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ORIGINAL ARTICLE

Emergence of tigecycline- and carbapenem-nonsusceptible *Klebsiella pneumoniae* ST11 clone in patients without exposure to tigecycline

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Abstract *Background/purpose:* Currently, tigecycline-nonsusceptible *Klebsiella pneumoniae* (TNSKP) is mainly reported to emerge following clinical use of tigecycline and is usually polyclonal. This study aimed to characterize TNSKP isolated from patients without prior tigecycline use.
Methods: Twenty-six TNSKP clinical isolates were collected, and carbapenemase and 16S rRNA methylase genes were identified by polymerase chain reaction and sequencing. Molecular typing was conducted by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Clinical data of patients in the carbapenem-susceptible TNSKP group and the tigecycline- and carbapenem-nonsusceptible *K. pneumoniae* (TCNSKP) group were compared.
Results: Of the 26 TNSKP isolates, eight contained both bla_{KPC-2} and 16S rRNA methylase genes. In the remaining 18 TNSKP isolates, no carbapenemase gene was detected, and only three had the 16S rRNA methylase gene. Among the 26 isolates, 24 distinct pulsotypes and 19 sequence types (STs) were identified by PFGE and MLST, respectively. Six of the eight TCNSKP were ST11, whereas the remaining 18 TNSKP isolates were assigned to 17 different STs. No patient received tigecycline prior to the isolation of TNSKP. By comparison, intensive care unit

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exposure, mechanical ventilation, prior β-lactam/β-lactamase use, and longer hospitalization were more common for the TCNSKP group than for the carbapenem-susceptible TNSKP group. *Conclusion:* TNSKP can occur without tigecycline use, and TCNSKP ST11 is predominant among them. Further, this report proposes potential risk factors for the occurrence of carbapenem-nonsusceptibility in TNSKP.

Introduction

Tigecycline, the first member of the glycylcyclines group of antibiotics with good *in vitro* activity against carbapenem-resistant *Klebsiella pneumoniae*,[1,2] may provide a potential treatment option as a last resort defense against these "superbugs". While tigecycline-nonsusceptible rates among *K. pneumoniae* isolates are generally low,[3] tigecycline-nonsusceptible *K. pneumoniae* (TNSKP) has been reported to occur following clinical use of the antibiotic.[4–7] Data regarding the clinical features of TNSKP, as well as the microbiological and the molecular characteristics of TNSKP, remain scarce. Previously, we reported tigecycline resistance mechanisms for 26 TNSKP isolates, and that efflux pumps AcrAB and OqxAB played an essential role in tigecycline resistance in *K. pneumoniae*.[8]

Although tigecycline has been available in China since the end of 2011, it is not commonly used in clinical practice because of its high price and limited indications for hospital-acquired infections. Herein, we report TNSKP infection or colonization in 26 patients who had not received tigecycline treatment previously and provide information concerning the predominance of ST11 among the tigecycline- and carbapenem-nonsusceptible *K. pneumoniae* (TCNSKP) isolates at Huashan Hospital in Shanghai, China. We also established the clinical and microbiological characteristics of the 26 TNSKP isolates, and investigated the factors that increase the risk of infection or colonization with TCNSKP.

Methods

Patients and isolates

Patients with TNSKP infection or colonization were identified among those admitted at Huashan Hospital, Fudan University, China. Only the first TNSKP was included in this study when more than one TNSKP isolate was isolated from the same patient. Twenty-six nonduplicated TNSKP isolates were identified during a study investigating tigecycline resistance mechanisms in *Enterobacteriaceae*.[8] Minimum inhibitory concentrations (MICs) of other antimicrobial agents were determined by the agar dilution methodology and interpreted following the recommendations of the Clinical and Laboratory Standards Institute (CLSI, Wayne, PA, USA).[9] *Escherichia coli* ATCC 25922 was used as the quality control strain.

Detection of β-lactamase and 16S rRNA methylase genes

All TNSKP were tested for the presence of various carbapenemase genes (bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM}, bla_{SPM}, and bla_{SIM}) and 16S rRNA methylase genes (*armA* and *rmtB*) by polymerase chain reaction and sequencing as described previously.[10,11]

Molecular typing

Pulsed-field gel electrophoresis (PFGE) was performed to determine genetic relatedness of the isolates using the restriction enzyme *Xba*I (TaKaRa Biotechnology Co. Ltd., Dalian, China). Multilocus sequence typing (MLST) was carried out as described earlier.[12] The PFGE banding patterns were compared via the BioNumerics version 6.6 software (Applied Maths, Sint-Martens-Latem, Belgium), and a similarity cutoff of 90% was used.[13]

Clinical data collection

Clinical information, including demographic data, duration of hospital stay, day of specimen collection, location of the patient, healthcare exposure within the prior 6 months, device use at the time of TNSKP isolation, utilization of antimicrobial agents prior to TNSKP isolation after the admission, underlying diseases, and outcomes (died or alive during hospital stay), were collected. The time of the TNSKP isolation was defined as the day of collection of the specimen in which TNSKP bacteria were detected.

Statistical analysis

Contingency data were analyzed by the Fisher's exact test, and continuous data were assessed by the Student *t* test. A p value < 0.05 was considered as statistically significant, and the odds ratio was calculated with confidence intervals of 95% (95% CI). Statistical analyses were carried out using SPSS 19.0 (SSPS Inc., Chicago, IL, USA).

Results

Of the 26 TNSKP isolates, eight (30.8%) were TCNSKP (meropenem MICs, 16—128 μg/mL; imipenem MICs, 4—128 μg/mL). These eight TCNSKP isolates were resistant to all antimicrobial agents tested except to colistin (MICs, 0.25—1 μg/

mL), and four were susceptible to fosfomycin. The remaining 18 TNSKP isolates were susceptible to meropenem, imipenem, fosfomycin, and colistin, and had a high rate of susceptibility to piperacillin/tazobactam (83.3%), aztreonam (72.2%), and cefepime (88.9%; Table 1). The most common sources of isolation were urine ($n = 13$), followed by sputum ($n = 8$), bile ($n = 4$), and blood ($n = 1$; Figure 1).

Of the 26 TNSKP isolates, the eight TCNSKP isolates carried bla_{KPC-2} and also harbored a 16S rRNA methylase gene (*armA*, $n = 2$ and *rmtB*, $n = 6$) in accordance with their aminoglycoside resistance phenotype. In the remaining 18 carbapenem-susceptible TNSKP isolates, polymerase chain reaction was negative for the carbapenemase genes tested, and only three possessed a 16S rRNA methylase gene (*rmtB*, $n = 2$ and *armA*, $n = 1$).

The 26 TNSKP isolates were assigned to 24 distinct pulsotypes and 19 distinct sequence types (STs; Figure 1), among which ST11 was the predominant ST type ($n = 7$, 26.9%), followed by ST37 ($n = 2$). Of the eight TCNSKP isolates, six were ST11, while the other two were ST690 (a single-locus variant of ST11) and ST39. Among the remaining 18 carbapenem-susceptible TNSKP isolates, 17 STs were identified, including three new STs designated in this study as STn1, STn2, and STn3, respectively (Figure 1).

Of the 26 patients with TNSKP, two outpatients were excluded since their clinical records were not available. Thus, the clinical data of the 24 patients were collected and grouped based on the TNSKP with carbapenem-resistance (TCNSKP group) or not (carbapenem-susceptible TNSKP group; Table 2).

Overall, 31 different antibiotics were administered after admission, prior to the isolation of TNSKP from the 26 patients, including β-lactams/β-lactamase inhibitors, fluoroquinolones, carbapenems, and cephalosporins. However, tigecycline was not given to any patient before TNSKP isolation.

Seven (87.5%) of the eight patients with TCNSKP had a history of intensive care unit (ICU) stay, whereas three (18.8%) patients in the carbapenem-susceptible TNSKP group had a history of ICU stay ($p < 0.01$; odds ratio, 30.33; 95% CI, 2.64–348.96; Table 3). Mechanical ventilation and β-lactam/β-lactamase inhibitors use were also significantly more common in the TCNSKP group (both $p = 0.02$). Moreover, the mean interval between patient admission to this hospital and the isolation of TNSKP was 36 days (range, 7–72 d) in the TCNSKP group and 16 days (range, 0–70 d) in the TNSKP group ($p = 0.01$), suggesting that longer hospitalization likely contributes to the isolation of TCNSKP.

Table 1 Antimicrobial susceptibility of tigecycline-nonsusceptible *Klebsiella pneumoniae* (TNSKP) isolates.

Patient	Isolate	MIC (μg/mL)										
		TGC	IPM	MEM	TZP	CTX	FEP	AMK	FOS	LEV	MIN	COL
1	TNSKP8	4	8	32	128/4	32	8	>128	4	4	>128	0.5
2	TNSKP25	4	4	32	128/4	128	>128	>128	8	32	4	1
3	TNSKP15	4	8	16	128/4	16	16	>128	>256	128	64	0.25
4	TNSKP7	4	8	32	128/4	128	64	>128	>256	128	32	1
5	TNSKP12	8	128	128	>128/4	128	64	>128	>256	>128	64	0.25
6	TNSKP17	8	8	64	>128/4	128	64	>128	8	128	>128	0.25
7	TNSKP16	8	8	64	>128/4	128	64	>128	>256	>128	64	0.25
8	TNSKP11	16	8	64	>128/4	128	128	>128	8	>128	>128	0.25
9	TNSKP2	16	≤0.06	≤0.06	16/4	16	8	0.5	8	16	>128	0.5
10	TNSKP14	4	≤0.06	≤0.06	16/4	0.5	0.25	0.5	4	0.5	32	0.25
11	TNSKP3	8	≤0.06	≤0.06	16/4	8	2	0.5	4	4	>128	0.5
12	TNSKP5	4	≤0.06	≤0.06	128/4	128	32	128	8	16	32	0.25
13	TNSKP20	16	≤0.06	≤0.06	>128/4	128	32	>128	>256	8	>128	0.5
14	TNSKP23	4	≤0.06	≤0.06	8/4	0.25	0.125	1	4	0.25	16	0.25
15	TNSKP1	4	≤0.06	≤0.06	16/4	0.5	0.25	0.5	8	2	32	0.25
16	TNSKP22	4	≤0.06	≤0.06	8/4	32	8	0.5	4	4	32	0.5
17	TNSKP24	4	≤0.06	≤0.06	2/4	8	8	1	4	1	8	0.5
18	TNSKP21	8	≤0.06	≤0.06	8/4	0.25	0.25	>128	4	32	>128	0.25
19	TNSKP9	8	≤0.06	≤0.06	8/4	0.125	0.125	1	4	>128	64	0.5
20	TNSKP13	4	≤0.06	≤0.06	16/4	8	2	>128	8	>128	>128	0.25
21	TNSKP26	4	≤0.06	≤0.06	2/4	≤0.06	≤0.06	1	2	≤0.06	4	0.5
22	TNSKP6	4	≤0.06	≤0.06	16/4	0.25	0.125	0.5	2	0.25	16	0.25
23	TNSKP18	8	≤0.06	≤0.06	8/4	0.25	0.25	1	8	0.5	64	1
24	TNSKP4	8	≤0.06	≤0.06	128/4	128	0.5	1	4	128	>128	0.25
25	TNSKP19	4	≤0.06	≤0.06	16/4	8	4	1	8	4	32	0.25
26	TNSKP10	4	≤0.06	≤0.06	16/4	1	0.5	1	8	128	32	0.5

Tigecycline minimum inhibitory concentrations (MICs) were determined by the broth microdilution method, and others were determined by the agar dilution method.

AMK = amikacin; COL = colistin; CTX = cefotaxime; FEP = cefepine; FOS = fosfomycin; IPM = imipenem; LEV = levofloxacin; MEM = meropenem; MIC = minimum inhibitory concentration; MIN = minocycline; TGC = tigecycline; TNSKP = tigecycline-nonsusceptible *Klebsiella pneumoniae*; TZP = piperacillin-tazobactam.

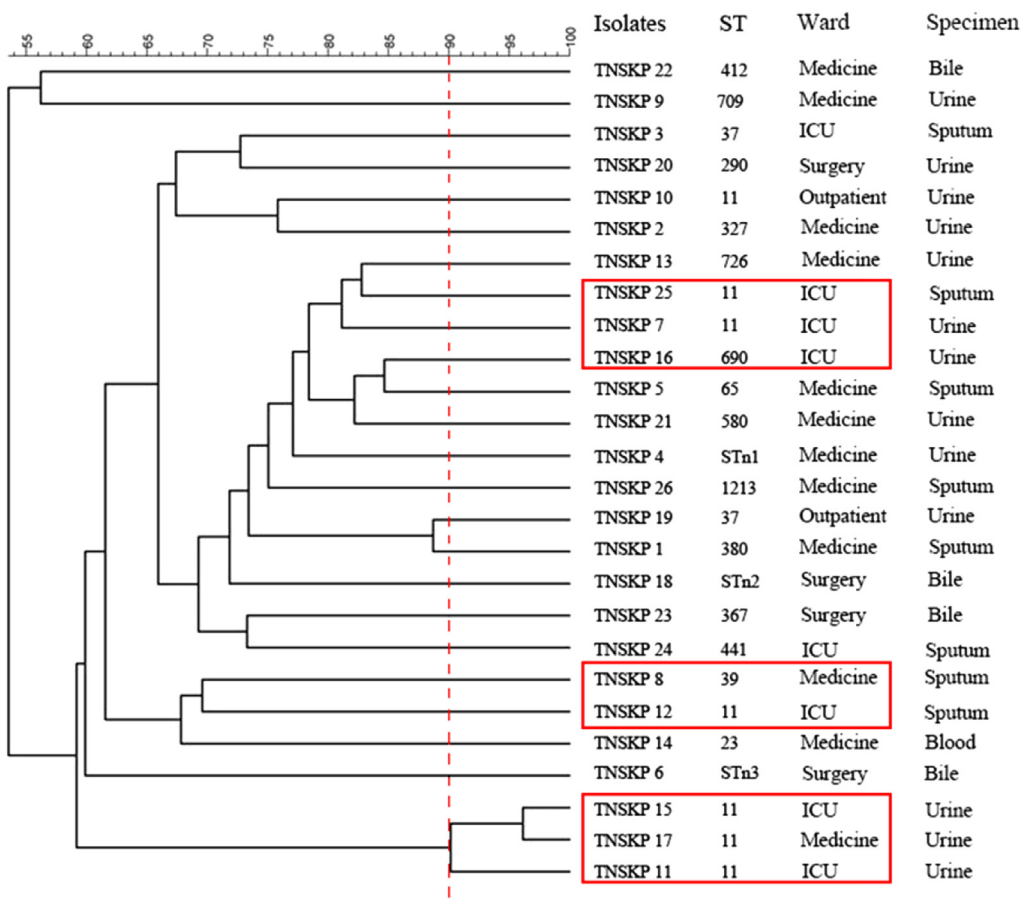

Figure 1. Pulsed-field gel electrophoresis (PFGE) dendrogram of 26 tigecycline-nonsusceptible *Klebsiella pneumoniae* (TNSKP) analyzed by BioNumerics, and the corresponding sequence types (STs) of the isolates. A similarity cutoff of 90% was used to group TNSKP (red dotted line). TNSKP with carbapenem resistance was indicated (red rectangle). ICU = intensive care unit; ST = sequence type.

Discussion

In the present study, tigecycline was not prescribed to any patient prior to TNSKP isolation, which was contrary to findings of a nested case-control study which revealed the receipt of tigecycline as the only independent predictor of subsequent isolation of a tigecycline-resistant *K. pneumoniae* isolate.[14] We hypothesized that spontaneous mutations or the use of other types of antibiotics might have contributed to the emergence of TNSKP in this examination. It is also possible that TNSKP existed and was disseminated by other patients who had been treated with tigecycline, although it is not commonly used in China.

Lin et al[15] reported that no major cluster of 36 TNSKP isolates was identified by PFGE, which was consistent with the results of our study. However, in the present investigation, MLST indicated predominance of ST11 among the TNSKP isolates, in particular TCNSKP isolates (6/8, 75.0%). Zhong et al[16] also established that *K. pneumoniae* ST11 was the predominant lineage among tigecycline- and carbapenem-nonsusceptible isolates. These findings indicated that ST11 exerts an important role in TNSKP, especially TCNSKP.

ST11 is the most common ST among *K. pneumoniae* carbapenemase (KPC)-2-producing *K. pneumoniae* in China[17] and some other regions.[18–21] This international epidemic of the *K. pneumoniae* ST11 clone may suggest the presence of selective advantages that facilitate the spread of carbapenem-resistance among TNSKP isolates. Therefore, TCNSKP ST11 may have the potential to be extensively disseminated. Tigecycline and polymyxin are usually recommended as last resort agents for the treatment of multidrug-resistant and/or extensively drug-resistant Gram-negative bacilli such as KPC-producing *K. pneumoniae*. Therefore, the emergence of TCNSKP is a real threat, especially for those regions and countries (such as China) where polymyxins are not available for clinical use.

Approaches to control the dissemination of TCNSKP are urgent. Based on the guidelines for multidrug-resistant Gram-negative bacteria,[22] we suggest: (1) measures such as enhanced antimicrobial stewardship and infection control compliance are needed to avoid the emergence and even outbreak of TCNSKP; (2) efforts such as reinforcement of hand hygiene and decolonization are important to reduce the spread of TCNSKP; and (3) active surveillance cultures should be performed in patients predisposed by the conditions mentioned above. In addition, routine microbiologic surveillance of newly admitted patients and outpatients may be not recommended,

Table 2 Clinical features of eight patients with tigecycline- and carbapenem-non-susceptible *Klebsiella pneumoniae* (TCNSKP) and 16 patients with carbapenem-susceptible tigecycline-nonsusceptible *K. pneumoniae* (TNSKP).

Isolates	Age (y)/sex	Underlying diseases	ICU stay	Device use	Duration of hospital stay (d)	Prior healthcare stay	Outcomes
TCNSKP							
TNSKP11	58/F	Craniotomy	YES	Ommaya reservoir	54	NO	Alive
TNSKP16	79/F	Guillain-Barre syndrome	YES	Mechanical ventilation, urinary catheter	77	NO	Alive
TNSKP15	53/M	Craniotomy	YES	Mechanical ventilation	48	YES	Alive
TNSKP7	40/F	Multiple organ dysfunction syndrome by drug poisoning	YES	Mechanical ventilation, urinary catheter, PICC	56	NO	Died
TNSKP12	57/M	Craniotomy	YES	Mechanical ventilation, urinary catheter, nasogastric tube, PICC	49	YES	Alive
TNSKP25	65/M	Craniotomy	YES	Mechanical ventilation, Ommaya reservoir	13	YES	Alive
TNSKP17	30/F	SLE nephritis	NO	—	21	YES	Alive
TNSKP8	88/M	Severe pneumonia, respiratory failure	YES	Mechanical ventilation	45	NO	Alive
Carbapenem-susceptible TNSKP							
TNSKP21	65/M	Complicated urinary tract infection	NO	—	22	YES	Alive
TNSKP9	51/M	Meningitis, severe pneumonia	YES	Urinary catheter	29	YES	Alive
TNSKP13	23/M	Tuberculous meningitis	NO	Urinary catheter	7	YES	Alive
TNSKP2	54/F	Complicated urinary tract infection	NO	PICC	31	YES	Alive
TNSKP4	79/F	Severe pneumonia	NO	—	19	YES	Alive
TNSKP5	49/M	Severe pneumonia	NO	—	17	YES	Alive
TNSKP1	56/M	Severe pneumonia	NO	—	22	YES	Alive
TNSKP26	95/M	Complicated urinary tract infection	NO	Urinary catheter	21	YES	Alive
TNSKP22	75/F	Pancreatic carcinoma	NO	PICC, T-tube drainage	120	NO	Died
TNSKP14	76/M	Complicated urinary tract infection	NO	Urinary catheter	72	NO	Alive
TNSKP6	65/M	Cholecystolithiasis	NO	T-tube drainage	10	NO	Alive
TNSKP18	71/F	Pancreatic carcinoma	NO	T-tube drainage	113	NO	Alive
TNSKP23	50/M	Cholangiocarcinoma	NO	T-tube drainage	27	NO	Alive
TNSKP20	71/M	Craniotomy	NO	Mechanical ventilation, urinary catheter, Ommaya reservoir	119	YES	Alive
TNSKP3	62/M	Craniotomy	YES	Mechanical ventilation, nasogastric tube	13	YES	Alive
TNSKP24	61/F	Craniotomy	YES	Mechanical ventilation	208	NO	Alive

ICU = intensive care unit; PICC = peripherally inserted central catheter; SLE = systemic lupus erythematosus; TCNSKP = tigecycline- and carbapenem-nonsusceptible *Klebsiella pneumoniae*; TNSKP = tigecycline-nonsusceptible *K. pneumoniae*.

because the rate of TNSKP and TCNSKP isolation is very low in *K. pneumoniae*.[8]

Of note, TNSKP10 (ST11) and TNSKP19 (ST37) isolates were from outpatients. Although TNSKP isolates may naturally exist in the community, we cannot rule out the possibility that these two are hospital-associated TNSKP, as the outpatients may have a history of hospitalization and then colonization by TNSKP. Actually, *K. pneumoniae* ST11 and ST37 have played an essential role in transfer and even nosocomial outbreak of carbapenem resistance (conferred by bla_{KPC} and bla_{NDM} respectively), which leads us to suspect that TNSKP with these two STs may disseminate in the community.

In conclusion, here we report the identification of TNSKP in 26 specimens isolated from patients without prior exposure to tigecycline, and the emergence of TCNSKP ST11 as the predominant clone in TNSKP. In addition, ICU exposure, mechanical ventilation, use of β-lactam/β-lactamase inhibitors, and longer hospitalization were established as possibly associated with the occurrence of carbapenem-nonsusceptibility in clinical TNSKP.

Table 3 Comparison of clinical features between patients with tigecycline- and carbapenem-nonsusceptible *K. pneumoniae* (TCNSKP) and those with carbapenem-susceptible tigecycline-nonsusceptible *K. pneumoniae* (TNSKP).

Risk factor	TCNSKP (n = 8)	Carbapenem-susceptible TNSKP (n = 16)	p	Odds ratio (95% CI)
Male sex (%)	4 (50)	12 (75)	0.36	0.33 (0.56—2.00)
Mean age (y)	58.8	62.7	0.60	
ICU exposure (%)	7 (87.5)	3 (18.8)	0.00	30.33 (2.64—348.92)
Interval of patient admission and TNSKP isolation, mean d (range)	36 (7—72)	16 (0—70)	0.01	
Previous hospitalization within 6 mo (%)	4 (50)	10 (62.5)	0.67	1.67 (0.30—9.27)
Recent surgery (%)	4 (50)	7 (43.8)	1.00	1.29 (0.23—7.05)
Device use (%)				
Central venous catheter	2 (25)	2 (12.5)	0.58	2.33 (0.26—20.66)
Mechanical ventilation	6 (75)	3 (18.8)	0.02	13.00 (1.70—99.38)
Urinary catheter	3 (37.5)	5 (31.3)	1.00	1.32 (0.22—7.82)
Prior corticosteroid use (%)	1 (12.5)	1 (6.3)	1.00	2.14 (0.12—39.47)
Antibiotic use (%)				
β-lactam/β-lactamase inhibitors	5 (62.5)	2 (12.5)	0.02	11.67 (1.49—91.54)
Carbapenems	4 (50)	5 (31.3)	0.41	2.20 (0.39—12.57)
Aztreonam	0	1 (6.3)	1.00	
1^{st}-/2^{nd}-generation cephalosporins	1 (12.5)	6 (37.5)	0.35	0.24 (0.02—2.44)
3^{rd}-/4^{th}-generation cephalosporins	4 (50)	4 (25)	0.36	3.00 (0.50—17.95)
Aminoglycosides	1 (12.5)	5 (31.3)	0.62	0.31 (0.03—3.29)
Fluoroquinolones	4 (50)	8 (50)	1.00	1.00 (0.18—5.46)
Fosfomycin	3 (37.5)	0	0.03	
Tetracyclines	2 (25)	1 (6.3)	0.25	5.00 (0.38—66.01)

ICU = intensive care unit; CI = confidence intervals; TCNSKP = tigecycline- and carbapenem-nonsusceptible *K. pneumoniae*; TNSKP = tigecycline-nonsusceptible *K. pneumoniae*.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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