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Case Report

A Case of Genetic Analysis from Colistin-Resistant and Carbapenem-Resistant Enterobacterales Bacteremia

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SUMMARY

In recent years, carbapenem-resistant Enterobacterales (CRE) has emerged as a more abundant and difficult-to-treat pathogen. Colistin seemed to be the last-resort antibiotic. We treated an eighty-year-old male who had colistin-resistant and carbapenem resistant *Klebsiella pneumoniae* bacteremia during hospitalization. Since there was no effective antibiotic available, the patient expired in 2019. We later used next-generation sequencing (NGS) on the patient's blood isolate to identify the resistant mechanisms of the pathogen. The bla_{OXA-48} resistant gene could indicate resistance to carbapenem. NGS technology became available in our research laboratory in 2024 to precisely detect antimicrobial resistance. In comparison with rapid BioFire FilmArray system, NGS could be used to precisely detect antimicrobial resistance and could be considered as one of the diagnostic tools to improve patient care.

1. Introduction

In recent years, carbapenem-resistant Enterobacterales (CRE) has emerged as a more abundant and difficult-to-treat pathogen. Hence, its prevention and treatment have become major issues. The risk factors for CRE may include environmental sources, bundled interventions, or intensive care unit admission.[1,2] In the clinical setting, colistin is the last-resort antibiotic for this type of bacteria. Colistin is an antimicrobial agent extracted from *Paenibacillus polymyxa* and belongs to the class of polymyxins. There are five polymyxins, viz., A, B, C, D, and E, of which polymyxin E (colistin) and polymyxin B are clinically relevant.[3] The major site of colistin action is the outer cell membrane of gram-negative bacteria, wherein it binds to lipopolysaccharides (LPSs) through electrostatic interactions between its α, *c*-diaminobutyric acid and the phosphate groups of the lipid A region of LPS. The phospholipid bilayer in Gram-negative bacteria loses its stability due to the action of colistin, which adds hydrophilic groups to fatty acid chains, causing changes in its integrity, failing to maintain cellular content, and cell lysis.[4] However, previous studies have demonstrated a rapid increase in the prevalence of colistin resistance in *Enterobacteriaceae*.[5] The mechanisms underlying colistin resistance remain unknown for some bacterial species. Furthermore, mutations or adaptation mechanisms in multidrug-resistant (MDR) strains elevate the difficulty. Transposable genetic elements (generally plasmids containing mobilized colistin resistance ***mcr*** genes) are the major cause of bacterial colistin resistance in the microbial world. Intrinsic resistance mechanisms may be mediated by chromosomal genes, and acquired resistance mechanisms may be mediated by plasmids, biofilm, and efflux pumps.[6]

A few antibiotics have been recently developed since 2019, including cefiderocol, ceftazidime-avibactam, ceftolozane-tazobactam, imipenem-relebactam, and meropenem-vaborbactam[7–10] with activity against carbapenem-resistant and multidrug-resistant bacilli, especially to treat colistin-resistant Enterobacterales.

Next-generation sequencing (NGS) has provided clinical microbiologists with powerful tools for detecting and monitoring antimicrobial resistance (AMR). The growing threat of AMR underscores the necessity of integrating genomics into efforts to combat this global health challenge. The genomic surveillance of AMR is vital for comprehending how resistance evolves, predicting effective treatments, and making informed decisions regarding patient care.

NGS for the clinical isolate form this patient was performed later in the referral laboratory after our patient passed away in 2019, and the aim of this case report is to emphasize the importance of using NGS to precisely detect antimicrobial resistance for research and making informed decisions regarding patient care.

The study was approved by MacKay Memorial Hospital under the protocol number 21MMHIS411e.

2. Case presentation

An 80-year-old man presented with a medical history of severe aortic stenosis. He underwent coronary artery bypass grafting (CABG) and aortic valve replacement (AVR). Additionally, he had type 2 diabetes mellitus, a suspected restrictive lung defect, and stenting for coronary artery disease. He was discharged from our ward in 2019 after CABG and AVR. After discharge, he experienced a cough with yellowish sputum for four days. He was sent to our emergency department, where his vital signs were as follows: temperature 36.4 °C,

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pulse rate 72 bpm, respiratory rate 22 cycles/min, blood pressure 102/76 mmHg, and SpO2 95%. The chest X-ray indicated left lower lobe pneumonia with pleural effusion. The patient was admitted to medical intensive care unit on 19 July, 2019 for intensive and aggressive care.

However, he developed ventricular fibrillation on Day 3 after admission. Emergent intubation was performed, mechanical ventilation support was provided, and defibrillation (150 Joule) was administered, resulting in the return of spontaneous circulation and gradual recovery of consciousness. On Day 37, he developed septic shock. Colistin, meropenem, and tigecycline were given as empirical antibiotic treatment. Sputum culture later revealed carbapenem-resistant *Acinetobacter baumannii*, which showed sensitivity to ceftazidime but resistance to colistin. Consequently, the antibiotic regimen was changed to ceftazidime based on sensitivity testing. Subsequent blood culture revealed colistin-resistant and carbapenem-resistant *Klebsiella pneumoniae*. Unfortunately, the patient succumbed to septic shock; and passed away on 14 September, 2019, 56 days after admission.

The blood culture from the clinical laboratory revealed resistance to ampicillin, trimethoprim/sulfamethoxazole, cefazolin, cefuroxime, cefotaxime, ceftazidime, levofloxacin, ciprofloxacin, ertapenem, imipenem and colistin. Following his demise, the isolate from blood was sent to be stored in the research laboratory for further investigation. We performed whole-genome sequencing.

We combined both short reads and long reads sequencing data to do the genome assembly. We used mixed genomic DNA and plasmid DNA to do the library by using ligation sequencing genomic DNA (SQK-LSK109) kit. Load the prepared library onto the Nanopore GridION Mk1 platform using the MinION flow cell to do the sequencing. Short reads (PE300 Pired-end 300 bps) were done by illumina MiSeq System. The bla_{OXA-48} resistant gene was the major finding in the sequence of NGS (Figure 1) and insertion sequence (IS-10A) on both sides in its surroundings. β-lactamase genes, including DHA-1, TEM, SHV-1, OXA-48 genes were detected by NGS. Efflux pump genes, including AcrA, AcrB, kpnE, kpnF, MdtK, TolC, oqxA, oqxB, crrA, crrB, genes were detected by NGS (Table 1). MCR 1–9 genes were not detected by NGS in this study. We used *Klebsiella pneumonia* MGH78578 as wild type to investigate colistin-resistant and carbapenem-resistant genes.

3. Discussion

The novelty of our study is that in our case report the blood isolate carried the bla_{OXA-48} gene of colistin-resistant and carbapenem-resistant Enterobacterales bacteremia, not the KPC gene. Increased consumption of colistin has led to patients being infected with colistin-resistant *Klebsiella pneumoniae* carbapenemase (KPC) – producing strains which have been reported globally.[11–13] The KPC-2 carbapenemase is more common in Taiwan. However, the OXA-48 carbapenemase is relatively rare as shown in previous studies including data from Taiwan.[12]

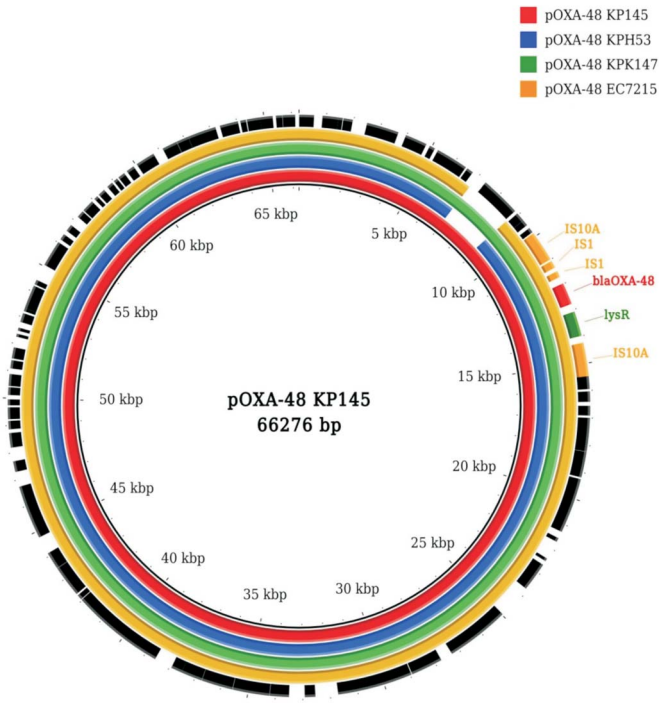

Figure 1. The sequence from the inner to the outer circles are pOXA-48 KP145, pOXA-48 KPH53, pOXA-48 KPK147 and pOXA-48 EC7215. pOXA-48 KP145: clinical blood isolate from MacKay Memorial Hospital by next generation sequencing. pOXA-48 KPH53, pOXA-48 KPK147, pOXA-48 EC7215: reference sequence obtained from the Official website of the National Institutes of Health (NIH).

Table 1
CP000647 similarity and gene function.

Gene	a.a. length	CP000647 similarity	Gene function*
pmrA	224	100%	Activation of LPS-modifying operon by mutations in TCSs
pmrB	366	99%	Activation of LPS-modifying operon by mutations in TCSs
phoP	224	100%	Activation of LPS-modifying operon by mutations in TCSs
phoQ	489	100%	Activation of LPS-modifying operon by mutations in TCSs
mgrB	48	98%	Inactivation of phoP/phoQ negative feedback regulator
lpxM	325	99.69%	Increased acylation of lipid A enhancing its modification with aminoarabinose
eptB	552	100%	Reduced the negative charge of LPS thereby decreasing the affnity of LPS to positively charged colistin
yciM	390	99.74%	Increase LPS production
AcrA	398	100%	Efflux pump
AcrB	1049	100.00%	Efflux pump
kpnE	121	100%	Efflux pump
kpnF	110	100%	Efflux pump
MdtK	458	99.56%	Efflux pump
TolC	492	97%	Efflux pump
oqxA	392	100.00%	Efflux pump
oqxB	1051	99.81%	Efflux pump
crrA	246	100%	Efflux pump
crrB	354	99.72%	Efflux pump

CP00067: the code for the standard strain.
LPS: lipopolysaccharide, TCSs: two component regulatory systems.
* Gene function was described in References 10–14.

Furthermore, this case is an 80-year-old man, elderly and frail with multiple co-morbidity. The elderly population is increasingly exposed to more and more antibiotics-resistance due to frequent healthcare exposure and prolonged and repeated hospitalizations, and this is a growing healthcare crisis.

This case report emphasizes the importance of identification of resistant genes from pathogens. NGS considered as one of the diagnostic tools shortens the time to detect the resistant genes in comparison with the traditional ways. Some of the bacterial mechanisms against colistin can be identified from previous research.[14–18] In the present case, we found that Enterobacterales harbors a mutation in the gene ***lpxM*** (Table 1), which increases the acylation of lipid A, improving its modification with aminoarabinose and preventing the addition of myristoyl group to this lipid A, causing sensitivity to polymyxin. Another mutation is in the gene ***mgrB***, which inactivates the PhoP/PhoQ negative feedback regulator. PhoP–PhoQ is a two-component system that regulates virulence, mediates adaptation to Mg^{2+}-limiting environments, and regulates numerous cellular activities in several gram-negative species. It consists of the inner membrane sensor PhoQ and the cytoplasmic regulator PhoP. These two components may be related to the major reason for the bacterial resistance against colistin. Enterobacterales also has other mutations in the genes ***pmrB***, ***AcrA***, ***MdtK***, ***TolC***, ***oqxB***, and ***crrB***, which cause the activation of the LPS-modifying operon and affect efflux pump separately (Table 1). These mutations may be related to a minor reason.

The $bla_{\text{OXA-48}}$ resistant gene could indicate the resistant mechanism of carbapenem (Figure 1). We referred the isolate for NGS testing to a referral laboratory. The clinical significance is the presence of insertion sequence (IS-10A) on the both sides of the Oxa-48 carbapenemase gene and its possibility of transferability.[19] Therefore, based on our findings, we can determine whether the NGS technology can be used extensively for research to identify bacterial resistance.

Compared to NGS, the rapid BioFire FilmArray system provided results within 3 hours from the blood culture, so BioFire FilmArray is performed for the blood isolates clinically (in 2024) since it was faster. The BioFire FilmArray system could detect OXA type, KPC type carbapenemase, or other metallo-β-lactamase (e.g. VIM, IMP, NDM) which harbor more resistant genes faster, but cannot detect whole sequences.[20]

The use of NGS for the precise detection of antibiotic resistant genes is worthy of further research. In comparison with BioFire FilmArray system, NGS could be used to precisely detect antimicrobial resistance and could be considered as one of the diagnostic tools to improve patient care.

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Conflicts of interest statement

The authors declare that they have no conflicts of interest.

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