Nested Case-Control Study of the Emergence of Tigecycline Resistance in Multidrug-Resistant *Klebsiella pneumoniae*

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We performed a nested case-control study (ratio of 1:4) on the emergence of tigecycline-resistant multidrug-resistant *Klebsiella pneumoniae* (TR-MDRKP) isolates among patients who initially presented with a tigecycline-susceptible MDRKP isolate. Out of 260 patients, 24 (9%) had a subsequent clinical culture positive for a TR-MDRKP isolate within the 90-day follow-up period. On logistic regression analyses, receipt of tigecycline (adjusted odds ratio [OR], 5.06; 95% confidence interval [CI], 1.80 to 14.23; $P = 0.002$) was the only independent predictor of subsequent isolation of a TR strain.

Tigecycline, a minocycline derivative, remains one of the few therapeutic options for the treatment of infections caused by multidrug-resistant *Klebsiella pneumoniae* (MDRKP) isolates—including *Klebsiella pneumoniae* carbapenemase (KPC) producers—and other Gram-negative organisms (1, 2). Of concern, tigecycline nonsusceptibility (i.e., tigecycline resistance [TR]) among *Klebsiella pneumoniae* isolates has been reported from different continents and ranged between 0% and 11.5% in a recent large study (3–6). Furthermore, several recent case studies identified patients who were treated with tigecycline for an initially tigecycline-susceptible MDRKP (TS-MDRKP) infection but from whom a TR-MDRKP strain was subsequently isolated (7–10). However, the potential of *in vivo* emergence of TR among MDRKP isolates has so far not been systematically investigated to our knowledge. We intended to study the rates of and risk factors for the *in vivo* emergence of TR among *Klebsiella pneumoniae* isolates in a large population of patients who initially presented with a TS-MDRKP.

We performed a nested case-control study on the subsequent emergence of TR among all patients from whom a TS-MDRKP strain was previously isolated at a tertiary care center between January 2008 and July 2011. MDRKP was defined as the presence of either an extended-spectrum β -lactamase (ESBL)- or KPC-producing *Klebsiella pneumoniae* isolate, determined as per Clinical and Laboratory Standards Institute (CLSI) guidelines on ESBL testing, carbapenem resistance patterns, and Hodge test results, respectively (11, 12). Food and Drug Administration (FDA) breakpoints were applied to interpret the results of tigecycline susceptibility testing using the disc diffusion method and Etest (susceptible if the zone diameter is ≥ 19 mm and the isolate MIC is ≤ 2 μ g/ml, respectively).

A case was defined as a patient from whom a TR-MDRKP was isolated between >48 h and <90 days after the day a TS-MDRKP strain was isolated (zero time). A control was defined as a patient from whom a TS-MDRKP strain was isolated during the study period but with no subsequent TR-MDRKP strains. Controls were matched to cases based on the isolate's resistance mechanism (ESBL versus KPC) but otherwise randomly selected in a 1:4 ratio. Cases were compared to controls with regard to a variety of demographic and clinical characteristics. We also recorded (i) all antibiotic exposures within 90 days before zero time and (ii) all antibiotic exposures between zero time and the day on which a

culture positive for a TR-MDRKP isolate was obtained (cases) or all antibiotic exposures within 90 days after zero time (controls). Patients with cultures positive for a TR-MDRKP organism any time before or on the day of isolation of a TS-MDRKP strain and patients with incomplete medical records or inadequate follow-up were excluded.

Bivariate analyses using the Pearson's chi-square test, Fisher's exact test, or the Mann-Whitney U test were performed to compare categorical and continuous variables as indicated. Logistic regression models were used to determine independent predictors of subsequent isolation of a TR-MDRKP isolate ($P < 0.05$); variables with a P value of <0.2 on bivariate analyses and plausible interaction terms were entered into the model.

TS-MDRKP strains were isolated from 276 patients during the observation period; 16 patients were excluded according to the criteria listed above. Among a total of 260 patients who were included into our cohort, 155 (60%) had a KPC isolate, and 105 (40%) had an ESBL isolate. Among these 260 patients, 24 (9%; 95% confidence interval [CI], 6.3 to 13.4%) had a subsequent clinical specimen culture positive for a TR-MDRKP isolate within our defined follow-up period; 18 (75%) of the 24 isolates were KPCs. These 24 case patients were compared to 96 matched individuals without subsequent TR-MDRKP ($n = 120$) in a nested case-control study. The median age of these patients was 73 years (range, 23 to 97 years), 59 (49%) were male, and the median Charlson comorbidity score was 3 (13). MDRKP was most commonly isolated from urine specimens at zero time (61%). Among the 96 control patients, 39 (41%) had at least one additional culture positive for TS-MDRKP within the follow-up period, whereas 57 patients had no subsequent MDRKP strain isolated.

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TABLE 1 Comparison of demographic and clinical characteristics between patients with and patients without subsequent isolation of MDRKP (*n* = 120)

Characteristic ^a	Result for:		OR (95% CI)	<i>P</i> value
	TR-MDRKP (<i>n</i> = 24)	No TR-MDRKP (<i>n</i> = 96)		
Age, median yr (range)	73 (54–92)	73 (23–97)		>0.2
Male, no. (%)	9 (38)	50 (52)	0.55 (0.22–1.38)	0.20
In ICU at ZT, no. (%)	6 (25)	24 (25)	1.00 (0.36–2.81)	>0.2
β-Lactam allergy, no. (%)	5 (21)	15 (16)	1.42 (0.46–4.39)	>0.2
Days of hospitalization, median no. (range)	28 (3–157)	23 (3–112)		>0.2
Days of hospitalization between ZT and end of observation period, no. (range)	12 (5–65)	14 (2–78)		>0.2
Charlson score, median (range)	3 (0–6)	3 (0–11)		>0.2
No. (%) of patients with:				
HIV/AIDS	1 (4)	7 (7)	0.55 (0.07–4.72)	>0.2
Diabetes mellitus	11 (46)	32 (33)	1.69 (0.68–4.20)	>0.2
COPD/asthma	5 (21)	25 (26)	0.75 (0.25–2.21)	>0.2
Congestive heart failure	6 (25)	16 (17)	1.67 (0.57–4.85)	>0.2
ESLD	0	6 (6)		>0.2
ESRD	1 (4)	14 (15)	0.26 (0.03–2.04)	>0.2
Current malignancy	6 (25)	22 (23)	1.12 (0.40–3.17)	>0.2
Connective tissue disease	1 (4)	2 (2)	2.04 (0.18–23.52)	>0.2
Peripheral vascular disease	3 (13)	9 (9)	1.38 (0.34–5.55)	>0.2
Dementia	2 (8)	10 (10)	0.78 (0.16–3.83)	>0.2
Surgery <12 mo	7 (29)	24 (25)	1.24 (0.46–3.34)	>0.2
Hospitalization <12 mo	15 (63)	70 (73)	0.62 (0.24–1.59)	>0.2
Stay in LTCF <12 mo	9 (38)	29 (30)	1.39 (0.55–3.53)	>0.2
No. (%) of patients with MDRKP isolated at ZT from:				
Blood ^b	3 (13)	7 (7)	1.82 (0.43–7.62)	>0.2
Urine ^b	14 (58)	59 (62)	0.88 (0.35–2.18)	>0.2
Respiratory tract ^b	4 (17)	13 (14)	1.28 (0.38–4.34)	>0.2
No. (%) of patients after ZT with:				
Foley catheter in place	16 (67)	55 (57)	1.49 (0.58–3.82)	>0.2
Temporary central venous or arterial device in place	9 (38)	51 (53)	0.53 (0.21–1.33)	0.17
No. (%) of patients with MDRKP isolate from ZT considered true pathogen	18 (75)	63 (66)	1.57 (0.57–4.34)	>0.2

^a Abbreviations: ICU, intensive care unit; ZT, zero time; COPD, chronic obstructive pulmonary disease; ESLD, end-stage liver disease; ESRD, end-stage renal disease; LTCF, long-term-care facility.

^b MDRKP may have been isolated from more than one site

Cases and controls were similar with regard to a variety of demographic and clinical characteristics, days of hospitalization, isolate source, clinical infection versus colonization with a TS-MDRKP isolate at zero time, presence of a medical device, and exposure to multiple antibiotic classes before and after zero time (Tables 1 and 2). In contrast, 18 (75%) of 24 cases versus only 32 (33%) of 96 controls were treated with tigecycline for a median duration of 9 days (range, 2 to 33 days) after zero time ($P < 0.001$ on bivariate analysis) (Table 2). On backward logistic regression analyses, receipt of tigecycline after zero time (adjusted odds ratio [OR], 5.06; 95% CI, 1.80 to 14.23; $P = 0.002$) was the only independent predictor of subsequent isolation of a TR strain.

MDRKP infections are associated with high attributable mortality rates of up to 50% (14); for patients with infections caused by KPC isolates, tigecycline remains one of the few therapeutic options besides aminoglycosides and colistin. Our study found a high rate (9%) of *in vivo* TR emergence among patients who initially presented with a TS-MDRKP strain in the previous 90 days; exposure to tigecycline was the only independent predictor of subsequent resistance. These results build on and expand our knowl-

edge from several recent case reports that describe the occurrence of TR-MDRKP among patients recently treated with tigecycline monotherapy or combination therapy (7, 8, 9, 10, 15). In our study, MDRKP isolation from the urinary tract or other body sites where low tigecycline concentrations are achieved was not associated with emergence of TR. This finding is interesting as achievable tigecycline concentrations in the urine are low (only 10% to 22% is excreted unaltered), and tigecycline treatment failure rates of up to 57% were observed in patients with urinary tract infections caused by KPC producers (16).

Molecular typing of initial and subsequent isolates was not performed in our study. Thus, the selection of a genetically distinct TR strain that was previously present below detection levels by tigecycline exposure cannot be excluded in our study; however, prior studies using molecular typing methodologies suggest the *in vivo* emergence of TR in previously drug-susceptible strains upon drug exposure (7). TR is mediated by the overexpression of the efflux pump AcrAB-TolC (17). Our retrospective study design did not include an algorithm that required standardized follow-up culturing of patients from whom TS-MDRKP was previously iso-

TABLE 2 Exposure to different antibiotic classes among patients with and among patients without subsequent isolation of TR-MDRKP ($n = 120$)

Characteristic ^a	Result for:		OR (95% CI)	P value
	TR-MDRKP (n = 24)	No TR-MDRKP (n = 96)		
Antibiotic exposure ≤90 days before ZT				
T/C or P/T	10 (42)	35 (37)	1.25 (0.50–3.10)	>0.2
3rd or “4th” generation cephalosporin	6 (25)	41 (43)	0.45 (0.16–1.23)	0.11
Carbapenem	7 (29)	16 (17)	2.06 (0.73–5.77)	>0.2
Aztreonam	3 (13)	6 (3)	2.14 (0.50–9.27)	>0.2
Fluoroquinolone	10 (42)	40 (42)	1.00 (0.40–2.48)	>0.2
Aminoglycoside	2 (8)	16 (17)	0.46 (0.10–2.13)	>0.2
Clindamycin	1 (4)	6 (6)	0.65 (0.08–5.69)	>0.2
Tigecycline	2 (8)	2 (2)	4.27 (0.57–32.02)	0.18
Polymyxin	0	2 (2)	–	>0.2
Vancomycin	12 (50)	55 (57)	0.75 (0.30–1.83)	>0.2
Linezolid	2 (8)	8 (8)	1.00 (0.20–5.05)	>0.2
Metronidazole	7 (29)	29 (30)	0.95 (0.36–2.54)	>0.2
Antibiotic exposure after ZT				
T/C or P/T	4 (17)	22 (23)	0.67 (0.21–2.18)	>0.2
3rd or “4th” generation cephalosporin	2 (8)	30 (31)	0.20 (0.04–0.91)	0.02
Carbapenem	7 (29)	33 (34)	0.79 (0.30–2.09)	>0.2
Aztreonam	2 (8)	6 (6)	1.36 (0.26–7.22)	>0.2
Fluoroquinolone	10 (42)	29 (30)	1.65 (0.66–4.15)	>0.2
Aminoglycoside	6 (25)	31 (32)	0.70 (0.25–1.94)	>0.2
Tigecycline	18 (75)	32 (33)	6.00 (2.17–16.59)	<0.001
Polymyxin	3 (13)	16 (17)	0.71 (0.19–2.68)	>0.2
Vancomycin	9 (38)	54 (56)	0.47 (0.19–1.17)	0.10
Linezolid	5 (21)	6 (6)	3.95 (1.09–14.28)	0.04
Metronidazole	7 (29)	33 (34)	0.79 (0.30–2.09)	>0.2

^a ZT, zero time; T/C, ticarcillin-clavulanate; P/T, piperacillin-tazobactam; “4th generation,” extremely broad spectrum of activity.

lated; despite the presence of these potential confounders, we believe our results to be of clinical relevance. Cautious tigecycline usage and close monitoring of tigecycline resistance patterns worldwide are warranted to preserve this agent as a potential future treatment alternative for MDRKP infections.

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