AAC Journals.ASM.org

Transfer of CMY-2 Cephalosporinase from *Escherichia coli* to Virulent *Klebsiella pneumoniae* Causing a Recurrent Liver Abscess

Yi-Tsung Lin,[a,b] Yi-Jiun Pan,[c] Tzu-Lung Lin,[c] Chang-Phone Fung,[a,b] Jin-Town Wang[c,d]

Division of Infectious Diseases, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan[a]; School of Medicine, National Yang-Ming University, Taipei, Taiwan[b]; Department of Microbiology, National Taiwan University College of Medicine, Taipei, Taiwan[c]; Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan[d]

A CMY-2-producing capsular type K2 *Klebsiella pneumoniae* strain (TVGHKP93) with multidrug resistance was isolated from a recurrent liver abscess in a patient who also carried a CMY-2-producing *Escherichia coli* strain (TVGHEC01) in the stool. TVGHKP93 retained its high virulence compared with that of the isogenic strain (TVGHKP60) with wild-type resistance from the first liver abscess. Our conjugation experiment showed the successful transfer of the bla_{CMY-2}-carrying plasmid from TVGHEC01 into TVGHKP60. The transconjugant showed both high virulence and the multidrug-resistant phenotype, as did TVGHKP93.

Klebsiella pneumoniae liver abscess (KPLA) has been increasingly reported in Asia and is considered to be endemic in Taiwan (1, 2). The capsular type of *K. pneumoniae* appears to be the major virulence factor (3, 4), and the K1 and K2 types were the most prevalent in KPLA (1). In KPLA, *K. pneumoniae* isolates demonstrate an almost unique antibiogram indicative of resistance to ampicillin only, and multidrug-resistant isolates have rarely been reported (5, 6). Currently, several investigations have provided evidence that KPLA is preceded by gastrointestinal colonization (7–10).

We identified an 84-year-old patient with diabetes who had recurrent KPLA. The strain (TVGHKP60) isolated from the first abscess in November 2012 was susceptible to all antibiotics tested, except for ampicillin, consistent with the natural resistance of *K. pneumoniae*. The patient received intravenous ceftriaxone for 3 weeks and recovered well. After discharge, he received oral cefuroxime for another 2 weeks. However, the patient had a recurrent liver abscess in January 2013, and a second *K. pneumoniae* strain with multidrug resistance (TVGHKP93) was isolated. The patient received intravenous ciprofloxacin for 3 weeks and was discharged uneventfully. Interestingly, a multidrug-resistant *Escherichia coli* strain (TVGHEC01) was isolated from the patient's stool during the recurrence episode. We further investigated the two *K. pneumoniae* strains and one *E. coli* strain from this case. The protocol was approved by the institutional review board of Taipei Veterans General Hospital.

Bacterial identification and antimicrobial susceptibility were determined using a Vitek2 system (bioMérieux, Marcy l'Etoile, France). The antimicrobial susceptibility was interpreted according to the guidelines of the CLSI (11) and is shown in Table 1. Pulsed-field gel electrophoresis DNA fingerprinting (12, 13) showed that the wild-type TVGHKP60 strain was nearly identical (only one band difference) to the multidrug-resistant TVGHKP93 strain. Capsular genotyping, detection of *rmpA/rmpA2*, molecular characterization of β-lactamases, and colony mucoviscosity were performed as previously described (14–16). Multilocus sequence typing (MLST) was performed on the TVGHKP60 and TVGHKP93 strains, and the results were analyzed as previously described (17). The TVGHKP60 and TVGHKP93 strains both belonged to capsular type K2 and sequence type (ST) 86. They showed hypermucoviscosity phenotypes and carried *rmpA* and *rmpA2* genes. Regarding the detection of β-lactamases, bla_{SHV-1} was detected in the TVGHKP60 strain, and bla_{SHV-1} and bla_{CMY-2} were detected in the TVGHKP93 strain. Interestingly, bla_{CMY-2} was also detected in the TVGHEC01 strain.

The *K. pneumoniae* strains were cultured in LB broth at 37°C for 16 h to obtain bacterial growth curves as described previously (18). The isogenic bla_{CMY-2}-producing strain (TVGHKP93) and the original strain (TVGHKP60) showed no significant differences in their growth rates. The *in vivo* virulence of the TVGHKP60 and TVGHKP93 strains was compared using a murine model of septicemia generated by intraperitoneal injection. Male 6- to 8-week-old C57/B6 mice were observed for 1 week after intraperitoneal inoculation of 100 CFU of *K. pneumoniae*. All animal care procedures and protocols were approved by the Institutional Animal Care and Use Committee of National Yang-Ming University. Upon intraperitoneal infection of mice, both strains showed hypervirulence with 50% lethal dose(LD_{50}) values of $<$100 CFU (Fig. 1). The clinical *K. pneumoniae* strain (capsular type K64) isolated from blood was used as the control and its LD_{50} was 10^7 CFU.

Plasmids were extracted from TVGHEC01 for high-throughput sequencing using the Illumina/Solexa GAII sequencing platform. Coding sequences were predicted and annotated with NCBI protein BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome). A circular map of a bla_{CMY-2}-carrying plasmid, designated pCMY2, with

Received 28 February 2015 **Returned for modification** 25 March 2015
Accepted 9 May 2015

Accepted manuscript posted online 18 May 2015

Citation Lin Y-T, Pan Y-J, Lin T-L, Fung C-P, Wang J-T. 2015. Transfer of CMY-2 cephalosporinase from *Escherichia coli* to virulent *Klebsiella pneumoniae* causing a recurrent liver abscess. Antimicrob Agents Chemother 59:5000–5002. doi:10.1128/AAC.00492-15.

Address correspondence to Jin-Town Wang, wangjt@ntu.edu.tw.

Y.-T.L. and Y.-J.P. contributed equally to this article.

doi:10.1128/AAC.00492-15

TABLE 1 Antimicrobial susceptibility test data for the strains in this study

Antibiotic(s)	MIC (mg/liter)[a]			
	K. pneumoniae TVGHKP60	*K. pneumoniae* TVGHKP93	*E. coli* TVGHEC01	*K. pneumoniae* transconjugant: TVGHKP60::bla_{CMY-2}
Ampicillin	≥32	≥32	≥32	≥32
Cefazolin	≤4	≥64	≥64	≥64
Cefuroxime	2	16	16	16
Cefoxitin	≤4	32	32	32
Ceftriaxone	≤1	8	8	8
Ceftazidime	≤1	16	4	16
Cefepime	≤1	≤1	≤1	≤1
Piperacillin-tazobactam	≤4	8	≤4	8
Gentamicin	≤1	≤1	≤1	≤1
Amikacin	≤2	≤2	≤2	≤2
Ciprofloxacin	≤0.25	≤0.25	≤0.25	≤0.25
Levofloxacin	≤0.12	≤0.12	≤0.12	≤0.12
Ertapenem	≤0.5	≤0.5	≤0.5	≤0.5
Imipenem	≤0.25	≤0.25	≤0.25	≤0.25
Trimethoprim-sulfamethoxazole	0.19	0.38	0.094	0.38

[a] The values are MIC correlates determined by the Vitek2 system, except for trimethoprim-sulfamethoxazole, which was determined by an Etest.

102,199 bp was obtained (GenBank accession no. LC019731). Nucleotide blast results revealed that pCMY2 exhibited a high level of similarity with a 108,660-bp *E. coli* plasmid, pC49-108 (GenBank accession no. KJ484638), which showed 94% coverage and shared 99% DNA sequence identity. Conjugation-related genes (associated with *tra* and *trb*) and type IV pili-associated genes were found in both plasmids. However, pC49-108 harbored the drug resistance genes *addA5*, *dhfrA17*, and $bla_{CTX-M-1}$, while pCMY2 carried an AmpC-type β-lactamase, bla_{CMY-2}, instead. The PCR results using four primer pairs located within the plasmid revealed that pCMY2 was present in both TVGHEC01 and TVGHKP93 but absent from the TVGHKP60. It is reasonable to consider that horizontal transfer of a bla_{CMY-2}-carrying resistance determinant from *E. coli* to *K. pneumoniae* developed in the patient's bowel.

Our attempts to mimic the natural transfer of the pCMY2 plasmid from TVGHEC01 into TVGHKP60 using conjugation experiments described previously (19) were successful. The conjugation frequency was determined to be 5.2×10^{-6}. The MICs for cefazolin, cefuroxime, cefoxitin, ceftriaxone, ceftazidime, and piperacillin-tazobactam increased in the transconjugant (TVGHKP60::bla_{CMY-2}) and were the same as those for TVGHKP93 (Table 1). The *in vivo* virulence assessment also confirmed that *K. pneumoniae* TVGHKP60::bla_{CMY-2} retained the hypervirulence characteristics (data not shown). These results implied that TVGHKP60 had acquired pCMY2 from TVGHEC01 in the patient's gut, leading to the formation of the hypervirulent and multidrug-resistant TVGHKP93 in the recurrent liver abscess.

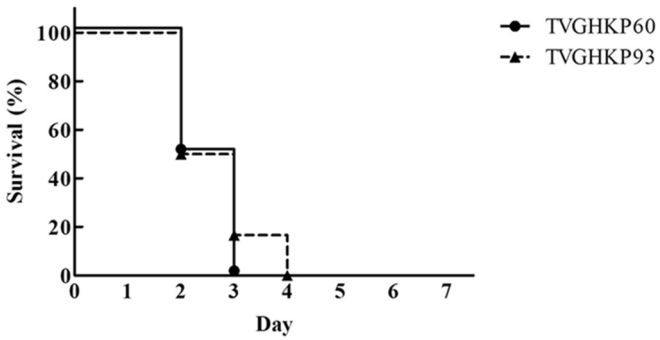

FIG 1 *In vivo* virulence study. Mouse lethality data following challenges with the TVGHKP60 and TVGHKP93 strains are presented. Male C57BL/6 mice ($n = 6$ from two independent experiments) were inoculated by intraperitoneal injection with 100 CFU of the TVGHKP60 and TVGHKP93 strains. Survival was assessed for 7 days following infection. The Kaplan-Meier method was used to evaluate the survival rate. Upon intraperitoneal infection, all mice from each group (TVGHKP60 versus TVGHKP93) were dead within 5 days (log rank test; $P = 0.6285$).

Capsular type K2 and ST86 are considered to be hypervirulent clones that can cause invasive diseases (20, 21). Our study first demonstrated that this virulent strain acquired the pCMY2 plasmid but still retained its virulence. The selective pressure by cephalosporins may predispose to the possible plasmid transfer in this case. Although increased antimicrobial resistance is generally associated with decreased virulence and fitness, evidence has also shown the opposite, and it is increasingly evident that the relationship is often of greater benefit to the pathogen, resulting in a growing public health problem (22).

A recent study showed that multidrug-resistant and hypervirulent populations of *K. pneumoniae* were mostly nonoverlapping, although two isolates with combined virulence and resistance features were detected (23). These results show that the threat of dual-risk *K. pneumoniae* strains, combining virulence and multidrug-resistance features, is becoming a reality. The multidrug-resistant and highly virulent TVGHKP93 strain derived from the wild-type TVGHKP60 strain serves as a good example to verify the relationship between virulence and resistance in *K. pneumoniae*.

With the increasing rate of drug-resistant *Enterobacteriaceae* colonizing the intestine, the possibility of interspecies transfer of drug resistance determinants into highly virulent *K. pneumoniae* increases. The acquisition of an important mechanism of antibiotic resistance, such as CMY-2, might suggest that virulent strains

may be a potential cause of nosocomial infections in the future (24).

Nucleotide sequence accession number. The complete nucleotide sequence of pCMY2 was deposited in GenBank under accession no. LC019731.

ACKNOWLEDGMENTS

This work was supported by grants from the Ministry of Science and Technology in Taiwan, Taipei Veterans General Hospital (V104B-001 and V104C-072), and Szu-Yuan Research Foundation of Internal Medicine.

We also thank Taipei Veterans General Hospital for providing experimental space and facilities in the Medical Science & Technology Building.

We declare no conflicts of interest.

REFERENCES

1. **Siu LK, Yeh KM, Lin JC, Fung CP, Chang FY.** 2012. *Klebsiella pneumoniae* liver abscess: a new invasive syndrome. Lancet Infect Dis **12:**881–887. http://dx.doi.org/10.1016/S1473-3099(12)70205-0.
2. **Tsai FC, Huang YT, Chang LY, Wang JT.** 2008. Pyogenic liver abscess as endemic disease, Taiwan. Emerg Infect Dis **14:**1592–1600. http://dx.doi.org/10.3201/eid1410.071254.
3. **Fung CP, Chang FY, Lee SC, Hu BS, Kuo BI, Liu CY, Ho M, Siu LK.** 2002. A global emerging disease of *Klebsiella pneumoniae* liver abscess: is serotype K1 an important factor for complicated endophthalmitis? Gut **50:**420–424. http://dx.doi.org/10.1136/gut.50.3.420.
4. **Fang CT, Chuang YP, Shun CT, Chang SC, Wang JT.** 2004. A novel virulence gene in *Klebsiella pneumoniae* strains causing primary liver abscess and septic metastatic complications. J Exp Med **199:**697–705. http://dx.doi.org/10.1084/jem.20030857.
5. **Hsueh PR, Wu JJ, Teng LJ, Chen YC, Yang PC, Ho SW, Luh KT.** 2002. Primary liver abscess caused by one clone of *Klebsiella pneumoniae* with two colonial morphotypes and resistotypes. Emerg Infect Dis **8:**100–102. http://dx.doi.org/10.3201/eid0801.010167.
6. **Su SC, Siu LK, Ma L, Yeh KM, Fung CP, Lin JC, Chang FY.** 2008. Community-acquired liver abscess caused by serotype K1 *Klebsiella pneumoniae* with CTX-M-15-type extended-spectrum beta-lactamase. Antimicrob Agents Chemother **52:**804–805. http://dx.doi.org/10.1128/AAC.01269-07.
7. **Tu YC, Lu MC, Chiang MK, Huang SP, Peng HL, Chang HY, Jan MS, Lai YC.** 2009. Genetic requirements for *Klebsiella pneumoniae*-induced liver abscess in an oral infection model. Infect Immun **77:**2657–2671. http://dx.doi.org/10.1128/IAI.01523-08.
8. **Chung DR, Lee H, Park MH, Jung SI, Chang HH, Kim YS, Son JS, Moon C, Kwon KT, Ryu SY, Shin SY, Ko KS, Kang CI, Peck KR, Song JH.** 2012. Fecal carriage of serotype K1 *Klebsiella pneumoniae* ST23 strains closely related to liver abscess isolates in Koreans living in Korea. Eur J Clin Microbiol Infect Dis **31:**481–486. http://dx.doi.org/10.1007/s10096-011-1334-7.
9. **Fung CP, Lin YT, Lin JC, Chen TL, Yeh KM, Chang FY, Chuang HC, Wu HS, Tseng CP, Siu LK.** 2012. *Klebsiella pneumoniae* in gastrointestinal tract and pyogenic liver abscess. Emerg Infect Dis **18:**1322–1325. http://dx.doi.org/10.3201/eid1808.111053.
10. **Lin YT, Liu CJ, Yeh YC, Chen TJ, Fung CP.** 2013. Ampicillin and amoxicillin use and the risk of *Klebsiella pneumoniae* liver abscess in Taiwan. J Infect Dis **208:**211–217. http://dx.doi.org/10.1093/infdis/jit157.
11. **Clinical and Laboratory Standards Institute.** 2012. Performance standards for antimicrobial susceptibility testing: 22nd informational supplement. CLSI document M100-S22. Clinical and Laboratory Standards Institute, Wayne, PA.
12. **Lin YT, Wang FD, Chan YJ, Fu YC, Fung CP.** 2014. Clinical and microbiological characteristics of tigecycline non-susceptible *Klebsiella pneumoniae* bacteremia in Taiwan. BMC Infect Dis **14:**1. http://dx.doi.org/10.1186/1471-2334-14-1.
13. **Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol **33:**2233–2239.
14. **Fang CT, Lai SY, Yi WC, Hsueh PR, Liu KL, Chang SC.** 2007. *Klebsiella pneumoniae* genotype K1: an emerging pathogen that causes septic ocular or central nervous system complications from pyogenic liver abscess. Clin Infect Dis **45:**284–293. http://dx.doi.org/10.1086/519262.
15. **Hsu CR, Lin TL, Chen YC, Chou HC, Wang JT.** 2011. The role of *Klebsiella pneumoniae rmpA* in capsular polysaccharide synthesis and virulence revisited. Microbiology **157:**3446–3457. http://dx.doi.org/10.1099/mic.0.050336-0.
16. **Yu WL, Ko WC, Cheng KC, Lee HC, Ke DS, Lee CC, Fung CP, Chuang YC.** 2006. Association between *rmpA* and *magA* genes and clinical syndromes caused by *Klebsiella pneumoniae* in Taiwan. Clin Infect Dis **42:**1351–1358. http://dx.doi.org/10.1086/503420.
17. **Diancourt L, Passet V, Verhoef J, Grimont PA, Brisse S.** 2005. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. J Clin Microbiol **43:**4178–4182. http://dx.doi.org/10.1128/JCM.43.8.4178-4182.2005.
18. **Lin WH, Kao CY, Yang DC, Tseng CC, Wu AB, Teng CH, Wang MC, Wu JJ.** 2014. Clinical and microbiological characteristics of *Klebsiella pneumoniae* from community-acquired recurrent urinary tract infections. Eur J Clin Microbiol Infect Dis **33:**1533–1539. http://dx.doi.org/10.1007/s10096-014-2100-4.
19. **Chuang YP, Fang CT, Lai SY, Chang SC, Wang JT.** 2006. Genetic determinants of capsular serotype K1 of *Klebsiella pneumoniae* causing primary pyogenic liver abscess. J Infect Dis **193:**645–654. http://dx.doi.org/10.1086/499968.
20. **Bialek-Davenet S, Criscuolo A, Ailloud F, Passet V, Nicolas-Chanoine MH, Decré D, Brisse S.** 2014. Development of a multiplex PCR assay for identification of *Klebsiella pneumoniae* hypervirulent clones of capsular serotype K2. J Med Microbiol **63:**1608–1614. http://dx.doi.org/10.1099/jmm.0.081448-0.
21. **Lin YT, Wang YP, Wang FD, Fung CP.** 2015. Community-onset *Klebsiella pneumoniae* pneumonia in Taiwan: clinical features of the disease and associated microbiological characteristics of isolates from pneumonia and nasopharynx. Front Microbiol **9:**122. http://dx.doi.org/10.3389/fmicb.2015.00122.
22. **Beceiro A, Tomas M, Bou G.** 2013. Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clin Microbiol Rev **26:**185–230. http://dx.doi.org/10.1128/CMR.00059-12.
23. **Bialek-Davenet S, Criscuolo A, Ailloud F, Passet V, Jones L, Delannoy-Vieillard AS, Garin B, Le Hello S, Arlet G, Nicolas-Chanoine MH, Decré D, Brisse S.** 2014. Genomic definition of hypervirulent and multidrug-resistant *Klebsiella pneumoniae* clonal groups. Emerg Infect Dis **20:**1812–1820. http://dx.doi.org/10.3201/eid2011.140206.
24. **Shon AS, Bajwa RP, Russo TA.** 2013. Hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*: a new and dangerous breed. Virulence **4:**107–118. http://dx.doi.org/10.4161/viru.22718.