First Report of Chronic Pulmonary Infection by KPC-3-Producing and Colistin-Resistant *Klebsiella pneumoniae* Sequence Type 258 (ST258) in an Adult Patient with Cystic Fibrosis

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The spread of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* continues to increase, and the possible development of KPC-producing *K. pneumoniae* infections in cystic fibrosis (CF) patients is a matter of concern. Here, we describe the establishment of a chronic lung infection due to a colistin-resistant KPC-producing *K. pneumoniae* isolate in an Italian CF patient.

CASE REPORT

A 57-year-old male cystic fibrosis (CF) patient (sweat test, Cl 100 meq/liter; genotype, F508del/F508del; forced expiratory volume in 1 s [FEV1] in good health, 31% of predicted normal value [31% pred]; weight, 56 kg; body mass index [BMI], 19.6; pancreatic insufficiency, CF-related diabetes) was first admitted to our department in 2011 for a severe pulmonary exacerbation. At that time, cultures of the sputum samples collected yielded colistin-susceptible (Col^S), carbapenem-resistant *Pseudomonas aeruginosa* (CRPA). Subsequently, several similar episodes occurred that required hospitalization. During these episodes, Col^S CRPA was repeatedly isolated from sputum cultures, and the administration of inhaled plus intravenous colistin was often required to achieve clinical stability. During interexacerbation intervals, long-term intermittent inhaled colistin was also administered with the aim of reducing the frequency of exacerbations.

In November 2013, intestinal carriage of Col^S *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* was detected by means of rectal swabbing. Concomitantly, Col^S KPC-producing *K. pneumoniae* started to grow from sputum cultures, together with Col^S CRPA. We initially considered the KPC-producing *K. pneumoniae* to be a respiratory colonizer in the context of a chronic Col^S CRPA lung infection; we therefore administered several courses of colistin to treat exacerbations and to reduce their frequency.

Beginning in March 2014, colistin-resistant (Col^R) KPC-producing *K. pneumoniae* grew from sputum cultures. Although concomitantly isolated CRPA was still Col^S, a lack of clinical response to colistin was observed after the first isolation of Col^R KPC-producing *K. pneumoniae*. Indeed, we started to consider the Col^R KPC-producing *K. pneumoniae* a true pathogen. Accordingly, the three subsequent exacerbations were treated with a Col^R KPC-producing *K. pneumoniae*-targeted regime of high-dose tigecycline (100 mg every 12 h after a loading dose of 200 mg) and meropenem (2 g every 6 h), achieving clinical improvements that allowed us to discharge the patient home at the end of each antibiotic course. However, despite these acute-phase successes, baseline respiratory function rapidly decreased after the first isolation of Col^R KPC-producing *K. pneumoniae*. At the time of this report, lung function tests revealed severe interexacerbation respiratory

impairment (FEV1, 23% pred), and the patient was undergoing evaluation for lung transplantation. The patient signed an informed consent for publishing this case report.

Six *Klebsiella pneumoniae* isolates consecutively isolated from the sputum samples of the patient from November 2013 to August 2014 were studied.

The Vitek 2 automated system (bioMérieux, Marcy l'Etoile, France) was used for identification of the isolates. In addition, quantitative cultures were performed for 5 of the 6 sputum samples mentioned above, by means of serial dilution of 500 μ l of sputum in 4.5 ml of 0.85% NaCl. Diluted samples were inoculated into standard MacConkey agar and chocolate medium and incubated at 35°C in an aerobic atmosphere, according to standard procedures (1). The number of CFU per milliliter of sputum was determined by colony counts. The results of quantitative cultures for KPC-producing *K. pneumoniae* and concomitantly isolated CRPA are shown in Table 1.

Antimicrobial susceptibility testing was performed by means of the Vitek 2 automated system. The MICs of meropenem, tigecycline, and colistin were also determined by the broth microdilution method suggested by EUCAST. Susceptibility results were interpreted according to the latest EUCAST criteria (version 4.0, 2014) (2).

According to the Vitek 2 results, the *K. pneumoniae* isolates were resistant to amoxicillin-clavulanate (MIC, >32 mg/liter),

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TABLE 1 Results from sputum cultures by quantitative methods

Organism ^a	No. of CFU/ml sputum in ^b :				
	November 2013	March 2014	April 2014	May 2014	August 2014
CRPA	>100,000	100,000	>100,000	>100,000	>100,000
KPC-producing <i>K. pneumoniae</i>	1,000	10,000	10,000	10,000	100,000

^a CRPA, carbapenem-resistant *Pseudomonas aeruginosa*; KPC, *Klebsiella pneumoniae* carbapenemase. All CRPA isolates were colistin susceptible, while KPC-producing *K. pneumoniae* isolates were colistin susceptible in November 2013 and February 2014 and colistin resistant afterwards.

^b True counts above 100,000 CFU/ml sputum were not measured. In February 2014, a qualitative culture was performed that was positive for both CRPA and KPC-producing *K. pneumoniae*.

extended-spectrum cephalosporins (MIC, >32 mg/liter), ertapenem (MIC, >16 mg/liter), imipenem (MIC, >16 mg/liter), meropenem (MIC, >16 mg/liter), amikacin (MIC, >64 mg/liter), ciprofloxacin (MIC, >4 mg/liter), fosfomycin (MIC, >64 mg/liter), and trimethoprim-sulfamethoxazole (MIC, >16/304 mg/liter). Their susceptibilities to gentamicin and tigecycline were intermediate (MICs, 4 mg/liter and 2 mg/liter, respectively), whereas the MIC of colistin was 2 mg/liter before March 2014 and rose to >16 mg/liter afterwards.

The results of the microdilution method for meropenem (MIC, >64 mg/liter) confirmed the susceptibility category obtained by the Vitek 2 system, while the tigecycline MIC (1 mg/liter) was 1 dilution lower than that observed with the automated system, placing the strains in the susceptible category, a phenomenon described previously by other authors (3, 4). The MICs of colistin were confirmed by the microdilution method, which is the only standardized method for testing colistin susceptibility. The Vitek 2 results showed high categorical agreement for colistin-susceptible and -resistant isolates.

Phenotypic testing for carbapenemase was performed with the Carba NP test (5), and the result was consistent with the type of carbapenemase produced: the color of wells containing imipenem plus ZnSO₄ and imipenem plus EDTA turned from red to yellow for all tested strains producing KPC-3. The identification of the KPC-3 gene was achieved by PCR and sequencing.

Genomic DNA was prepared, digested with XbaI (New England BioLabs, Inc., Ipswich, MA), and subjected to pulsed-field gel electrophoresis (PFGE) with the CHEF DRII device (Bio-Rad, Milan, Italy) as described previously (6). All six isolates exhibited the same PFGE macrorestriction profile, revealing chronic colonization with the same clone.

Multilocus sequence typing (MLST) was performed as described elsewhere (6). The isolates were found to belong to sequence type 258 (ST258) by using the *Klebsiella pneumoniae* MLST Database (<http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html>).

Our report describes the establishment of a Col^R KPC-producing *K. pneumoniae* chronic lung infection in a 57-year-old Italian CF patient. Previously, Leão et al. reported on two Brazilian female CF patients whose sputum cultures yielded KPC-2-producing *K. pneumoniae* during pulmonary exacerbations (7). However, no KPC-producing strains grew from subsequent sputum cultures. Conversely, KPC-producing *K. pneumoniae* was persis-

tently isolated from sputum cultures collected from our patient since November 2013. The same clone, as attested by the identical PFGE profiles of the isolates, was initially Col^S and then Col^R.

Chronic lung infection with this clone was defined as the persistent growth of the same organism in sputum cultures collected from our patient for at least 6 consecutive months, reflecting standard definitions that have been developed for *P. aeruginosa* (8–10). Indeed, no tailored clinical definitions have been provided so far for chronic lung infection due to *Enterobacteriaceae* such as *K. pneumoniae* and *Escherichia coli*. This lack of definitions reflects the fact that these organisms have classically been considered to have a minor chronic pathogenic role in CF, since their long-term persistence in the lung of CF patients has seldom been described (11–13). However, in a recent study by Barilova et al., the prevalence of persistent *E. coli* isolation (i.e., for longer than 6 months) from sputum samples was as high as 11% among 176 German CF patients, thus supporting a previously unrecognized role of members of the *Enterobacteriaceae* as possible CF pathogens (14). Although the author stressed the role of an adaptive strain shift toward either a mucoid or a small-colony-variant (SCV) phenotype as a predictor of long-term lung persistence, as many as 13 of 19 long-term *E. coli* strains in their study (68%) did not undergo these phenotypic changes. Similarly, in the present case, a long-term increase in KPC-producing *K. pneumoniae* log CFU counts per milliliter of sputum occurred without a shift toward either a mucoid or an SCV phenotype being detected. These data suggest that the development of Col^R KPC-producing *K. pneumoniae* chronic lung infection in CF patients may occur without notable limitation by the necessity of adaptive changes, at least in the strain phenotype. Whether or not specific genotypic changes are associated with long-term persistence of KPC-producing *K. pneumoniae* in the CF lung niche deserves further investigation through dedicated molecular studies. This is also true for genotypic changes in the virulence of KPC-producing *K. pneumoniae*, since phenotypes known to be associated with increased virulence, such as hypermucoviscosity, were not observed in our patient's isolates. However, it should be noted that enhanced virulence might be unnecessary for KPC-producing *K. pneumoniae* to cause disease. Indeed, despite showing a low virulence in animal models, the widespread ST258 clone is a well-known cause of infection in hospitalized patients, even in subjects without a severe degree of immunodeficiency (15–17).

In our patient, the pathogenic role of the long-term persistent Col^R KPC-producing *K. pneumoniae* was strongly supported by the reduced clinical response to colistin, which was observed despite the fact that all concomitantly isolated CRPA isolates continued to be Col^S. Further supporting this hypothesis, clinical improvements during the most recent exacerbations occurred after the administration of a Col^R KPC-producing *K. pneumoniae*-targeted regimen of high-dose meropenem and tigecycline, which did not include antimicrobials active against Col^S CRPA. The favorable impact of this antibiotic choice was in line with the improved outcome observed in non-CF patients with severe KPC-producing *K. pneumoniae* infections treated with meropenem-based combined regimens (16, 17).

We could not clearly establish where and when the patient actually acquired KPC-producing *K. pneumoniae*, since he was admitted to different hospitals and wards before being screened for intestinal KPC-producing *K. pneumoniae* carriage. Regarding this concern, massive efforts are made in our hospital to ensure all

possible infection control measures, and no other CF patient has been found to be colonized and/or infected with KPC-producing *K. pneumoniae* to date. However, colonization and/or infection of other CF patients is unfortunately expected in the near future, since the prevalence of KPC-producing *K. pneumoniae* in Italy is increasing, with a high percentage of isolates also being Col^R (18–20). This possibility is rather worrisome, since these organisms are often resistant to almost all the antibiotics administered to CF patients (i.e., fluoroquinolones, third-generation cephalosporins, aminoglycosides, carbapenems, and colistin). In addition, colistin resistance itself has been proven to be associated with increased mortality in the case of KPC-producing *K. pneumoniae* infections, further fueling concerns regarding the spread of Col^R KPC-producing *K. pneumoniae* (21).

Finally, although an increased periprocedural mortality has been reported in other-than-lung transplant recipients colonized by KPC-producing *K. pneumoniae*, whether or not KPC-producing *K. pneumoniae* carriage may significantly impair survival after lung transplantation in our and other CF patients remains unknown (22).

In conclusion, our report provides evidence of a chronic lung infection due to Col^R KPC-producing *K. pneumoniae* in an Italian CF patient. Because of the dramatic shortage of antibiotics active against these rods, all possible hospital-based infection control measures should be followed to avoid the spread of Col^R KPC-producing *K. pneumoniae* to other CF patients.

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REFERENCES

- Saiman L, Schidlow D, Smith A (ed). 1994. Concepts in care: microbiology and infectious diseases in cystic fibrosis, vol 5. Cystic Fibrosis Foundation, Bethesda, MD.
- European Committee on Antimicrobial Susceptibility Testing. 2014. Breakpoint tables for interpretation of MIC's and zone diameters, version 4.0. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_4.0.pdf.
- Marchaim D, Pogue JM, Tzuman O, Hayakawa K, Lephart PR, Salimnia H, Painter T, Zervos MJ, Johnson LE, Perri MB, Hartman P, Thyagarajan RV, Major S, Goodell M, Fakih MG, Washer LL, Newton DW, Malani AN, Wholehan JM, Mody L, Kaye KS. 2014. Major variation in MICs of tigecycline in Gram-negative bacilli as a function of testing method. *J Clin Microbiol* 52:1617–1621. <http://dx.doi.org/10.1128/JCM.00001-14>.
- Lat A, Clock SA, Wu F, Whittier S, Della-Latta P, Fauntleroy K, Jenkins SG, Saiman L, Kubin CJ. 2011. Comparison of polymyxin B, tigecycline, cefepime, and meropenem MICs for KPC-producing *Klebsiella pneumoniae* by broth microdilution, Vitek 2, and Etest. *J Clin Microbiol* 49:1795–1798. <http://dx.doi.org/10.1128/JCM.02534-10>.
- Dortet L, Poirel L, Nordmann P. 2012. Rapid detection of carbapenemase-producing *Pseudomonas* spp. *J Clin Microbiol* 50:3773–3776. <http://dx.doi.org/10.1128/JCM.01597-12>.
- Marchese A, Coppo E, Barbieri R, Debbia E. 2010. Emergence of KPC-2 carbapenemase-producing *Klebsiella pneumoniae* strains and spread of an isolate of sequence type 258 in the neuro-rehabilitation unit of an Italian hospital. *J Chemother* 22:212–214. <http://dx.doi.org/10.1179/joc.2010.22.3.212>.
- Leão RS, Pereira RH, Folescu TW, Albano RM, Santos EA, Junior LG, Marques EA. 2011. KPC-2 carbapenemase-producing *Klebsiella pneumoniae* isolates from patients with cystic fibrosis. *J Cyst Fibros* 10:140–142. <http://dx.doi.org/10.1016/j.jcf.2010.12.003>.
- Hoiby N. 1977. *Pseudomonas aeruginosa* infection in cystic fibrosis. Diagnostic and prognostic significance of *Pseudomonas aeruginosa* precipitins determined by means of crossed immunoelectrophoresis. A survey. *Acta Pathol Microbiol Scand Suppl* 262:1–96.
- Lee TW, Brownlee KG, Conway SP, Denton M, Littlewood JM. 2003. Evaluation of a new definition for chronic *Pseudomonas aeruginosa* infection in cystic fibrosis patients. *J Cyst Fibros* 2:29–34. [http://dx.doi.org/10.1016/S1569-1993\(02\)00141-8](http://dx.doi.org/10.1016/S1569-1993(02)00141-8).
- Pressler T, Bohmova C, Conway S, Dumcius S, Hjelte L, Hoiby N, Kollberg H, Tümmler B, Vavrova V. 2011. Chronic *Pseudomonas aeruginosa* infection definition: EuroCareCF Working Group report. *J Cyst Fibros* 10(Suppl 2):S75–S78. [http://dx.doi.org/10.1016/S1569-1993\(11\)60011-8](http://dx.doi.org/10.1016/S1569-1993(11)60011-8).
- Hoiby N. 1974. Epidemiological investigations of the respiratory tract bacteriology in patients with cystic fibrosis. *Acta Pathol Microbiol Scand B Microbiol Immunol* 82:541–550.
- Maccone AB, Pier GB, Pennington JE, Matthews WJ, Jr, Goldmann DA. 1981. Mucoid *Escherichia coli* in cystic fibrosis. *N Engl J Med* 304:1445–1449. <http://dx.doi.org/10.1056/NEJM198106113042401>.
- Crement L, Caroff N, Giraudeau C, Reynaud A, Caillon J, Corvec S. 2013. Detection of clonally related *Escherichia coli* isolates producing different CMY beta-lactamases from a cystic fibrosis patient. *J Antimicrob Chemother* 68:1032–1035. <http://dx.doi.org/10.1093/jac/dks520>.
- Barilova P, Tchesnokova V, Dübbers A, Küster P, Peters G, Dobrindt U, Sokurenko EV, Kahl BC. 2014. Prevalence and persistence of *Escherichia coli* in the airways of cystic fibrosis patients—an unrecognized CF pathogen? *Int J Med Microbiol* 304:415–421. <http://dx.doi.org/10.1016/j.ijmm.2014.02.008>.
- Tzouveleakis LS, Miriagou V, Kotsakis SD, Spyridopoulou K, Athanasiou E, Karagouni E, Tzelepi E, Daikos GL. 2013. KPC-producing, multidrug-resistant *Klebsiella pneumoniae* sequence type 258 as a typical opportunistic pathogen. *Antimicrob Agents Chemother* 57:5144–5146. <http://dx.doi.org/10.1128/AAC.01052-13>.
- Tumbarello M, Viale P, Viscoli C, Trecarichi EM, Tumietto F, Marchese A, Spanu T, Ambretti S, Ginocchio F, Cristini F, Losito AR, Tedeschi S, Cauda R, Bassetti M. 2012. Predictors of mortality in bloodstream infections caused by *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*: importance of combination therapy. *Clin Infect Dis* 55:943–950. <http://dx.doi.org/10.1093/cid/cis588>.
- Qureshi ZA, Paterson DL, Potoski BA, Kilayko MC, Sandovsky G, Sordillo E, Polsky B, Adams-Haduch JM, Doi Y. 2012. Treatment outcome of bacteremia due to KPC-producing *Klebsiella pneumoniae*: superiority of combination antimicrobial regimens. *Antimicrob Agents Chemother* 56:2108–2113. <http://dx.doi.org/10.1128/AAC.06268-11>.
- Munoz-Price LS, Poirel L, Bonomo RA, Schwab MJ, Daikos GL, Cormican M, Cornaglia G, Garau J, Gniadkowski M, Hayden MK, Kumarasamy K, Livermore DM, Maya JJ, Nordmann P, Patel JB, Paterson DL, Pitout J, Villegas MV, Wang H, Woodford N, Quinn JP. 2013. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. *Lancet Infect Dis* 13:785–796. [http://dx.doi.org/10.1016/S1473-3099\(13\)70190-7](http://dx.doi.org/10.1016/S1473-3099(13)70190-7).
- Nordmann P, Naas T, Poirel L. 2011. Global spread of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis* 17:1791–1798. <http://dx.doi.org/10.3201/eid1710.110655>.
- Giani T, Pini B, Arena F, Conte V, Bracco S, Migliavacca R, AMCLICRE Survey Participants, Pantosti A, Pagani L, Luzzaro F, Rossolini GM. 2013. Epidemic diffusion of KPC carbapenemase-producing *Klebsiella pneumoniae* in Italy: results of the first countrywide survey, 15 May to 30 June 2011. *Euro Surveill* 18(22):pii=20489. <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20489>.
- Capone A, Giannella M, Fortini D, Giordano A, Meledandri M, Ballardini M, Venditti M, Bordini E, Capozzi D, Balice MP, Tarasi A, Parisi G, Lappa A, Carattoli A, Petrosillo N, SEERBIO-GRAB Network. 2013. High rate of colistin resistance among patients with carbapenem-resistant *Klebsiella pneumoniae* infection accounts for an excess of mortality. *Clin Microbiol Infect* 19:E23–E30. <http://dx.doi.org/10.1111/1469-0691.12070>.
- Lübbert C, Becker-Rux D, Rodloff AC, Laudi S, Busch T, Bartels M, Kaisers UX. 2014. Colonization of liver transplant recipients with KPC-producing *Klebsiella pneumoniae* is associated with high infection rates and excess mortality: a case-control analysis. *Infection* 42:309–316. <http://dx.doi.org/10.1007/s15010-013-0547-3>.