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Publication Date

2018

DOI

10.1016/j.idcr.2017.11.007

Peer reviewed

IDCases 11 (2018) 9–11

Contents lists available at ScienceDirect

IDCases

journal homepage: www.elsevier.com/locate/idcases

Case report

Unusual carbapenem resistant but ceftriaxone and cefepime susceptible *Klebsiella oxytoca* isolated from a blood culture: Case report and whole-genome sequencing investigation

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ARTICLE INFO

Keywords:
Carbapenem resistance
Klebsiella oxytoca
blaOXY
Porin mutation
Efflux system
Whole-genome sequencing

ABSTRACT

A carbapenem resistant but ceftriaxone and cefepime susceptible *Klebsiella oxytoca* was isolated from the blood of a patient with polymicrobial bacteremia after 2 weeks of ertapenem treatment. Whole-genome sequencing identified no carbapenemase gene nor plasmid, but only $bla_{OXY\text{-}2\text{-}8}$ gene with a mutation in the promoter that's been reported to increase its expression. Two other specific carbapenem resistance mechanisms including mutated porin genes and the AcrAB-TolC efflux system genes were also identified. Clinicians need to be aware of such unusual antibiogram and should not assume carbapenems are always broader spectrum antibiotics than expanded-spectrum cephalosporins.

Introduction

Klebsiella oxytoca is associated with blood stream, intra-abdominal and urinary tract infections. The rise of carbapenem resistant *Klebsiella oxytoca* (CRKO) has become part of the urgent threat of carbapenem resistant Enterobacteriaceae (CRE) epidemic, and most CRKO carry plasmid-mediated carbapenemase genes that are highly transmissible [1–4]. Here we report a case of bacteremia with an unusual CRKO that is susceptible to ceftriaxone and cefepime.

Case report

A patient with history of recent tricuspid valve endocarditis with septic pulmonary emboli, as well as a large, obstructing staghorn calculus developed polymicrobial bacteremia including *Escherichia coli* and ESBL *Klebsiella pneumoniae* five days after suffering an iatrogenic ureteral perforation during a failed percutaneous nephrolithotomy (*day 0*). Empiric vancomycin and piperacillin-tazobactam were initiated and changed to ertapenem monotherapy when blood culture identification and susceptibilities were available. Subsequent blood cultures grew vancomycin resistant *Enterococcus faecium (days 11, 13)*, which prompted addition of daptomycin. After 2 weeks of ertapenem, the patient continued to be clinically septic, and ertapenem was switched to meropenem to broaden coverage. Three days after changing to meropenem (*day 25*), blood cultures grew CRKO, *Stenotrophomonas maltophilia*, and *Enterococcus faecalis*. Amikacin was added to cover the CRKO, trimethoprim-sulfamethoxazole was started for the *Stenotrophomonas maltophilia*, and daptomycin and meropenem were continued. The CRKO isolate had an unusual antibiogram (Table 1), as it was resistant to cefazolin, piperacillin/tazobactam, ertapenem and meropenem, and intermediate to imipenem but susceptible to aztreonam and most 3rd and 4th generation cephalosporins including ceftriaxone, ceftazidime, and cefepime except cefotaxime (resistant) and ceftolozane/tazobactam (intermediate). It was susceptible to ceftazidime/avibactam. Due to this susceptibility pattern, meropenem was switched to cefepime on *day 29*, after which no *Enterobacteriaceae* has been isolated to date. The patient didn't have recent travel history.

Investigation

To investigate the carbapenem resistance mechanism of this CRKO isolate, the modified carbapenem inactivation method (CIM) [5] and

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https://doi.org/10.1016/j.idcr.2017.11.007
Received 25 October 2017; Received in revised form 20 November 2017; Accepted 21 November 2017

Table 1
Drug Susceptibility Results of CRKO (MIC = minimal inhibitory concentration).

Drugs	Susceptibility Results	Test Methods
Amikacin	MIC < = 8 Susceptible	BD Phoenix™ Automated System
Ampicillin	MIC > 16 Resistant	BD Phoenix™ Automated System
Aztreonam	MIC < = 2 Susceptible	BD Phoenix™ Automated System
Ampicillin/Sulbactam	MIC > 16/8 Resistant	BD Phoenix™ Automated System
Cefepime	MIC = 2 Susceptible	BD Phoenix™ Automated System
Cefepime	MIC = 2 Susceptible	Manual Broth Microdilution
Cefoxitin	MIC > 16 Resistant	BD Phoenix™ Automated System
Ciprofloxacin	MIC > 2 Resistant	BD Phoenix™ Automated System
Ceftriaxone	MIC < = 1 Susceptible	BD Phoenix™ Automated System
Ceftriaxone	MIC = 1 Susceptible	Manual Broth Microdilution
Ceftolozane/Tazobactam	MIC = 4/4 Intermediate	Manual Broth Microdilution
Cefotaxime	Zone size = 20 Resistant	Manual Disk Diffusion
Ceftazidime	MIC = 2 Susceptible	Manual Broth Microdilution
Ceftazidime/avibactam	MIC < = 2 Susceptible	Manual Broth Microdilution
Cefazolin	MIC > 16 Resistant	BD Phoenix™ Automated System
Cefazolin	MIC > 32 Resistant	Manual Broth Microdilution
Colistin	MIC < = 2 Susceptible	Manual Broth Microdilution
Ertapenem	MIC > 2 Resistant	BD Phoenix™ Automated System
Ertapenem	MIC > 4 Resistant	Manual Broth Microdilution
ESBL	Negative	BD Phoenix™ Automated System
Gentamicin	MIC < = 2 Susceptible	BD Phoenix™ Automated System
Imipenem	MIC = 2 Intermediate	Manual Broth Microdilution
Meropenem	MIC > 8 Resistant	BD Phoenix™ Automated System
Meropenem	MIC = 4 Resistant	Manual Broth Microdilution
Piperacillin/Tazo	MIC > 64/4 Resistant	BD Phoenix™ Automated System
Piperacillin/Tazo	MIC > 64/4 Resistant	Manual Broth Microdilution
Sulfameth/trimeth	MIC > 2/38 Resistant	BD Phoenix™ Automated System
Tetracycline	MIC = 8 Intermediate	BD Phoenix™ Automated System

the modified Hodge test (MHT) [5] were performed and both were negative. Additionally, the isolate was negative for carbapenemase genes using the Cepheid GeneXpert CARBA-R (for KPC, NDM, IMP, VIM, OXA-48) and a laboratory developed real-time PCR (for KPC, NDM, IMP, VIM, SME, OXA-48) [6]. To further characterize the molecular mechanism of resistance, whole-genome sequencing (WGS) was performed using Illumina MiSeq system [7] and the WGS data was deposited to NCBI (accession # PCMV01000000). Analysis using PlasmidFinder [8] suggested that the isolate did not harbor a plasmid. The only β-lactamase gene identified by the WGS using ResFinder [9] was a chromosomally encoded $bla_{\text{OXY-2-8}}$ gene with a GATA[G → A]T mutation in the −10 box of its promoter, which has been reported to cause overexpression of the gene and > 100 fold increase in enzyme activity [10]. Compared to the originally reported $bla_{\text{OXY-2-8}}$ gene, there are four single-nucleotide polymorphisms (SNPs) resulting in amino acid substitutions S23G, D38A, N134T, and A239T. Several reports have shown residual carbapenemase activities of chromosomal OXY-2 enzymes including K1 [11–14] that may also have ESBL-like properties [14]. The $bla_{\text{OXY-2-8}}$ gene was only recently identified (NCBI Reference Sequence: NG_049857.1) and the characteristics of this new enzyme is unknown. More studies are undergoing to verify the residual carbapenemase activities of the OXY-2-8 enzyme in this isolate.

WGS also identified genes involved in the AcrAB-TolC efflux system including *acr*A, *acr*B, *tol*C [15] and their regulatory genes including *acr*R, *ram*A, *mar*A, *rob*A, *msb*A, and *sox*R, etc [16,17]. Interestingly, we identified SNPs in the *acr*R gene (Y114F and V165I) associated with increased AcrAB expression in *K. pneumoniae* [18]. We also found numerous missense mutations in the porin genes *omp*K35 and *omp*K36 compared to the wild type genes, apparently leading to nonfunctional porins, which had been associated with carbapenem resistance [15,19].

Discussion

Currently, most reported CRKO contain plasmids carrying carbapenemase [1–4] or ESBL genes [20]. To our knowledge, this is the first report of CRKO without a plasmid, or carbapenemase or non-OXY-related ESBL genes. Overexpression of both $bla_{\text{OXY-2-8}}$ and the AcrAB-TolC efflux system, as well as loss of function in key porins, most likely contributed to carbapenem resistance in this isolate. The unusual antibiogram of this isolate (i.e. resistant to carbapenems but susceptible to cefepime and ceftriaxone) led to the change of treatment regimen from meropenem to cefepime, which treated the CRKO blood stream infections. Clinicians need to be aware of such an unusual CRE, as more plasmid- and carbapenemase-free CRE may be on the horizon. Despite their reputations in general clinical medicine, carbapenems may not always be broader spectrum antibiotics than expanded-spectrum cephalosporins.

Disclosure

The authors declare that they have no competing interests.

Acknowledgement

Not applicable.

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