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Genome Note

Extended-spectrum β-lactamase-producing *Proteus mirabilis* with multidrug resistance isolated from raw chicken in Singapore: Genotypic and phenotypic analysis

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ABSTRACT

Objectives: Proteus mirabilis is ubiquitous in soil and water. It is an important catheter-associated urinary tract pathogen and has reportedly been associated with antimicrobial-resistant infections. This study reports the draft genome of a multidrug-resistant *P. mirabilis* isolated from raw retail chicken meat in Singapore.

Methods: The *P. mirabilis* strain was isolated on Brilliance™ ESBL Agar and was screened for antimicrobial susceptibility against 29 antimicrobial agents using a MicroScan® Neg MIC Panel Type 44. The double-disk synergy test (DDST) was used for confirmation of extended-spectrum β-lactamase (ESBL) production. Genomic DNA from the pure culture isolate was extracted and was sent for sequencing based on Illumina HiSeq 2500 technology. Further bioinformatics analysis was performed using online tools available at the Center for Genomic Epidemiology.

Results: Species identification of the isolate was performed by KmerFinder. Antimicrobial susceptibility testing of the isolate showed multidrug resistance to broad-spectrum β-lactams, fluoroquinolones and aminoglycosides, among others. ESBL production was confirmed by the DDST. A total of 29 antimicrobial resistance genes were detected by ResFinder.

Conclusion: To the best of our knowledge, this is the first report of the whole-genome sequence of a multidrug-resistant *P. mirabilis* producing an ESBL from raw chicken meat in Singapore. This indicates that raw meat in Singapore can be a reservoir for drug-resistant pathogens.

1. Introduction

Proteus mirabilis is a Gram-negative bacterium with swarming motility and urease activity that has a close association with infection following catheterisation. The urease activity of *P. mirabilis* generates ammonium and lowers the pH. Alkaline conditions can lead to the formation of crystalline biofilms on catheters as well as stones in the urinary system [1,2]. Catheter-associated urinary tract infection is one of top four healthcare-associated infections in the USA, which are often polymicrobial and difficult to treat [3].

The emergence of antimicrobial resistance (AMR) in *P. mirabilis* has raised concern for treatment failure. The prevalence of multidrug-resistant *P. mirabilis* isolates producing extended-spectrum β-lactamases (ESBLs) is increasing worldwide [4]. Previously, *P. mirabilis* was found to be the second most prevalent species, after *Escherichia coli*, among ESBL-producing Enterobacteriaceae in chicken meat in Singapore [5]. However, to the best of our knowledge, there is no general genomic information on these ESBL-producing *P. mirabilis* isolates.

In this study, a *P. mirabilis* isolate was obtained from raw chicken in a supermarket in Singapore. The whole genome of the *P. mirabilis*

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isolate was sequenced to determine the presence of AMR genes and other genetic characteristics. This study provides an insight into the genetic basis of multidrug resistance in *P. mirabilis*.

2. Methods

P. mirabilis strain E12ESBLY1 was isolated from chilled retail chicken in a supermarket in Singapore in 2017. A sample (10 g) was homogenised with 90 g of Universal Pre-enrichment Broth in a stomacher at 230 rpm for 1 min. The homogenate was incubated overnight at 37 °C and was then streaked on Brilliance™ ESBL Agar (Thermo Fisher Scientific). A single colony was picked after 24 h of incubation. The double-disk synergy test (DDST) was performed to confirm ESBL production as previously described [6]. Antimicrobial susceptibility testing against 29 antimicrobial agents was performed by the broth microdilution method to determine minimum inhibitory concentrations (MICs) using a MicroScan® Neg MIC Panel Type 44 (Beckman Coulter, Inc., Brea, CA, USA) following the manufacturer's instructions. Genomic DNA was extracted using a QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. An Illumina HiSeq 2500 sequencer (Illumina Inc., San Diego, CA, USA) was used for whole-genome sequencing (WGS). Raw reads were assembled using Assembler 1.2 [7]. Species identification of the isolate was performed by KmerFinder 3.1 [8], and AMR genes were identified using ResFinder[9].

3. Results

The whole genome of *P. mirabilis* strain E12ESBLY1 is 4 158 866 bp in size with a GC content of 39.2% and N50 an value of 186 772. The genome assembly comprises 163 contigs with 3975 coding sequences (CDS) and 93 RNAs. KmerFinder indicated that the

Table 1
Results of antimicrobial susceptibility testing and corresponding antimicrobial resistance (AMR) genes detected in *Proteus mirabilis* strain E12ESBLY1.

Antimicrobial class/agent	MIC (μg/mL)	Interpretation[a]	AMR gene(s)
Penicillins			
Ampicillin	>16	R	bla_{TEM-1B}, bla_{OXA-1}, $bla_{CTX-M-65}$
Piperacillin	>64	R	
Cephalosporins			
Cefalotin	>16	R	
Cefotaxime	>32	R	
Cefuroxime	>16	R	
Ceftazidime	<1	S	
Cefepime	>16	R	
Cefoxitin	<8	S	
Monobactams			
Aztreonam	>16	R	
Carbapenems			
Imipenem	2	S [E]/I [C]	
Meropenem	<1	S	
Ertapenem	<0.5	S	
Doripenem	<1	S	
Polymyxins			
Colistin	>4	R	
Tetracyclines			
Tetracycline	>8	R	*tet(A)*, *tet(C)*-like, *tet(J)*-like
Tigecycline	2	I	
Minocycline	>8	R	
Quinolones			
Ciprofloxacin	>2	R	*aac(6')-Ib*-cr
Levofloxacin	>4	R	
Nalidixic acid	>16	R	
Amphenicols			
Chloramphenicol	>16	R	*cat*-like, *catB3*-like, *cmlA1*-like, *floR*-like
Aminoglycosides			
Amikacin	<8	S	*aac(3)-Id*, *aac(3)-IVa*-like, *aac(6')-Ib*-cr, *aadA1*-like, *aadA2*, *aadA7*,
Gentamicin	>8	R	*aph(3')-Ia*, *aph(4)-Ia*, *strA*, *strB*
Tobramycin	>8	R	
Combinations			
Amoxicillin/clavulanic acid	<8/4	S	
Piperacillin/tazobactam	<8	S	
Trimethoprim/sulfamethoxazole	>4/76	R	*dfrA1*, *dfrA15*, *dfrA32*, *sul2*, *sul3*
Nitrofurantoin	>64	R	
Fosfomycin	>64	R	*fosA3*
Macrolide[b]	NA	NA	*ere(A)*-like, *erm(42)*-like
Rifampicin[b]	NA	NA	*arr-3*

MIC, minimum inhibitory concentration; EUCAST, European Committee on Antimicrobial Susceptibility Testing; CLSI, Clinical and Laboratory Standards Institute; R, resistant; S, susceptible; I, intermediate; NA, not available.

[a] Interpretation is based on EUCAST v.3.1 and CLSI standards. Interpretation results without a marker indicate that the results are consistent according to EUCAST and CLSI, whereas [E] indicates that the interpretation result is based on EUCAST and [C] indicates that the interpretation result is based on CLSI.

[b] Not included in the MIC test.

closest genome to strain E12ESBLY1 is *P. mirabilis* strain T18 (NZ_CP017085.1) with 91.43% query coverage. The DDST showed a 'keyhole'-shaped inhibition zone, indicating the production of a β-lactamase. The MICs of the 29 antimicrobial agents tested are shown in Table 1. The isolate exhibited a broad spectrum of resistance phenotypically, including resistance to β-lactams (penicillins, cephalosporins, monobactams), tetracyclines, quinolones, aminoglycosides, chloramphenicol, fosfomycin, nitrofurantoin, colistin and the combination trimethoprim/sulfamethoxazole. Correspondingly, a total of 29 AMR genes were revealed by ResFinder. Ten of them are known to be responsible for resistance to aminoglycosides, including *aac(3)-Id*, *aac(3)-IVa*-like, *aac(6')-Ib*-cr, *aadA1*-like, *aadA2*, *aadA7*, *aph(3')-Ia*, *aph(4)-Ia*, *strA* and *strB*. Among these, the *aac(6')-Ib*-cr gene is also related to quinolone resistance. Three β-lactam resistance genes (bla_{TEM-1B}, bla_{OXA-1} and $bla_{CTX-M-65}$), three tetracycline resistance genes [*tet(A)*, *tet(C)*-like and *tet(J)*-like], four phenicol resistance genes (*cat*-like, *catB3*-like, *cmlA1*-like and *floR*-like), three trimethoprim resistance genes (*dfrA1*, *dfrA15* and *dfrA32*), two sulfonamide resistance genes (*sul2* and *sul3*) and one fosfomycin resistance gene (*fosA3*) were observed. Besides these, the macrolide resistance genes *ere(A)*-like and *erm(42)*-like as well as the rifampicin resistance gene *arr-3* were also detected, however these two antimicrobial agents were not included in the antimicrobial susceptibility testing in this study. With the exception of intrinsic nitrofurantoin and colistin resistance, AMR genes corresponding to all of the other resistance phenotypes can be found in the genome. All of the AMR genes detected in this isolate show a corresponding resistant phenotype, except that macrolide and rifampicin were not included in the MIC testing panel but the resistance genes were found.

4. Discussion

This study revealed that *P. mirabilis* isolated from raw chicken meat in Singapore can have a broad spectrum of AMR, including various common antimicrobial classes. There is only one recorded human clinical *P. mirabilis* isolate from Singapore with WGS records published in the National Center for Biotechnology Information (NCBI) (accession no. SRR7371340). The current study is the first WGS record of *P. mirabilis* isolated from food in Singapore. Its genetic basis of multidrug resistance may have reference value in the future.

The OXA group of β-lactamases belong to class D β-lactamases, also known as oxacillinases. The bla_{OXA-1} gene is often identified in ampicillin-resistant enterobacteria such as *E. coli*, *Salmonella* etc. [10]. In addition, bla_{OXA-1} has frequently been detected together with ESBL genes and this phenomenon was also observed in the current study. The co-existence of bla_{TEM-1B}, bla_{OXA-1} and $bla_{CTX-M-65}$ corresponds with the characteristic phenotype of ESBLs, i.e. broad β-lactam resistance including penicillins, cephalosporins and monobactams. Besides resistance to the β-lactam class, other resistance phenotypes also show agreement with the genotype. However, macrolide and rifampicin were not included in the MIC testing panel although their resistance genes were detected. Most *Proteus* spp. are naturally resistant to nitrofurantoin [11] and colistin [12] and thus it is explainable that no related resistance genes for these antimicrobials were found in this isolate. The intrinsic resistance together with other acquired drug resistance determinants carried by this *P. mirabilis* isolate greatly narrows the choice of antimicrobial agents for treatment. This may also raise concern for public health as consumption of undercooked meat and cross-contamination between raw and ready-to-eat food may provide the possibility of transmission of antimicrobial-resistant bacteria from food to humans. Its potential transferability to humans and impact on public health needs to be assessed in further studies.

Nucleotide sequence accession no

The whole genome raw reads were deposited in ENA under project PRJEB34176 with accession no. ERR3500178.

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Competing interest

None declared.

Ethical approval

Not required.

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