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BRIEF REPORT

Risk Factors and Outcomes for Carbapenem-Resistant *Klebsiella pneumoniae* Isolation, Stratified by Its Multilocus Sequence Typing: ST258 Versus Non-ST258

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A "high risk" clone of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) identified by multilocus sequence typing (MLST) as sequence type (ST) 258 has disseminated worldwide. As the molecular epidemiology of the CRE pandemic continues to evolve, the clinical impact of non-ST258 strains is less well defined. We conducted an epidemiological investigation of CRKP based on strains MLST. Among 68 CRKP patients, 61 were ST258 and 7 belonged to non-ST258. *Klebsiella pneumoniae* ST258 strains were significantly associated with bla_{KPC} production and with resistance to an increased number of antimicrobials. Clinical outcomes were not different. Based on this analysis, one cannot rely solely on the presence of bla_{KPC} in order to diagnose CRKP.

Keywords. CRE; KPC; MDRO; outcome; risk factors.

In less than a decade, a transposon (*Tn*4401)-mediated outbreak of bla_{KPC}-producing *Klebsiella pneumoniae* has disseminated worldwide [1–3]. In most regions, a predominant carbapenem-resistant *K pneumoniae* (CRKP) strain, designated as ST258 by multilocus sequence typing (MLST), and harboring bla_{KPC} [1–4] is found. Therefore, the epidemiological investigations on the CRKP pandemic focuses almost exclusively on bla_{KPC}-producing ST258 CRKP strains [1, 2]. However, resistance to carbapenems is also seen among other sequence types (STs) of *K pneumoniae*, and it is mediated by various other mechanisms of resistance (non-bla_{KPC}) [5]. Carbapenemases such as bla_{VIM}, bla_{IMP}, bla_{NDM}, and bla_{OXA-48} rapidly emerged in clinical strains of *K pneumoniae* in the United States [6–9] and worldwide [10–12]. Moreover, other mechanisms of resistances to carbapenems, not mediated through carbapenemase production but through production of extended-spectrum β-lactamases (ESBLs) and plasmid-borne AmpCs combined with loss of outer-membrane porins [13, 14] or efflux pumps [15, 16], have been reported as well among CRKP.

Many of the diagnostic techniques that are used to investigate CRKP focus on phenotypic tests such as the modified Hodge tests (MHT), which are much less accurate at detecting carbapenemases other than bla_{KPC}, or limited genotypic (polymerase chain reaction [PCR]) tests to bla_{KPC} only [4, 17, 18]. This might result in under diagnosis of CRKP, due to the continued dissemination of non-bla_{KPC}-producing strains. The evolving epidemiology of CRKP necessitates continued study of its epidemiology (ie, patients' characteristics and infection outcomes), stratified upon the molecular characteristics of circulating strains. In this study, our aims were to compare the characteristics and outcomes of CRKP ST258 carriers (ie, carriers of the predominant strain) to carriers of other STs defined using MLST.

METHODS

Carbapenem-resistant *K pneumoniae* isolates from adult patients, obtained from September 2008 to September 2009, were analyzed at the Detroit Medical Center (DMC). Carbapenem-resistant *K pneumoniae* cases consisted of all patients who had an isolate discovered in a clinical sample, sent from all inpatient and outpatient facilities that submit specimens to DMC Microbiology Laboratory [19, 20]. Active surveillance screening cultures were not routinely performed during the study period, and they were excluded from the analysis. Cultures from all anatomic sites were collected, and both infected and "colonized-only" patients were enrolled [21]. For patients who had >1 CRKP isolate during the study period, only the first episode of CRKP isolation was analyzed [19, 20]. More than 200 parameters were retrieved from patient charts including demographics, background and comorbid conditions, recent exposures to antimicrobials and to healthcare procedures and environments, acute severity of illness indices, therapeutic regimens, and outcomes [19, 20]. Patient characteristics were compared between groups using Fisher's exact test.

Received 21 September 2015; accepted 16 December 2015.

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DOI: 10.1093/ofid/ofv213

Table 1. Representative Parameters of the Univariate Analysis Comparing Epidemiological Features and Outcomes of Patients With Carbapenem-Resistant *Klebsiella pneumoniae* (CRKP) Clone ST258 to Other CRKP Clones

Parameter	ST258 (n = 61), N (%)	Non-ST258 (n = 7), N (%)	*P* Value
Demographics			
Age, years, mean ± SD	65.4 ± 15.3	63.9 ± 15.3	.8
Elderly (over 65 y)	39 (63.9)	3 (42.9)	.4
Female gender	28 (45.9)	5 (71.4)	.3
Functional Status and Comorbidities on Admission			
Residence at any long-term care facility before admission	43 (74.1)	6 (85.7)	.50
Dependent functional status upon admission [31]	47 (87.0)	5 (83.3)	>.99
Cognitive impairment upon admission	26 (48.1)	4 (66.7)	.67
Charlson's combined condition score, mean ± SD [32]	7.8 ± 3.4	8.7 ± 4.4	.61
Exposure to Healthcare Settings and Antibiotics Before CRKP Isolation			
Time at risk: days from admission to culture, median (IQR)	9 (1–19)	8 (2–16)	.64
Previous hospitalization in the past 3 mo	46 (80.7)	5 (83.3)	>.99
Regular visits to outpatient clinic	22 (38.6)	4 (57.1)	.43
Invasive procedure in past 3 mo	49 (89.1)	6 (100.0)	.39
Chronic permanent devices (eg, tracheotomy, gastrostomy, tunneled central-line, urinary catheter, pacemaker/defibrillator, external fixator)	48 (88.9)	6 (85.7)	.8
Received antibiotics (any type) in preceding 3 mo	46 (98)	5 (83)	.22
Received carbapenems in preceding 3 mo	11 (25)	2 (50)	.3
Microbiology			
Body Site of Isolation			
Blood	14 (23)	0 (0)	.33
Respiratory	15 (24.6)	3 (42.9)	.37
Urine	23 (37.7)	3 (42.9)	>.99
Wound	8 (13.1)	1 (14.3)	>.99
CSF	1 (1.6)	0	>.99
Infection (as opposed to asymptomatic colonization)	39 (78)	4 (66.7)	.62
Colistin resistance	12 (20)	2 (28.6)	.63
Tigecycline resistance	12 (20.7)	0	.33
Tobramycin resistance	**57 (100)**	**3 (50)**	**.001**
Ciprofloxacin resistance	**54 (98.2)**	**3 (60)**	**.02**
Trimethoprim/ sulfamethoxazole resistance	49 (86)	4 (67)	.24
Positive modified Hodge test	57 (98)	7 (100)	>.99
PCR bla_{KPC}-positive	**55 (98.2)**	**5 (71.4)**	**.03**
rep-PCR typing			
Clone 1	**42 (78.6)**	**1 (14.3)**	**.002**
Clone 2	12 (21.4)	0	.33
Clone 3	**0**	**3 (42.9)**	**<.001**
Clone 4	**0**	**3 (42.9)**	**<.001**
Severity of Illness Indices at Time of Isolation			
McCabe score, mean ± SD [33]	2.04 ± 0.66	1.83 ± 0.41	.31
Severe sepsis/septic shock/multiorgan failure	8 (20.5)	2 (33.3)	.6
Received vasopressors	**3 (7.7)**	**3 (50)**	**.02**
Acutely transferred to an intensive care unit	7 (14.3)	3 (42.9)	.1
Necessitated intubation	**4 (11.1)**	**3 (50)**	**.05**
Infectious clinical syndrome			
Colonization, no infection	11 (25)	2 (33.3)	.64
Central-line infection	5 (11.4)	0	>.99
Pneumonia	10 (22.7)	2 (33.3)	.62
UTI	7 (15.9)	2 (33.3)	.29
Skin/soft tissue infection	7 (15.9)	0	.58
Bone/joint infection	1 (2.3)	0	>.99
CNS	1 (2.3)	0	>.99
Bacteremia without focus	2 (4.5)	0	>.99
Antimicrobial therapy			
Hours to appropriate therapy, median (IQR)	97 (76–123)	72 (24–120)	.71
Appropriate therapy options were available	55 (92)	7 (100)	.43

Table 1 continued.

Parameter	ST258 (n = 61), N (%)	Non-ST258 (n = 7), N (%)	*P* Value
Outcomes			
In-hospital mortality	14 (26.4)	3 (50)	.34
90-days mortality	18 (35.3)	4 (66.7)	.19
For Survivors of Index Hospitalization Only			
Functional status deterioration	23 (67.6)	2 (66.7)	>.99
Discharged to a long-term care facility after being admitted from home	27 (77.1)	1 (100)	>.99
Additional hospitalizations in the 3 mo after the isolation	27 (52.9)	4 (66.7)	.68
Invasive procedure or surgery in the 3 mo after the isolation	25 (53.2)	4 (80.0)	.37
Additional isolations of CRKP ("bacteriological failure")	28 (50.9)	4 (57.1)	>.99
Total length of hospital stay (in days), median (IQR)	27 (14–40)	18 (12–23)	.18
Length of stay (days) from culture to discharge, excluding the dead, median (IQR)	9 (4–21)	10 (5–12)	.7

Highlighted in bold are statistically significant ($P < .05$) associations.

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; IQR, interquartile range; PCR, polymerase chain reaction; SD, standard deviation; UTI, urinary tract infection.

Bacteria were identified to the species level, and susceptibilities were determined to predefined antimicrobials, based on an automated system (MicroScan; Siemens AG, Munich, Germany) and in accordance with Clinical and Laboratory Standards Institute criteria [17]. Susceptibilities to colistin and tigecycline were determined by using E-tests (bioMérieux Co., Paris, France). Extended-spectrum β-lactamases, after being identified in the automated system, were confirmed with a disc diffusion test [17]. All *K pneumoniae* that were resistant to 1 or more expanded-spectrum cephalosporin (ie, 3rd or 4th generation) and had a minimum inhibitory concentration ≥2 mg/L to ertapenem were screened for carbapenemase-production with the MHT [17]. Subsequently, CRKPs were tested by PCR specifically for the presence of bla_{KPC}, at the Research Services of the Veterans Affairs Medical Center (Cleveland, OH), with previously characterized *K pneumoniae* carbapenemase (KPC)-producing *K pneumoniae* strains used as controls [22, 23]. Strains that lacked the bla_{KPC} initially were tested for the presence of other carbapenemases (bla_{IMP}, bla_{VIM}, bla_{OXA}, bla_{NDM}) and bla_{KPC} again, with a different PCR-based methodology at DMC microbiology laboratory (Verigene; Nanosphere; Northbrook, Illinois, USA). Multilocus sequence typing was conducted to determine ST according to standard protocols using a simplified protocol and universal sequence primers (http://bigsdb.web.pasteur.fr) (Pasteur Institute, France) [24]. Sequence type with 6 of 7 shared alleles (single locus variants) were considered to be within a single clonal complex. Repetitive extragenic palindromic PCR (rep-PCR) using an automated system (DiversiLab, bioMérieux, Paris, France) was performed as previously detailed [20]. Isolates with band patterns ≥95% similarity were considered genetically related [23]. Institutional review boards of all participating centers approved the study before its initiation [20].

RESULTS

Seventy CRKPs isolated from 69 patients were typed using MLST. One patient had 2 CRKP strains isolated from a wound on the same day: one was ST258 and the other ST248. This patient was excluded from the analysis. As displayed in the Table 1, 61 patients with CRKP ST258 were compared with 7 patients with other STs (3 with ST514, 2 with ST11, 1 with ST13, and 1 with ST248). All but 1 strain had a positive MHT, indicating the presence of a carbapenemase; the strain with a false-negative MHT was ST258 type. In 55 of 56 ST258 strains that were tested (98%) with bla_{KPC} PCR, the presence of bla_{KPC} was confirmed, vs 5 (71%) bla_{KPC} producers among the non-ST258 strains ($P = .03$ by Fisher's exact test). In the additional multiplex PCR test analysis (Verigene), the 2 non-ST258 strains that did not harbor bla_{KPC} had no other carbapenemase (ie, bla_{IMP}, bla_{VIM}, bla_{OXA}, bla_{NDM}). In 1 strain, a $bla_{CTX\text{-}M}$ ESBL was detected.

As displayed in the Table 1, patients with CRKP other than ST258 were younger and had more severe indices of acute illness (eg, necessitated vasopressors and acute tracheal intubation during the acute disease course). In addition, ST258 strains were resistant to more classes of antimicrobials (eg, tobramycin, ciprofloxacin) than other strains.

Outcomes did not differ significantly between patients with carriage of a CRKP ST258 strain versus other CRKP strains in this study. Due to low numbers of carriers of non-ST258 strains, multivariable risk factors and/or outcomes analyses were not performed. All but 2 ST258 isolates clustered into 2 related groups by rep-PCR. One group (Figure 1, Key 6–17) was rep-PCR type A, which has been previously reported [25] and likely corresponds to clade 1 of ST258 [26–28]. The second group (Figure 1, Key 21–62) was rep-PCR type B, which likely corresponds to clade 2.

DISCUSSION

The epidemiology of CRKP is continually evolving, and it now consists of non-ST258 circulating strains that possess distinct epidemiological features. Despite the low number of patients with non-ST258 CRKP that were enrolled, this analysis clearly

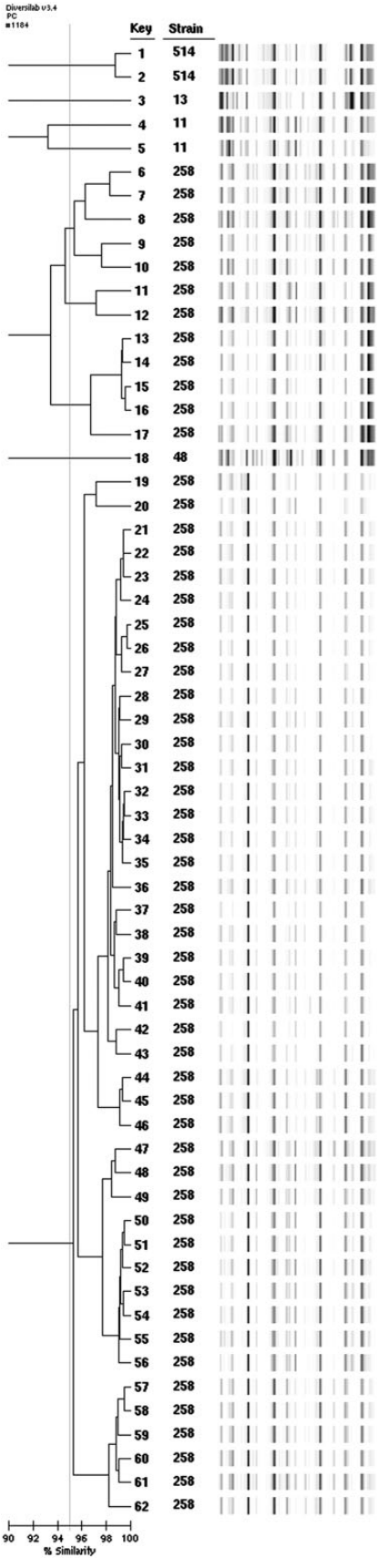

Figure 1. Multilocus sequence typing analysis of carbapenem-resistant *Klebsiella pneumoniae* strains, Detroit Medical Center, Michigan, 2008–2009.

stresses that these strains are associated with non-bla_{KPC} status. It is notable that 2 of the non-ST258 strains were identified as ST11, which are single-locus variants of ST258 (ST11) and are thought to be precursors of bla_{KPC}-producing *K pneumoniae* ST258 (1 isolate produced bla_{KPC} and 1 did not) [27].

We acknowledge that this investigation was conducted on strains isolated in 2008–2009, when CRKP was just emerging and disseminating at DMC and Southeast Michigan [19, 20, 29]. However, in 2013, 125 (15%) of 846 patient-unique *K pneumoniae* clinical isolations at DMC were CRKP; of those, 26 isolates (ie, 21% of all CRKPs) had a negative MHT, which might represent false-negative tests due to the presence of carbapenemases other than KPC. This observation suggests increased diversity in the current molecular epidemiology of CRKP at DMC (and possibly elsewhere [30]). This focused investigation also suggests that one cannot assume that bla_{KPC} is always responsible for a CRKP phenotype. Therefore, attempts to detect CRKP by the phenotypic MHT without using a broad range of PCR probes seeking to detect other carbapenemases, including but not limited to bla_{KPC}, are limited. However, when strains who were non-bla_{KPC} producers were tested for the presence of other known carbapenemases, it proved to be negative as well. Other mechanisms (eg, ESBLs such as bla_{CTX-M} coupled with porin loss mutations [14]) might be involved in current CRKP epidemiology. Nvertheless, the routine practice in many clinical microbiology laboratories [4] might not fit the current epidemiology of CRKP anymore.

In this analysis, patients with ST258 strains, considered to be a high-risk clone because of its recognized negative clinical impact, were frequently found to have poor clinical outcomes and high rates of antimicrobial resistance. However, it is notable that poor outcomes and high prevalence of resistance was identified among the small sample of non-ST258 cases as well, including in-hospital mortality among 3 of 7 non-ST258 cases (Table 1). This underscores the fact that the epidemiological significance and clinical impact of non-ST258 strains cannot be dismissed [31]. Although our high numbers of ST258 strains limits our ability to fully describe the epidemiologic patterns of non-ST258 strains, patients′ background characteristics (ie, demographics, chronic medical conditions, exposures to healthcare settings) appeared to be largely similar between groups, implying that carbapenem-resistant *Enterobacteriaceae* may affect a similar population regardless of the ST, at least in the United States.

We previously described a contemporary outbreak of a colistin-resistant CRKP at this same hospital [29]. Multilocus sequence typing grouped all of these (original) strains to the ST-258 group, whereas both pulsed-field gel electrophoresis and rep-PCR determined that 2 clonally related groups were involved (similar to the 2 rep-PCR groups seen within ST258 strains from this analysis) [29]. Since patient-to-patient transmission was demonstrated in a detailed "transmission

opportunities analysis" [29], reflecting clonal spread, this might imply that in epidemic settings, MLST might be a suitable molecular tool to use in outbreak investigations of CRKP.

CONCLUSIONS

To conclude, our focused analysis did not identify risk factors or outcomes that were unique to the ST258 CRKP. However, the microbiological features of these strains were significantly different, because ST258 strains are associated with bla_{KPC} production and with increased resistances to multiple classes of antimicrobials. Therefore, investigations of CRKP should include the molecular detection of bla_{KPC} and other carbapenemase genes, as well as genetic typing. Moreover, bla_{KPC} detection should not be used as the sole method to diagnose CRKPs carriage.

Acknowledgments

Disclaimer. Funding organizations were not involved in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Veterans Administration.

Financial support. E. T. M. is supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award Number K01AI099006. K. S. K. is supported by the National Institute of Allergy and Infectious Diseases (DMID Protocol Number: 10-0065). F. P. is supported by the VISN 10 Geriatric Research, Education, and Clinical Centers at the Veterans Affairs Medical Center and the Cleveland Translational Science Award (UL1TR000439). R. A. B. acknowledges support from the National Institutes of Health under award numbers R01AI072219, R01AI063517, and R01AI100560 and by funds and/or facilities provided by the Louis Stokes Cleveland Department of Veterans Affairs Medical Center and the VISN 10 Geriatric Research, Education and Clinical Care Center (VISN 10) of the Department of Veterans Affairs.

Potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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