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Molecular Detection of the *Arr-2* Gene in *Escherichia coli* and *Klebsiella pneumoniae* Resistant to Rifampicin in Abidjan, Côte D'Ivoire

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VG, NKG and JAD designed the study, wrote the protocol. Authors JMR, SMD and MD provided the material and equipment. Authors AAT, FK and MBO contributed to the writing and editing of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to demonstrate the presence of rifampicin resistance arr-2 gene in certain enterobacteria of clinical origin from various biological products.
Place and Duration of Study: National Reference Center for Antibiotics of Pasteur Institute of Côte d'Ivoire, and research unit on emerging tropical infectious diseases of Aix-Marseille University,

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between January to July 2017.
Methodology: The strains were isolated from different biological samples and identified by biochemical tests and MALDI-TOF MS mass spectrometry. The Antibiotic susceptibility testing was performed by diffusion on Mueller- Hinton (MH) agar.
Results: All strains were resistant to rifampicin (100%) and amoxicillin (100%), but sensitive to imipenem and colistin. Conventional PCR using specific primers detected the *arr-2* gene in four strains, including 3 strains of *Klebsiella pneumoniae* and one strain of *Escherichia coli*. Molecular typing of the strains by MLST showed that the *E. coli* strain had ST 5 and the three strains of *K. pneumoniae* had ST 307, ST 309, ST 273 respectively.
Conclusion: This article is the first report that highlights the presence of the arr-2 gene in enterobacteria in Côte d'Ivoire.

Keywords: Escherichia coli; *Klebsiella pneumoniae; rifampicin; aar-2 gene; Abidjan.*

1. INTRODUCTION

Rifampicin is an antibiotic used in the treatment of tuberculosis [1], meningitis [2] and against Staphylococcus aureus infections [3,4]. It works by binding to the β-subunit of the RNA polymerase and inhibits the transition from initiation to elongation during transcription [5].

Resistance to rifampicin was mainly due to the mutations in the *rpoB* gene that encodes the β-subunit synthesis of bacterial RNA polymerase, resulting in decreased binding of R-RNA polymerase to rifampicin [6]. Presently, several others resistance mechanisms are identified, including efflux systems [7], glycosylation [8,9], phosphorylation [10] and inactivation of the antibiotic by ADP-ribosyltransferase (Arr) [11]. The first *arr-1* resistance gene has been described in *Mycobacterium smegmatis* [12] and subsequently the *arr-2* and *arr-3* genes have been described, carried by class I integrons in gram-negative bacilli in Europe and Asia [13,14, 15]. In particular, the arr-2 gene is present on several transposons and integrons found in strains of *Pseudomonas aeruginosa* [16], *Escherichia coli* [17], *Klebsiella pneumoniae* [13] and *Acinetobacter baumannii* [18].

The objective of the present work was to highlight the presence of the *arr-2* gene in rifampicin-resistant clinical enterobacteria isolated from Abidjan.

2. MATERIALS AND METHODS

2.1 Bacterial Strains

The strains that were used in this study were 153 in number mainly of Enterobacteriaceae producing Extended-Spectrum Beta-Lactamase (ESBL) and those that did not. They were isolated from different biological samples, pre-identified by biochemical tests and stored at the Center of Biological Resources (CeReB) of the Pasteur Institute of Côte d'Ivoire. Their identification was confirmed by MALDI-TOF MS (Bruker) mass spectrometry.

2.2 Antibiotic Susceptibility Test

Antibiotic susceptibility was determined from young colonies grown on Mac Conkey agar (BioMérieux SA, France) for 24 hr. The antibiotics used to perform the antibiogram were: amoxicillin (25 µg), amoxicillin + clavulanic acid (20 µg + 10 µg), ticarcillin + clavulanic acid (75 µg + 10µg), cefotaxime (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), aztreonam (30 µg), imipenem (10 µg), ertapenem (10 µg), amikacin (30 µg), gentamicin (15 µg), ciprofloxacin (5 µg), fosfomycin (50 µg), colistin (50 µg), cotrimoxazole (25 µg), rifampicin (30 µg). Mueller-Hinton agar growth medium was used for diffusion according to the method described by the Antibiogram Committee of the French Society of Microbiology (CA-SFM, 2013).

2.3 Total DNA Extraction

Extraction of the total DNA from the strains was performed from 24 h young colonies using the G2 buffer contained in the EZ1 DNA Tissue Kit (QIAGEN). Using a vortex, a colony of the strain was homogenized in 200 mL of buffer G2. The homogenate was then introduced into the EZ1 automated for extraction. The DNA thus extracted was stored at -20°C.

2.4 Gene Detection by Conventional PCR

Conventional PCR was performed for detection of *arr-2* using the following primers: arr-2_Forward_AATTACAAGCAGGTGCAAGGA and arr-2_Reverse_ TTCAATGACGTGTAAACCACG. The reaction

was carried out in a reaction volume of 25 µL consisted of 12.5 µL of Master Mix (Quantitect Probe PCR master mix, Qiagen), 1 µl of the primer mixture (Eurogentec), 6.5 µL of UP water and 5µL of total DNA. The amplification consisted of an initial denaturation step of the double-stranded DNA for 15 min at 95°C. This step was followed by 35 amplification cycles comprising denaturation at 94°C for 1 min, hybridization at 55°C for 50 sec, elongation at 72°C for 2 min and final elongation of 7 min at 72°C.

2.5 Visualization of Amplified Genes

The PCR products were analysed using 1.5% agarose gel electrophoresis prepared with 0.5% Tris-Borate-EDTA (TBE). The BenchTop pGEM^R DNA Markers Cat.# G7521 (Promega, France) molecular weight marker were used and the migration lasted for 20 minutes at a voltage of 135 V. The visualization of the DNA bands were done on a transilluminator.

2.6 PCR, Sequencing and Molecular Typing by MLST of Resistant Strains

To determine the typical sequences of the resistant strains, a conventional PCR using specific primers of the house-keeping genes per species was carried out. The functions of these genes and the sequences of the primers were summarized in Tables 1 and 2. The purified PCR products were sequenced using the BigDye® kit (Invitrogen, Life Technologies, North America) following the manufacturer's user's guide, in the automated ABI PRISM 3730xl Genetic Analyzer. Sequence analysis was performed on the MLST websites of the Pasteur Institut of Paris and MLST Warwick in order to assign a standard sequence number according to the allelic profiles of the different strains.

3. RESULTS

3.1 Antibiotics Susceptibility

All strains showed resistance 100% to rifampicin. For the resistance to amoxicillin alone, it was 100%, while the resistance rate to amoxicillin/ clavulanic acid was 99.3%. The susceptibility test showed high resistance to third-generation cephalosporins, cefotaxime (85.6%), ceftriaxone (81%) and cefoxitin (80.4%) respectively. The trimethoprim-sulfamethoxazole and fosfomycin resistance levels were 86.3% and 16.3%, respectively. The aminoglycoside resistance rate was 80.4% and 81% of the strains resistant to ciprofloxacin. However, all strains were sensitive to imipenem and colistin.

3.2 Distribution of Rifampicin Resistance Genes and MLST

The *arr2* gene was detected in four strains, 1 *E. coli* and 3 *K. pneumoniae* (Fig. 1). Table 3 presents the epidemiological profile of the 4 strains carrying the *arr-2* gene. Fig. 2 shows the visualization of the 7 house-keeping genes of the *E. coli* and *K. pneumoniae* species after electrophoresis. Strain typing by MLST analysis showed that *E. coli* strain had ST 5 and all three strains of *K. pneumoniae* had ST 273, ST 307 and ST 309 respectively.

Table 1. Primers used in MLST for *E. coli*

Gene (function)	Primer sequence (5' ▶ 3')	Amplicon size (bp)
adk (adenylate kinase)	ATTCTGCTTGGCGCTCCGGG (F) CCGTCAACTTTCGCGTATTT (R)	583
fumC (fumarate hydratase)	TCACAGGTCGCCAGCGCTTC (F) GTACGCAGCGAAAAAGATTC (R)	806
icd isocitrate/isopropylmalate déshydrogénase)	ATGGAAAGTAAAGTAGTTGTTCCGGCACA (F) GGACGCAGCAGGATCTGTT (R)	878
purA (adenylosuccinate déshydrogénase)	CGCGCTGATGAAAGAGATGA (F) CATACGGTAAGCCACGCAGA (R)	816
gyrB (DNA gyrase)	TCGGCGACACGGATGACGGC (F) ATCAGGCCTTCACGCGCATC (R)	911
recA (ATP/GTP binding motif)	CGCATTCGCTTTACCCTGACC (F) TCGTCGAAATCTACGGACCGGA (R)	780
mdh (malate déshydrogénase)	ATGAAAGTCGCAGTCCTCGGCGCTGCTGGCGG (F) TTAACGAACTCCTGCCCCAGAGCGATATCTTTCTT (R)	932

Table 2. Primers used in MLST for *K. pneumoniae*

Gene (function)	Primer sequence(5'►3')	Amplicon size (bp)
rpoB (beta-subunit of RNA polymerase)	GTTTTCCCAGTCACGACGTTGTAGGCGAAATGGCWGAGAACCA(F) TTGTGAGCGGATAACAATTTCGAGTCTTCGAAGTTGTAACC (R)	501
gapA (Glyceraldehyde-3-phosphate dehydrogenase)	GTTTTCCCAGTCACGACGTTGTATGAAATATGACTCCACTCACGG (F) TTGTGAGCGGATAACAATTTCCTTCAGAAGCGGCTTTGATGGCTT (R)	450
mdh (malate dehydrogenase)	GTTTTCCCAGTCACGACGTTGTACCCAACTCGCTTCAGGTTCAG (F) TTGTGAGCGGATAACAATTTCCCGTTTTTCCCCAGCAGCAG (R)	477
pgi (phosphoglucose isomerase)	GTTTTCCCAGTCACGACGTTGTAGAGAAAAACCTGCCTGTACTGCTGGC (F) TTGTGAGCGGATAACAATTTCCGCGCCACGCTTTATAGCGGTTAAT (R)	432
phoE (phosphorine E)	GTTTTCCCAGTCACGACGTTGTAACCTACCGCAACACCGACTTCTTCGG (F) TTGTGAGCGGATAACAATTTCTGATCAGAACTGGTAGGTGAT (R)	420
inf B (translation initiation factor 2)	GTTTTCCCAGTCACGACGTTGTACTCGCTGCTGGACTATATTCG (F) TTGTGAGCGGATAACAATTTCCGCTTTCAGCTCAAGAACTTC (R)	318
tonB (periplasmic energy transducer)	GTTTTCCCAGTCACGACGTTGTACTTTATACCTCGGTACATCAGGTT (F) TTGTGAGCGGATAACAATTTCATTCGCCGGCTGRGCRGAGAG (R)	414

Table 3. Epidemiologic profile of the 4 strains carrying the arr-2 gene

Strains	Services	Biological sample	Typical sequences
E. coli 725YO/15	Neurology	Blood	ST 5
K. pneumoniae 868Y/13	Pediatrics	Suppuration	ST 307
K. pneumoniae 1141Y/15	Neonatal maternity	Urine	ST 309
K. pneumoniae 654UB/15	pneumonology	sputum	ST 273

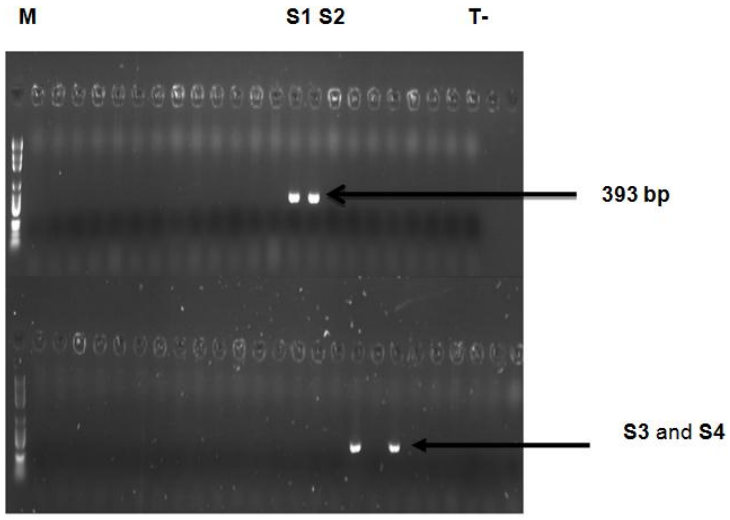

Fig. 1. Electrophoresis of *arr2* genes

M: Molecular weight marker
T-: negative control
S1: K. Pneumoniae 868Y/13
S2 : K. Pneumoniae 1141Y/15
S3 : E. coli 725YO/15
S4 : K. Pneumoniae 654UB/15

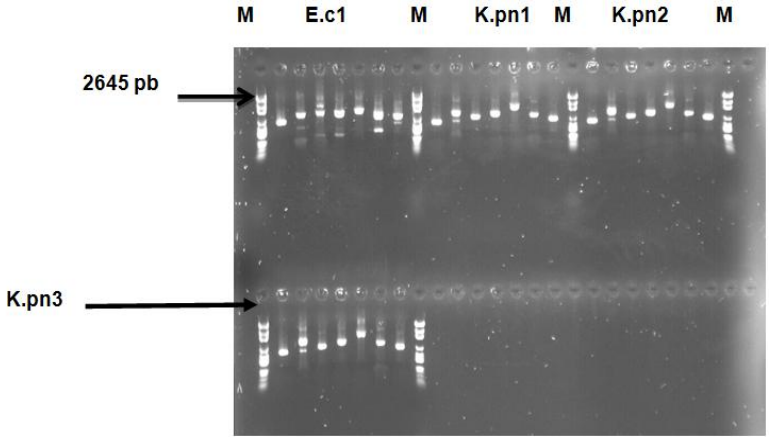

Fig. 2. Visualization of 7 house-keeping genes of *E. coli* and *K. peumoniae*

E.c1 : E. coli 725YO/15
K.pn1 : K. Pneumoniae 868Y/13
K.pn2 : K. Pneumoniae 1141Y/15;
K.pn3 : K. Pneumoniae 654UB/15

4. DISCUSSION

Resistance to rifampicin is a serious public health problem [19] because it is the first-line drug for the treatment of tuberculosis [20] and the prevention of meningitis [2]. Rifampicin resistance induced by the *arr-2* gene has been described in several bacterial strains including *P. aeruginosa* [16], *E. coli* [17], *K. pneumoniae* [13] and *A. baumannii* [18]. Detection of the *arr-2* gene in strains of *K. pneumoniae* and *E. coli* is the first of its kind in Côte d'Ivoire could explain rifampicin resistance observed in strains. However, the presence of this gene only in four strains while the resistance rate was 100%, suggests that strains use others mechanisms of resistance to rifampicin such as efflux systems [7], glycosylation [8,9], phosphorylation [10] or

resistance may be due to other resistance genes such as arr-3 [13].

Moreover, the typing of strains with the *arr-2* gene by MLST analysis showed that an *E. coli* strain had ST 5. This sequence (ST 5) was found in an *E. coli* strain isolated from stool from a patient in Douala, Cameroon, which also carried the β-lactam resistance genes *blaNDM-1* and *blaOXA-1*. The patient had been hospitalized for a month in Douala for an inflammatory syndrome associated with renal failure before his transfer to Paris [21].

Also, the three strains of *K. pneumoniae* carrying the *arr-2* gene had ST 273, ST 307, and ST 309, respectively. These different standard sequences have also been obtained elsewhere in the world. The type 273 sequence was found in a strain of *K. pneumoniae*, isolated from a patient with pneumonia in the Philippines, which carried the *blaNDM-7* gene [22]. *K. pneumoniae* ST 273 has been reported in Italy, Norway, Russia and the United Kingdom. These studies reported that ST273 isolates carried various carbapenemase genes including *blaKPC*, *blaNDM-1* and *blaVIM*, and ST 273 is known to have the epidemic potential [23,24].

Regarding the ST 307 sequence, work done in Italy showed that strains of *K. pneumoniae* isolated from three Italian hospital centers and which carried the *KPC-2*, *KPC-3* and *blaCTXM-15* genes had ST 307 [25]. ST 307 was first described in 2008 in the multi-locus sequence typing database (MLST) (an unpublished isolate) and has since been described in 2013 in the United States [26]. It was initially associated with *CTX-M-15* expanded spectrum β-lactamase production. The acquisition of a KPC enzyme is posterior to that of CTX-M-15, as Kp ST 307 CTX-M-15 producer was previously reported at a high rate in Italy, Korea, Pakistan, Morocco and in domestic animals from Japan [27-28].

As for the type 309 sequence, it was found in strains of *K. pneumoniae* isolated from hospitalized patients in northern China [29].

4. CONCLUSION

This study has highlighted the high resistance to rifampicin in enterobacteria of clinical origin in Côte d'Ivoire. The *arr-2* resistance gene was detected in only four strains, suggesting that there would be other mechanisms of rifampicin resistance used by the strains that should be explored. Multilocus sequence typing (MLST) has made it possible to genetically characterize resistant strains and highlight the circulation of various clones around the world.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Baysarowich J, Koteva K, Hughes DW, Ejim L, Griffiths E, Zhang K, Junop M, Wright GD. Rifamycin antibiotic resistance by ADP-ribosylation: Structure and diversity of Arr. PNAS. 2008;105: 12.
2. Taha MK, Zarantonelli ML, Ruckly C, Giorgini D, Alonso JM. Rifampin-resistance *Neisseria meningitidis*. Emerg. Infect. Dis. 2006;12:859-860.
3. Kapusnik JE, Parenti F, Sande MA. The use of rifampicin in staphylococcal infections-a review. J. Antimicrob. Chemother. 1984;13:61-66.
4. Leung MJ, Kell AD, Collignon P. Antibiotic guidelines for meningococcal prophylaxis. Med. J. Aust. 1998;169:396.
5. McClure WR, Cech CL. On the mechanism of rifampicin inhibition of RNA synthesis. J. Biol. Chem. 1978;253:8949-8956.
6. Williams DL, Waguespack C, Eisenach K, Crawford JT, Portaels F, Salfinger M, Nolan CM, Abe C, Sticht-Groh V, Gillis TP. Characterization of rifampin-resistance in pathogenic mycobacteria. Antimicrob. Agents Chemother. 1994;38:2380-2386.
7. Chandrasekaran S, Lalithakumari D. Plasmid-mediated rifampicin resistance in *Pseudomonas fluorescens*. J. Med. Microbiol. 1998;47:197-200.
8. Tanaka Y, Yazawa K, Dabbs ER, Nishikawa K, Komaki H, Mikami Y, Miyaji M, Morisaki N, Iwasaki S. Different rifampicin inactivation mechanisms in *Nocardia* and related taxa. Microbiol. Immunol. 1996;40:1-4.
9. Yazawa K, Mikami Y, Maeda A, Akao M, Morisaki N, Iwasaki S. Inactivation of rifampin by *Nocardia brasiliensis*. Antimicrob. Agents Chemother. 1993; 37:1313-1317.
10. Yazawa K, Mikami Y, Maeda A, Morisaki N, Iwasaki S. Phosphorylative inactivation

of rifampicin by *Nocardia otitidiscaviarum*. J. Antimicrob. Chemother. 1993;33:1127-1135.

11. Dabbs ER, Yazawa K, Mikami Y, Miyaji M, Morisaki N, Iwasaki S, Furihata K. Ribosylation by mycobacterial strains as a new mechanism of rifampin inactivation. Antimicrob. Agents Chemother. 1995;39: 1007-1009.
12. Quan S, Venter H, Dabbs ER. Ribosylative inactivation of rifampin by *Mycobacterium smegmatis* is a principal contributor to its low susceptibility to this antibiotic. Antimicrob. Agents Chemother. 1997;41: 2456-2460.
13. Arlet G, Nadjar D, Herrmann JL, Donay JL, Rouveau M, Lagrange PH, Philippon A. Plasmid-mediated rifampin resistance encoded by an *arr-2*-like gene cassette in *Klebsiella pneumoniae* producing an AAC-1 class C β-lactamase. Antimicrob. Agents Chemother. 2001;45:2971-2972.
14. Lee K, Yum JH, Yong D, Lee HM, Kim HD, Docquier JD, Rossolini GM, Chong Y. Novel acquired metallo-lactamase gene, *bla*SIM-1, in a class 1 integron from *Acinetobacter baumannii* clinical isolates from Korea. Antimicrob. Agents Chemother. 2005;49:4485-4491.
15. Mammeri H, Van De Loo M, Poirel L, Martinez-Martinez L, Nordmann P. Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. Antimicrob. Agents Chemother. 2005;49: 71-76.
16. Tribuddharat C, Fennewald P. Integron-mediated rifampin resistance in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. 1999;43:960-962.
17. Naas T, Mikami Y, Imai T, Poirel L, Nordmann P. Characterization of In53, a class1 plasmid- and composite transposon-located integron of *Escherichia coli* which carries an unusual array of gene cassettes. J Bacteriol. 2001;183:235-249.
18. Houang ET, Chu YW, Lo WS, Chu KY, Cheng AF. Epidemiology of rifampin ADP-ribosyltransferase (arr-2) and metallo-beta-lactamase (blaIMP-4) gene cassettes in class 1 integrons in *Acinetobacte*r strains isolated from blood cultures in 1997 to 2000. Antimicrob Agents Chemother. 2003;47:1382-1390.
19. Sam IC, Drobniewski F, More P, Kemp M, Brown T. *Mycobacterium tuberculosis* and rifampin resistance, United Kingdom. Emerg. Infect. Dis. 2006;12:752–759.
20. Grosset JH. Present status for chemotherapy for tuberculosis. Rev. Infect. Dis. 1989;11:347–352.
21. Smits SL, Leeuwen MV, Schapendonk CME, Schürch AC, Bodewes R, Haagmans BL, Osterhaus ADME. New Delhi Metallo-β-Lactamase 4-producing *Escherichia coli* in Cameroon. Emerg Infectious Diseases. 2012;18:9.
22. Chou A, Roa M, Evangelista MA, Sulit AK, Lagamayo E, Torres BC, Klinzing DC, Daroy MLG, NavoaNg G, Sucgang R, Zechiedrich L. Emergence of *Klebsiella pneumoniae* ST273 Carrying blaNDM-7 and ST656 Carrying blaNDM-1 in Manila, Philippines. Microbial Drug Resistance. 2016;22:7.
23. Samuelsen O, Toleman MA, Hasseltvedt V, Fuursted K, Leegaard TM, Walsh TR, Sundsfjord A, Giske CG, Molecular characterization of VIM-producing *Klebsiella pneumoniae* from Scandinavia reveals genetic related ness with international clonal complexes encoding transferable multidrug resistance. Clin. Microbiol. Infect. 2011;17:1811-1816.
24. Ageevets VA, Partina IV, Lisitsyna ES, Ilina EN, Lobzin YV, Shlyapnikov SA, Sidorenko SV. Emergence of carbapenemase-producing Gram-negative bacteria in Saint Petersburg, Russia. Int. J. Antimicrob. Agents. 2014;44:152-155.
25. Villa LCF, Fortini D, Brisse S, Passet V, Bonura C, Endimiani A, Mammina C, Ocampo AM, Jimenez JN, Doumith M, Woodford N, Hopkins K, Carattoli A. Diversity, virulence, and antimicrobial resistance of the KPC producing *Klebsiella pneumoniae* ST307 clone. Microbial Genomics. 2017;3.
26. Castanheira M, Farrell SE, Wanger A, Rolston KV, Jones RN. Rapid expansion of KPC-2-producing *Klebsiella pneumoniae* isolates in two Texas hospitals due to clonal spread of ST258 and ST307 lineages. Microb Drug Resist. 2013; 19:295-297.
27. Girlich D, Bouihat N, Poirel L, Benouda A, Nordmann P. High rate of faecal carriage of extended-spectrum b-lactamase and OXA-48 carbapenemase producing *Enterobacteriaceae* at a university hospital

in Morocco. Clin Microbiol Infect. 2014; 20:350–354.

28. Park DJ, Yu JK, Park KG, Park YJ. Genotypes of ciprofloxacin resistant *Klebsiella pneumoniae* in Korea and their characteristics according to the genetic lineages. Microb Drug Resist. 2015;21: 622-630.

29. Zhao J, ChenJ, ZhaoM, Qiu X, Chen X, Zhang W, SunR, Ogutu JO, Zhang F. Multilocus Sequence Types and Virulence Determinants of Hypermucoviscosity-Positive *Klebsiella pneumoniae* Isolated from Community-Acquired Infection Cases in Harbin, North China. J. Infect. Dis. 2016; 69:357-360.

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