Lipid A modification-induced colistin-resistant *Klebsiella variicola* from healthy adults

Sun Ju Kim
Sungkyunkwan University School of Medicine
Jeongwoo Jo
Sungkyunkwan University School of Medicine
Kwan Soo Ko (ksko@skkku.edu)
Sungkyunkwan University School o f Medicine https://orcid.org/0000-0002-0978-1937

Short Report

Keywords: Klebsiella variicola, colistin resistance, lipid A

Posted Date: December 1st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1116232/v1

Abstract

We identified two *Klebsiella variicola* isolates from fecal samples: one is colistin-resistant, and another is colistin-susceptible. The colistin-resistant *K. variicola* isolate showed no mutations in *phoPQ, pmrAB*, and *mgrB*, and *crrAB* and *mcr* were not identified. However, its *phoQ* and *pbgP* expression was significantly higher and amino-arabinosylated lipid A with hexa-acylated species in lipopolysaccharide was identified.

Introduction

Colistin is a long-known drug, but is now considered one of the last resorts for treating bacterial infections caused by multidrug-resistant gram-negative pathogens. Colistin resistance in *Klebsiella pneumoniae* has emerged and is now a great concern in clinical settings as well as in public health (Gipson et al., 2020). However, colistin resistance in other species of the *K. pneumoniae* complex has not been actively explored. *Klebsiella variicola*, formerly known as a benign endosymbiont, has become an emerging pathogen that can cause life-threatening infections in immunocompromised individuals (Rodríguez-Medina et al., 2019). In this study, we investigated the resistance mechanism of a colistin-resistant *K. variicola* isolate obtained from a healthy adult in South Korea.

Materials And Methods

We identified two *K. variicola* isolates in the fecal samples of healthy Korean adults, collected between June and September 2014 (Joo et al., 2018). Species identification was performed based on 16S rRNA and *rpoB* sequences, and the isolates were designated 464-1 and 475-3, respectively. Antimicrobial susceptibility testing was performed using the broth microdilution method according to the CLSI guidelines (2018). For colistin, we further performed a disk diffusion assay using Sensi-Disc™ (Becton, Dickinson and Company, Sparks, MD, USA) and the Epsilometer test (E-test) using ETEST® strip (bioMérieux, Marcy l'Etoile, France).

Based on the whole genome sequences of *K. variicola* GJ1 (GCF_001989495.1), we designed PCR and sequencing primers for colistin resistance-associated genes, *phoPQ*, *pmrAB*, *mgrB*, and *crrB* (Wirhgt et al., 2015). The relative expression of *pmrB*, *phoQ*, and *pbgP* was measured by quantitative real-time PCR (qPCR), as described previously, using *rpoB* as the reference gene (Kim and Ko, 2018).

We investigated the structural differences in the lipid A of the lipopolysaccharide (LPS) of the colistin-resistant and colistin-susceptible *K. variicola* isolates. Lipid A sample for a MALDI-TOF MS analysis was prepared as previously described (Yi and Hackett, 2000). MALDI-TOF analyses were performed on the MALDI TOF-TOF 5800 system (AB SCIEX, USA). The raw *m/z* values were processed and analyzed using Mass-Up (López-Fernández et al., 2015). The baseline value was corrected using TopHat transform, and the curve was standardized by total ion current and smoothed by Savitzky-Golay filtering.

Results And Discussion

Both *K. variicola* isolates were susceptible to imipenem (MICs, 0.5 mg/L), ciprofloxacin (MICs, ≤0.06 mg/L), cefepime (MICs, ≤0.06 and 0.12 mg/L), gentamicin (MICs, 0.5 mg/L), and tetracycline (MICs, 1 mg/L), and resistant to rifampin (MICs, 16 and 32 mg/L). While 464-1 was resistant to colistin (MIC, 32 mg/L), 475-3 was susceptible to it (MIC, 1 mg/L). The results of the disk diffusion assay and E-test were consistent with those of the broth microdilution method. The colistin-resistant isolate 464-1 showed no zone of inhibition in the disk diffusion assay, and an MIC of 16 mg/L in the E-test. The isolate 475-3 showed a colistin MIC of 0.19.

No nucleotide variation was identified between colistin-resistant and colistin-susceptible isolates in *phoPQ*, *pmrAB*, and *mgrB*. *crrAB* was not detected in either *K. variicola* isolates. Additionally, *mcr-1* was not detected in the isolates. The expression of *pmrB* did not differ significantly between the colistin-resistant and colistin-susceptible isolates, but *phoQ* expression was significantly higher in the colistin-resistant isolate 464-1 than in the colistin-susceptible isolate 475-3, despite there being no differences in the sequences of the two isolates (Figure 1). *pbgP* expression also increased significantly in the colistin-resistant isolate.

The colistin-susceptible isolate 475-3 showed two distinctive peaks at *m/z* 1824 and 1840, each representing two glucosamines, phosphates, myristates (C_{14}) with four R-3-hydroxy-myristoyl acyl chains, and a hexa-acylated lipid A consisting of two glucosamines, phosphates, four R-3-hydroxy-myristoyl acyl chains, and one myristate (C_{14}) with 2-hydroxymyristates ($C_{14:OH}$) (Figure 2). These moieties have also been reported in *K. pneumoniae* (Leung et al., 2017). Contrastingly, the colistin-resistant isolate 464-1 showed strong intensities between *m/z* 1400 and 1900 with the two highest peaks at *m/z* 1643 and 1744, representing amino-arabinosylated lipid A with hexa-acylated species (Trent et al., 2001; Vorachek-Warren et al., 2002).

Colistin resistance in *K. variicola* has rarely been studied. Recently, colistin-resistant hypervirulent *K. variicola* isolates (MICs, 8 mg/L and 16 mg/L) have been reported in China and Chile, respectively (Lu et al., 2018; Morales-León et al., 2021). In these isolates, amino acid substitutions in PhoP or PmrB have been suggested to mediate colistin resistance. In a colistin-resistant mutant, mutations in *phoP* have been confirmed to be associated with colistin resistance in *K. variicola in vitro* (Janssen et al., 2020). This previous study identified lipid A modifications, such as the hydroxylation of an acyl chain, addition of 4-amino-4-deoxy-L-arabinose, and acylation with palmitate, in the colistin-resistant *K. variicola* mutant.

Here, we did not find any mutations in *pmrAB*, *phoPQ*, and *mgrB*, which are known to be associated with colistin resistance in *K. pneumoniae*, in the colistin-resistant *K. variicola* isolate. However, an upregulation of *phoQ* and *pbgP* expression was identified in the colistin-resistant *K. variicola* isolate, along with lipid A modifications. Thus, lipid A modification due to the two-component regulatory system and an overexpression of the *pbgP* operon conferred colistin resistance in the *K. variicola* isolate. Moreover, it has been reported that reduced colistin susceptibility occurs due to lipid A modification without amino acid changes in the proteins encoded by *pmrAB*, *phoPQ*, *mgrB*, and *crrAB* in *K. pneumoniae*.[6] This may be attributed to adaptive resistance, which involves an increase in antibiotic resistance because of

alterations in protein expression levels triggered by environmental conditions, such as nutrient conditions, stress, and sub-inhibitory antibiotic concentrations (Skida et al., 2011). Alternatively, genetic changes in other genes may lead to the upregulation of genes associated with colistin resistance.

In this study, we identified a colistin-resistant *K. variicola* isolate and compared it with a colistin-susceptible *K. variicola* isolate. We found that colistin resistance in *K. variicola* was mediated by the modification of lipid A, which was associated with an overexpression of the two-component regulatory system and the *pbgP* operon, as observed in the closest related pathogen, *K. pneumoniae*. Although the isolate was obtained from fecal samples of healthy adults, colistin-resistant *K. variicola* challenges public health as an opportunistic pathogen.

Declarations

Acknowledgments

This study was approved by the Institutional Review Board at Kangbuk Samsung Hospital (IRB No. 2014-08-035) and the requirement for informed consent was waived as we used remaining stool samples without any personal. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Science and ICT (grant number, 2019R1A2C2004879).

Author Contributions: SJK and KSK designed experiments; SJK and JJ collected samples; SJK and JJ conducted experiments; SJK, JJ, and KSK conducted data analysis and drafted the manuscript.

Conflicts of Interest: The authors declare no conflict of interest. All the authors read and approved the final manuscript.

References

1. Clinical and Laboratory Standards Institute (CLSI) (2018) Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. CLSI supplement M100. CLSI 2018, Wayne, Pa, USA
2. Gipson KS, Nickerson KP, Drenkard E, Llanos-Chea A, Dogiparthi SK, Lanter BB, Hibbler RM, Yonker LM, Hurley BP, Faherty CS (2020) The Great ESKAPE: Exploring the crossroads of bile and antibiotic resistance in bacterial pathogens. Infect Immun 88:e00865–e00819
3. Janssen AB, Doorduijn DJ, Mills G, Rogers MRC, Bonten MJM, Rooijakkers SHM, Willems RJL, Bengoechea JA, van Schaik W (2020) Evolution of colistin resistance in the *Klebsiella pneumoniae* complex follows multiple evolutionary trajectories with variable effects on fitness and virulence characteristics. Antimicrob Agents Chemother 65:e01958–e01920
4. Joo EJ, Kim SJ, Baek M, Choi Y, Seo J, Yeom JS, Ko KS (2018) Fecal carriage of antimicrobial-resistant Enterobacteriaceae in healthy Korean adults. J Microbiol Biotechnol 28:1178–1184

5. Kim SJ, Ko KS (2018) Diverse genetic alterations responsible for post-exposure colistin resistance in populations of the same strain of *Klebsiella pneumoniae*. Int J Antimicrob Agents 52:425–429
6. Leung LM, Cooper VS, Rasko DA, Guo Q, Pacey MP, McElheny CL, Mettus RT, Yoon SH, Goodlett DR, Ernst RK, Doi Y (2017) Structural modification of LPS in colistin-resistant, KPC-producing *Klebsiella pneumoniae*. J Antimicrob Chemother 72:3035–3042
7. López-Fernández H, Santos HM, Capelo JL, Fdez-Riverola F, Glez-Peña D, Reboiro-Jato M (2015) Mass-Up: an all-in-one open software application for MALDI-TOF mass spectrometry knowledge discovery. BMC Bioinformatics 16:318
8. Lu Y, Feng Y, McNally A, Zong Z (2018) Occurrence of colistin-resistant hypervirulent *Klebsiella variicola*. J Antimicrob Chemother 73:3001–3004
9. Morales-León F, Opazo-Capurro A, Caro C, Lincopan N, Cardenas-Arias A, Esposito F, Illesca V, Rioseco ML, Domínguez-Yévenes M, Lima CA, Bello-Toledo H, González-Rocha G (2021) Hypervirulent and hypermucoviscous extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* and *Klebsiella variicola* in Chile. Virulence 12:35-44
10. Rodríguez-Medina N, Barrios-Camacho H, Duran-Bedolla J, Garza-Ramos U (2019) *Klebsiella variicola*: an emerging pathogen in humans. Emerg Microbes Infect 8:973–988
11. Skiada A, Markogiannakis A, Plachouras D, Daikos GL (2011) Adaptive resistance to cationic compounds in *Pseudomonas aeruginosa*. Int J Antimicrob Agents 37:187-193.Trent MS, Ribeiro AA, Lin S, Cotter RJ, Raetz CRH. (2001) An inner membrane enzyme in Salmonella and Escherichia coli that transfers 4-amino-4-deoxy-L-arabinose to lipid A: induction on polymyxin-resistant mutants and role of a novel lipid-linked donor. J Biol Chem 276:43122-43131
12. Vorachek-Warren MK, Ramirez S, Cotter RJ, Raetz CR (2002) A triple mutant of *Escherichia coli* lacking secondary acyl chains on lipid A. J Biol Chem 277:14194–14205
13. Wright MS, Suzuki Y, Jones MB, Marshall SH, Rudin SD, van Duin D, Kaye K, Jacobs MR, Bonomo RA, Adams MD (2015) Genomic and transcriptomic analyses of colistin-resistant clinical isolates of *Klebsiella pneumoniae* reveal multiple pathways of resistance. Antimicrob Agents Chemother 59:536–543
14. Yi EC, Hackett M (2000) Rapid isolation method for lipopolysaccharide and lipid A from gram-negative bacteria. Analyst 125:651–656

Figures

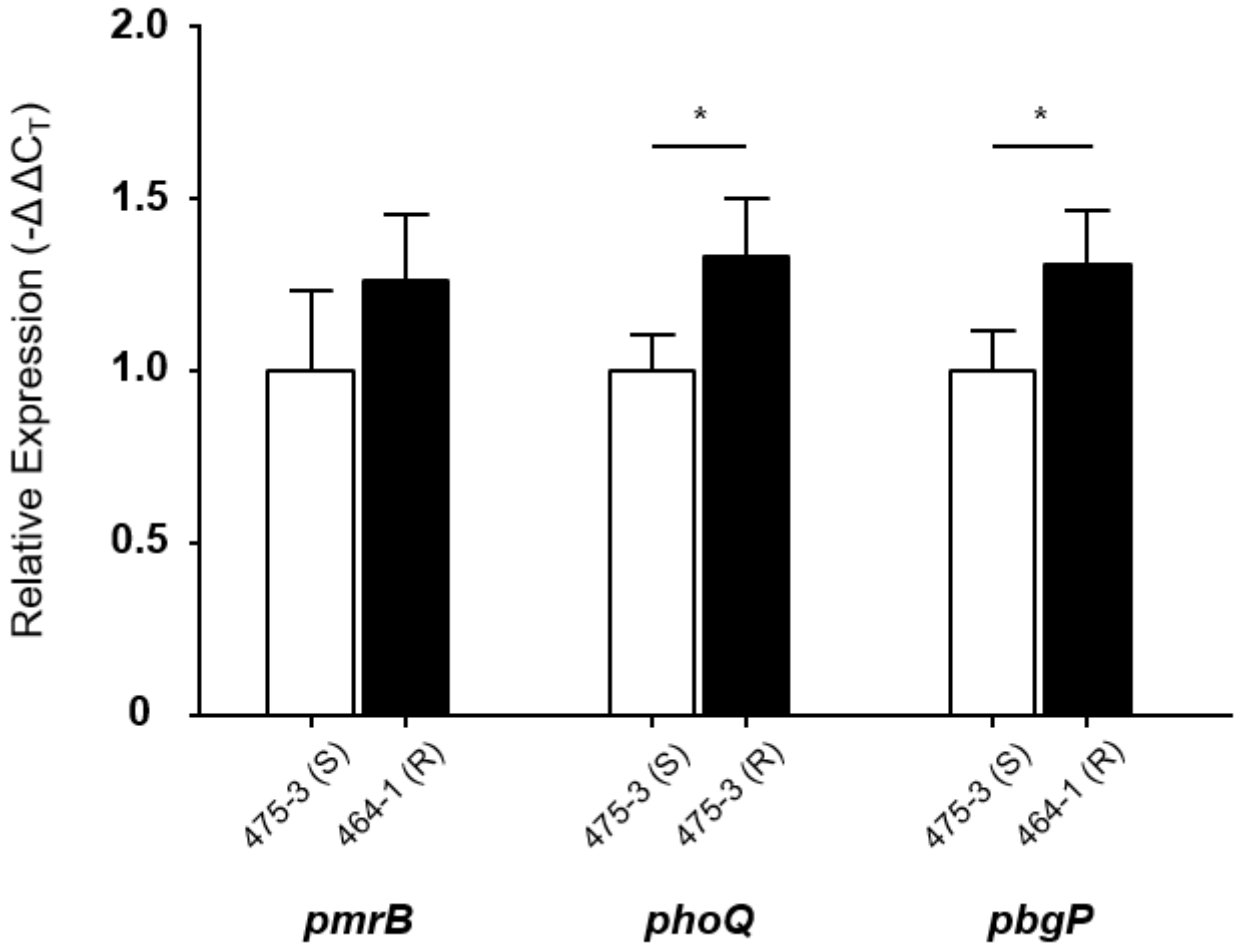

Figure 1

Quantification of pmrB, phoQ, and pbgP expression in colistin-susceptible and colistin-resistant K. variicola isolates. The experiments were repeated with three independent cultures, and rpoB was used as the reference gene. The fold changes were calculated using the comparative threshold cycle (-ΔΔCT) method. Statistical significance was determined using Student's unpaired t-test, and statistical analysis was performed using Prism version 3.00 for Windows (GraphPad Software, San Diego, CA, USA). S, colistin-susceptible; R, colistin-resistant; *P < 0.05.

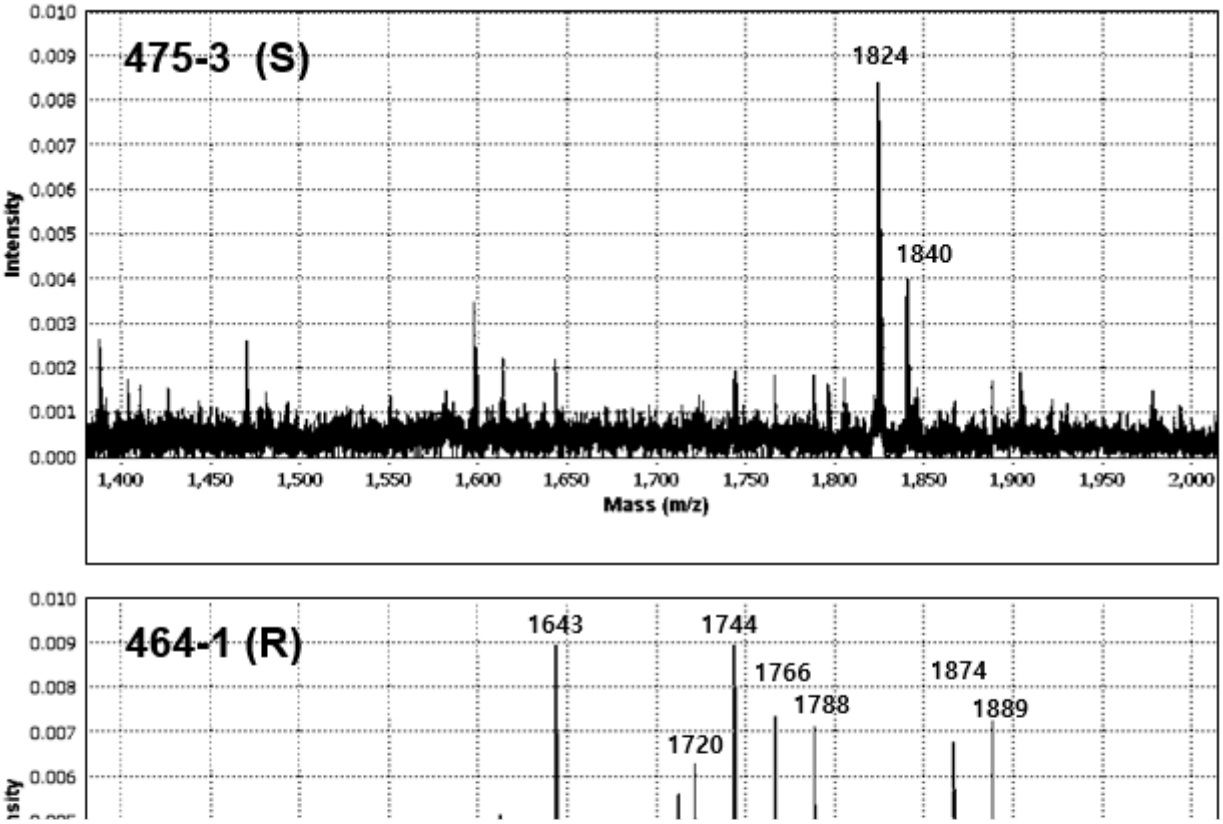

Figure 2

Negative-ion mode matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS) spectra of the lipid A moieties of lipopolysaccharide isolates obtained from the two Klebsiella variicola isolates, 464-1 and 475-3. S, colistin-susceptible; R, colistin-resistant.