Data Article

Data on the phylogenetic typing, integron gene cassette array analysis, multi-drug resistance analysis and correlation between antimicrobial resistance determinants in *Klebsiella* strains

Hao Wu, Mingyu Wang, Yuqing Liu, Xinhua Wang, Yunkun Wang, Jinxing Lu, Hai Xu

State Key Laboratory of Microbial Technology, School of Life Sciences, Shandong University, Jinan 250100, Shandong, PR China

Shandong Key Laboratory of Animal Disease Control and Breeding, Institute of Animal Science and Veterinary Medicine, Shandong Academy of Agricultural Sciences, Jinan 250100, Shandong, PR China

School of Environmental Science and Engineering, Shandong University, Jinan 250100, Shandong, PR China

State Key Laboratory for Infectious Disease Prevention and Control, and National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, PR China

**A R T I C L E   I N F O**

**Article history:**
Received 6 June 2016
Received in revised form 4 July 2016
Accepted 11 July 2016
Available online 3 August 2016

**Keywords:**
Antimicrobial resistant *Klebsiella* species
Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)
Gene cassette arrays of integron
Statistical analysis

**A B S T R A C T**

The antimicrobial resistance of *Klebsiella* species in the poultry industry is becoming a public concern. In support our recent publication “Characterization of antimicrobial resistance in *Klebsiella* species isolated from chicken broilers” (Wu et al., 2016) [1], multilocus sequence typing (MLST) and *gyrA* PCR-RFLP assays were conducted to identify the genetic relationships between and phylogenetic groups of the 90 antimicrobial resistant *Klebsiella* species isolated from a commercial broiler slaughter plant in Shandong, China. In addition, PCR-RFLP was performed to identify different gene cassette arrays in class 1 and 2 integrons, and the correlations between different antimicrobial resistance determinants were analyzed.

© 2016 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

DOI of original article: http://dx.doi.org/10.1016/j.ifoodmicro.2016.06.001
*Corresponding author.

E-mail addresses: wuhao3075@163.com (H. Wu), wangmingyu@sdu.edu.cn (M. Wang), liuiuqing@163.com (Y. Liu), xinhuawang@sdu.edu.cn (X. Wang), ykwang@sdu.edu.cn (Y. Wang), lujinxing@icdc.cn (J. Lu), haixu@sdu.edu.cn (H. Xu).

http://dx.doi.org/10.1016/j.dib.2016.07.016

2352-2409/© 2016 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
Specifications Table

| Subject area | Microbiology |
|--------------|--------------|
| More specific subject area | Food safety, antibiotic resistance |
| Type of data | Table, figure |
| How data was acquired | PCR, sequencing and statistical analysis |
| Data format | Analyzed |
| Experimental factors | Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), chi-square tests using SPSS |
| Experimental features | Identification of phylogenetic groups and different gene cassette arrays in class 1 and 2 integrons of *Klebsiella* species, analysis of the correlations between different antimicrobial resistance determinants |
| Data source location | Jinan, Shandong province of China. |
| Data accessibility | The data is available with this article |

Value of the data

- The gyrA PCR-RFLP assay and MLST analysis in the *Klebsiella* isolates indicate the relationship of epidemiology of drug resistant bacteria in between clinical and poultry industry.
- The PCR-PFLP by *Eco*RII can be applied as a tool for detection of gene cassette arrays of integron 1 or 2.
- The statistical data and finding of a significant association of antimicrobial resistance determinants can be used as references for the investigation of other drug resistant bacteria.

1. Data

MLST was performed using seven housekeeping genes (*rpoB, gapA, mdh, pgi, phoE, infB, and tonB*), and primers of those genes for PCR amplification and sequencing were designed (Table 1) [2]. gyrA PCR-RFLP profiles showed nearly all (89/90) of the isolates were identified as KpI-type and only one isolate was KpIII (Fig. 1). Antimicrobial susceptibility to nine antimicrobial agents was tested for the 90 *Klebsiella* isolates [1]. Among the isolates, 96.7% of them were resistant to more than three tested antimicrobial agents as well as 91.1% were resistant to more than three beta-lactam antibiotics (Fig. 2). A significant association between different antimicrobial resistance determinants was analyzed (Table 2). PCR-PFLP patterns of gene cassette arrays for integron 1 or 2 were performed (Fig. 3), and the detailed description was in the original article [1].

2. Experimental design, materials and methods

2.1. PCR Program

PCRs were prepared as follows: a final volume of 25 μl containing 1 μM of each primer, 0.2 mM dNTPs, 1.5 mM MgCl₂, and 1 unit of *Taq* polymerase (TransGen Biotech, Beijing, China). The conditions used for amplification were as described by the original article [1].

2.2. Primers designed for the MLST analysis of *Klebsiella* isolates

The primer pairs for seven housekeeping genes (*rpoB, gapA, mdh, pgi, phoE, infB, and tonB*) were designed for PCR amplification and sequencing (Table 1), as described previously [2].
### 2.3. Molecular identification by PCR-RFLP analysis of the gyra gene

PCR-RFLP patterns were obtained by the restriction analysis of a 441-bp PCR fragment of the gyra gene using the restriction enzymes HincII, TaqI, or HaeIII (Fig. 1) [3,4]. According to this approach, Klebsiella strains can be classified into the KpI, KpII, and KpIII phylogenetic groups.

### 2.4. Statistics analysis

According to the prevalence of antimicrobial resistance genes among 90 Klebsiella isolates [1], the number of antimicrobial resistance strains (Fig. 2a) and the percentage of tested strains resistant to

---

#### Table 1

| Locus | Putative function of gene | Primer sequence (5′–3′) | No. of alleles | Amplicon size (bp) | Melting temp (°C) |
|-------|---------------------------|-------------------------|---------------|-------------------|------------------|
| rpoB  | Beta-subunit of RNA polymerase B | VIC3 VIC2 GGCAGA ATGGCWAAGAACCA | 4 | 501 | 51 |
| gapA  | Glyceraldehyde 3-phosphate dehydrogenase | gapA173 gapA1810 5 | 450 | 60 |
| mdh   | Malate dehydrogenase | mdhB867 mdhB10 4 | 477 | 50 |
| pgi   | Phosphogluco isomerase | pgi1F pgi1R pgi2F pgi2R | 5 | 432 | 50 |
| phoE  | Phosphoprotein E | phoE604.II phoE604.II | 9 | 420 | 50 |
| infB  | Translation initiation factor 2 | infB1F infB1R infB2F infB2R | 6 | 318 | 50 |
| tonB  | Periplasmic energy transducer | tonB1F tonB2R | 17 | 414 | 50 |

*a sequencing primers were the same as the PCR primers for rpoB, gapA, mdh, phoE, and tonB, while pgi2F/2R and infB2F/1R were the sequencing primers for pgi and infB, respectively.

---

![Fig. 1. PCR-RFLP profiles of the gyra gene identified in the 90 Klebsiella isolates using HincII, TaqI, and HaeIII. Lane 1, 3, 5, 7 for Kpi (89 isolates) and lanes 2, 4, 6, 8 for Kpii (one isolate). Lanes 1 and 2, the 441-bp PCR product of the gyra gene. Lanes 3 and 4, HincII restriction profiles (298- and 143-bp fragments). Lanes 5 and 6, TaqI restriction profiles (197-, 142-, and 93-bp fragments). Lane 7, HaeIII restriction profile (175-, 129-, 92-, and 45-bp fragments). Lane 8, HaeIII restriction profile (175-, 174-, and 92-bp fragments). M, molecular size marker.

---

2.3. Molecular identification by PCR-RFLP analysis of the gyra gene

gyra PCR-RFLP patterns were obtained by the restriction analysis of a 441-bp PCR fragment of the gyra gene using the restriction enzymes HincII, TaqI, or HaeIII (Fig. 1) [3,4]. According to this approach, Klebsiella strains can be classified into the Kpi, Kpii, and Kpiii phylogenetic groups.

2.4. Statistics analysis

According to the prevalence of antimicrobial resistance genes among 90 Klebsiella isolates [1], the number of antimicrobial resistance strains (Fig. 2a) and the percentage of tested strains resistant to
Fig. 2. Antimicrobial resistance to different antibiotics of 90 *Klebsiella* isolates. (a) The percentage of tested strains resistant to different numbers of antibiotics. (b) The percentage of tested strains resistant to different beta-lactam antibiotic groups. CAZ, ceftazidime; CFP, cefoperazone; CTX, cefotaxime; CPE, cefepime; AMP, ampicillin; KAN, kanamycin; CHL, chloramphenicol; TET, tetracycline; CIP, ciprofloxacin.

Table 2

The correlation between different antimicrobial resistance determinants.

| Antimicrobial resistant *Klebsiella* isolates | Strain(s) containing antimicrobial resistance determinants | p-value  |
|---------------------------------------------|----------------------------------------------------------|----------|
| 90 Both PMQR and ESBL                       | PMQR, ESBL                                              | 0.0001   |
| 77 Both ESBL and Integron 1                 | ESBL, Integron 1                                         | 0.0003   |
| 76 Both PMQR and Integron 1                 | PMQR, Integron 1                                         |          |
| 71 Transconjugants from antimicrobial resistant *Klebsiella* isolates | PMQR, Integron 1                                         | 0.0001   |
| 86 Transconjugants from antimicrobial resistant *Klebsiella* isolates | PMQR, Integron 1                                         | 0.0045   |

ESBL, extended-spectrum beta-lactamase gene; PMQR, plasmid-mediated quinolone resistance gene; integrons 1, class 1 integron.
different numbers of beta-lactam antibiotic groups were analyzed (Fig. 2b). Also the statistical analysis of the correlation between different antimicrobial resistance determinants was performed by chi-square tests using SPSS (SPSS 19.0 for Windows; SPSS Inc., Chicago, IL, USA), and a \( p \)-value < 0.05 was considered to be statistically significant (Table 2).

2.5. Identification of integron gene cassette arrays

The gene cassette arrays of class 1 and 2 integrons were analyzed (Fig. 3) by a PCR-RFLP method as described previously [5].

Acknowledgments

This work was supported by the Grants from the National Science and Technology Major Project (2013ZX10004217-003), the National Natural Science Foundation of China (31170112), the Fundamental Research Funds of Shandong University (2015JC025), and the State Key Laboratory of Microbial Technology Open Projects Fund (M2015-10), Shandong University. We would like to thank the team of curators of the Institut Pasteur MLST and whole genome MLST databases for curating the data and making them publicly available at http://bigsdb.web.pasteur.fr/.

Transparency document. Supporting material

Transparency data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.07.016.

References

[1] H. Wu, M. Wang, Y. Liu, X. Wang, Y. Wang, J. Lu, H. Xu, Characterization of antimicrobial resistance in Klebsiella species isolated from chicken broilers, Int. J. Food Microbiol. 232 (2016) 95–102. http://dx.doi.org/10.1016/j.ijfoodmicro.2016.06.001.
[2] L. Diancourt, V. Passet, J. Verhoef, P.A. Grimont, S. Brisse, Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates, J. Clin. Microbiol. 43 (2005) 4178–4182.

[3] S. Brisse, E. van Duijkeren, Identification and antimicrobial susceptibility of 100 *Klebsiella* animal clinical isolates, Vet. Microbiol. 105 (2005) 307–312.

[4] S. Brisse, T. van Himbergen, K. Kusters, J. Verhoef, Development of a rapid identification method for *Klebsiella pneumoniae* phylogenetic groups and analysis of 420 clinical isolates, Clin. Microbiol. Infect. 10 (2004) 942–945.

[5] X. Guo, R. Xia, N. Han, H. Xu, Genetic diversity analyses of class 1 integrons and their associated antimicrobial resistance genes in *Enterobacteriaceae* strains recovered from aquatic habitats in China, Lett. Appl. Microbiol. 52 (2011) 667–675.