several virulence factors, such as secreted exoproteases and exotoxins, type IV pili, and Type II and III secretion systems (Romero *et al.*, 2018).

Increased resistance to frequently used healthcare disinfectants is linked to the multidrug efflux pump *qacG* gene, which is another multidrug efflux pump gene found in both Gram-positive and Gram-negative bacteria that gives resistance to a variety of antimicrobial treatments (Behera *et al.*, 2023). DHA-1 is a member of DHA family.

The earliest and most common method of bacterial resistance to chloramphenicol is enzymatic inactivation, which involves acetylating the medication using several chloramphenicol acetyltransferases. Azidamfenicol, thiamphenicol, and chloramphenicol can all be rendered inactive by *cats* (Van Hoek *et al.,* 2011). *cat A2* gene found previously in *M. Morganii* KT (Chen *et al.*, 2012). *fosA8*gene identified in the chromosome of *Lecleriaadecarboxylata*. Lately detected in *E. coli* which isolated from urine (Poirel *et al.,* 2019). Recently described in *M. Morganii* UM869 (Behera *et al.*, 2023). *ArnT* (Glycosyltransferase) is necessary for bacteria to resist antibiotic peptides such as colistin A and colistin B (Tavares-Carreón*etal.*, 2016). gyrB, Fluoroquinolone resistance in *M.morganii* is caused by a point mutation (Nasri- Yaiche *et al.*, 2014 ).

## Conclusion

The treatment of *M. morganii* infections is becoming more difficult as a result of the bacterium's acquisition and accumulation of intrinsic and acquired multidrug resistance genes.

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