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RESEARCH ARTICLE

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Prevalence of *pks* gene cluster and characteristics of *Klebsiella pneumoniae*-induced bloodstream infections

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Background: The emerging *pks*-positive (*pks*$^+$) strains have aroused great public concern recently. Colibactin, encoded by *pks* gene cluster, has been reported to be involved in DNA damage and increased virulence. Little is known about its prevalence among *Klebsiella pneumoniae*-induced bloodstream infections (BSIs). Therefore, the aim of this study was to investigate the prevalence of *pks* gene cluster, and molecular and clinical characteristics of *K pneumoniae*-induced BSIs.

Methods: A total of 190 non-duplicate *K pneumoniae* bloodstream isolates were collected at a university hospital in China from March 2016 to March 2018. Molecular characteristics including capsular types, virulence, and *pks* genes were detected by polymerase chain reaction (PCR). Clinical characteristics and antimicrobial susceptibility were also investigated.

Results: Overall, 21.6% (41/190) of *K pneumoniae* bloodstream isolates were hypervirulent *K pneumoniae* (hvKP). The prevalence of *pks* gene cluster was 26.8% (51/190). The positive rates of K1, K57, and genes associated with hypervirulence, that is, *rmpA*, *wcaG*, *mrkD*, *allS*, *ybtS*, *kfu*, and *iucA*, were significantly higher in the *pks*$^+$ isolates than the *pks*-negative (*pks*$^-$) isolates ($P < 0.05$), while the *pks*$^+$ isolates were significantly less resistant to 11 antimicrobial agents than the *pks*$^-$ isolates. Multivariate analysis showed diabetes mellitus, and K1 and K20 capsular types as independent risk factors for *pks*$^+$ *K pneumoniae* bloodstream infections.

Conclusions: The *pks*$^+$ *K pneumoniae* was prevalent in individuals with bloodstream infections in mainland China. The high rates of hypervirulent determinants among *pks*$^+$ *K pneumoniae* revealed the potential pathogenicity of this emerging gene cluster. Diabetes mellitus, and K1 and K20 capsular types were identified as independent risk factors associated with *pks*$^+$ *K pneumoniae* bloodstream infections. This study highlights the significance of clinical awareness and epidemic surveillance of *pks*$^+$ strains.

KEYWORDS
bloodstream infections, hypervirulent, *Klebsiella pneumoniae*, molecular characteristic, *pks* gene cluster

1 | INTRODUCTION

Klebsiella pneumoniae is one of the most important pathogens responsible for bloodstream infections, second only to *Escherichia coli*.[1,2] Recently, a new variant termed hypervirulent *K pneumoniae* (hvKP) has been reported in Taiwan.[3] Compared with classic *K pneumoniae* (cKP), hvKP is characterized by the hypermucoviscous phenotype and hypervirulent factors. Alarmingly, hvKP strains are

capable of inducing severe, invasive, community-acquired infection in immunocompetent individuals with a propensity for causing metastatic spread to distant sites, which constitutes a serious threat to public health.

The *pks* gene cluster, originally identified in extraintestinal pathogenic *E coli*,[4] encodes enzymes responsible for the synthesis of colibactin, a genotoxin that has been shown to induce double-strand DNA breaks, cell cycle arrest, and cell death and contribute to increased virulence. It was shown that the presence of the *pks* genes is strongly correlated with bacteremia in *E coli*.[5] In a mouse model of septicemia, the colibactin-producing *E coli* strains were reported to be associated with significantly lower survival rate.[6] Several studies showed that inactivation of *pks* genes reduce the ability of *E coli* strains to colonize the intestinal tract and consequently to translocate to the blood.[7,8] Recently, the *pks* gene cluster was also found in *K pneumoniae*. It was reported that *pks*-encoding colibactin was related to the *K pneumoniae* hypervirulence in meningitis model.[9] On the basis of these researches, we speculated that there may be a potential correlation between the *pks* gene cluster, virulence, and *K pneumoniae*-induced bloodstream infections (BSIs). However, little reports are available regarding the characteristics of *K pneumonia* bloodstream strains caused by hvKP, and even less focused on colibactin-producing *K pneumoniae*. Thus, the aim of this study was to investigate the prevalence of the *pks* gene cluster, and clinical and molecular characteristics of *K pneumoniae*-induced BSIs.

2 | MATERIALS AND METHODS

2.1 | Isolates

A total of 190non-repetitive *K pneumonia* bloodstream isolates were collected from March 2016 to March 2018. Relevant clinical data were also retrieved. The detection of *K pneumoniae* in blood cultures within 48 hours after admission was defined as community-acquired BSIs. Correspondingly, the development of bacteremia over 48 hours into inpatient admission was defined as hospital-acquired BSIs, including infections correlated with the presence of medical devices.[10,11] The primary site of BSIs was identified if a localized infection was present before or coincident with the detection of bacteremia.[12] Laboratory data were obtained on the day of the first positive episode isolated from blood.

2.2 | Detection of the *pks* gene cluster, capsular types, and virulence genes

The presence of *pks* gene cluster, capsular types, and virulence genes were detected by polymerase chain reaction (PCR) as previously described.[13] Genomic DNA of *K pneumonia* was extracted by boiling method. Briefly, 3-5 colonies from an overnight culture of *K pneumonia* was suspended in 200 μL of sterile distilled water and boiled at 95°C for 10 minutes and then centrifuged at 13 000 *g* for 10 minutes to remove cellular debris. The supernatant was used as template for amplifications. The PCR products were visualized by 1% agarose gel electrophoresis. Strains positive for *p-rmpA* and *iucA* were designated as hvKP. For *pks*-positive strains that were negative for K1, K2, K5, K20, K54, and K57, their capsular types were identified by PCR amplification and sequencing of *wzi* gene as previously described.[14] The primers used in this study are listed in Table 1.

2.3 | Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out by bioMerieux VITEK-2 (bioMerieux). The minimum inhibitory concentrations (MICs) of antimicrobial agents were interpreted according to the guidelines established by the Clinical and Laboratory Standards Institute (CLSI).[15] A panel of 20 antimicrobial agents was tested, including ampicillin-sulbactam, piperacillin-tazobactam, cefoperazone-sulbactam, cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, cefotan, aztreonam, ertapenem, imipenem, meropenem, tobramycin, amikacin, gentamicin, levofloxacin, ciprofloxacin, trimethoprim-sulfamethoxazole, and furantoin. *K pneumoniae* ATCC 700603 and *Staphylococcus aureus* ATCC 25923 were included in each experiment as controls.

2.4 | Statistical analysis

Categorical variables were analyzed by using chi-square test or Fisher's exact test. For continuous variables, Student's *t* test or the Mann-Whitney *U* test was used to analyze the data, as appropriate. Logistic regression was employed to identify risk factors for pks^+ *K pneumoniae*-induced BSIs. All variables with *P* values<0.1 were incorporated into a multivariate model using a backward approach. All data analysis was performed by SPSS software (version 25.0). A *P* value < 0.05 was considered statistically significant.

2.5 | Ethics statement

Permission for collecting the information in the medical records of the patients and the *K pneumoniae* isolates for research purposes was approved by the Ethics Committee of Xiangya Hospital Central South University.

3 | RESULTS

3.1 | Prevalence of *pks* gene cluster, capsular types, and virulence gene distribution

In this study, the colibactin system markers *clbB* and *clbN* were simultaneously detected in 26.8% (51/190) isolates, which were considered as pks^+ *K pneumoniae*.[5,16] The results of two additional colibactin genes *clbA* and *clbQ* were consistent with those for *clbB* and *clbN*. A total of 43 isolates tested positive for K1, K2, K5, K20, K54, and K57 capsular types. Capsular types K1, K2, K5, K20, K54, and K57 comprised 4.7% (9/190), 11.6% (22/190), 0.5% (1/190), 1.0% (2/190), 1.0% (2/190), and 3.7% (7/190) of all *K pneumoniae* strains, respectively. Statistical analysis indicated that the positive

TABLE 1 Primers used in this study

Primer name	DNA sequence (5′-3′)	Amplicon size (bp)
Capsular serotypes		
K1	F: GGTGCTCTTTACATCATTGC	1283
	R:GCAATGGCCATTTGCGTTAG	
K2	F:GACCCGATATTCATACTTGACAGAG	641
	R:CCTGAAGTAAAATCGTAAATAGATGGC	
K5	F:TGGTAGTGATGCTCGCGA	741
	R:CCTGAACCCACCCCAATC	
K20	F:CGGTGCTACAGTGCATCATT	280
	R:GTTATACGATGCTCAGTCGC	
K54	F:CATTAGCTCAGTGGTTGGCT	881
	R:GCTTGACAAACACCATAGCAG	
K57	F:CTCAGGGCTAGAAGTGTCAT	1037
	R:CACTAACCCAGAAAGTCGAG	
Wzi	F:GTGCCGCGAGCGCTTTCTATCTTGGTA TTCC	580
	R:GAGAGCCACTGGTTCCAGAA[C/T]TT[C/G]ACCGC	
Virulence genes		
p-rmpA	F:CATAAGAGTATTGGTTGACAG	461
	R:CTTGCATGAGCCATCTTTCA	
wcaG	F: GGTTGGKTCAGCAATCGTA	169
	R:ACTATTCCGCCAACTTTTGC	
mrkD	F:AAGCTATCGCTGTACTTCCGGCA	340
	R:GGCGTTGGCGCTCAGATAGG	
allS	F:CATTACGCACCTTTGTCAGC	764
	R:GAATGTGTCGGCGATCAGCTT	
ybtS	F:GACGGAAACAGCACGGTAAA	242
	R:GAGCATAATAAGGCGAAAGA	
kfu	F:GGCCTTTGTCCAGAGCTACG	638
	R:GGGTCTGGCGCAGAGTATGC	
iucA	F:GCATAGGCGGATACGAACAT	556
	R:CACAGGGCAATTGCTTACCT	
Pks gene cluster		
clbA	F:CTAGATTATCCGTGGCGATTC	1311
	R:CAGATACACAGATACCATTCA	
clbB	F:GATTTGGATACTGGCGATAACCG	579
	R:CCATTTCCCGTTTGAGCACAC	
clbN	F:GTTTTGCTCGCCAGATAGTCATTC	733
	R:CAGTTCGGGTATGTGTGGAAGG	
clbQ	F:CTTGTATAGTTACACAACTATTTC	821
	R:TTATCCTGTTAGCTTTCGTTC	

rates of K1 and K57 capsular types in pks^+ strains were significantly higher than the pks^- strains ($P < 0.05$). The capsular type of remaining 25 pks^+ isolates was further determined by *wzi* amplification and sequencing. One isolate was PCR-negative, and the other 24 isolates were identified as K14, K23, K24, K25, K27, K80, and 17 distinct *wzi* allelic types, respectively. The *wzi* sequences are provided in Supplemental Material 1.

Seven virulence genes were detected including *p-rmpA*, *wcaG*, *mrkD*, *allS*, *ybtS*, *kfu*, and *iucA*. Compared with the pks^- strains, the pks^+ strains had significantly higher positive rates of all the tested virulence genes ($P < 0.05$). As determined by positive *p-rmpA* and *iucA*, 21.6% (41/190) of *K pneumoniae* bloodstream isolates were hvKP. The *pks*-positive rate was significantly higher than *pks*-negative rate among hvKP isolates. More details regarding virulence factors are shown in Table 2.

TABLE 2 Capsular types and virulence gene distribution of *pks*-positive and *pks*-negative *K pneumoniae* bloodstream isolates

Virulence factors	*pks*-positive isolates (n = 51)	*pks*-negative isolates (n = 139)	*P* value
Capsular types			
K1	9 (17.6%)	0	0.000*
K2	7 (13.7%)	15 (10.8%)	0.575
K5	0	1 (0.7%)	1.000
K20	2 (3.9%)	0	0.071
K54	2 (3.9%)	0	0.071
K57	6 (11.8%)	1 (0.7%)	0.000*
Virulence genes			
p-rmpA	30 (58.8%)	21 (15.1%)	0.000*
wcaG	20 (39.2%)	4 (2.8%)	0.000*
mrkD	51 (100%)	125 (89.9%)	0.019*
allS	38 (74.5%)	52 (37.4%)	0.000*
ybtS	41 (80.4%)	65 (47.0%)	0.000*
kfu	21 (41.2%)	25 (18.0%)	0.001*
iucA	32 (62.7%)	23 (16.5%)	0.000*
HvKP	28 (54.9%)	13 (9.4%)	0.000*

*A *P* value < 0.05 was considered to be statistically significant.

TABLE 3 Antimicrobial resistance of *pks*-positive and *pks*-negative *K pneumoniae* bloodstream isolates

Antimicrobial agent	*pks*-positive isolates (n = 51)	*pks*-negative isolates (n = 139)	*P* value
Ampicillin-sulbactam	20 (39.2%)	71 (51.1%)	0.097
Piperacillin-tazobactam	8 (15.7%)	46 (33.1%)	0.013*
Cefoperazone-sulbactam	10 (19.6%)	51 (36.7%)	0.017*
Cefazolin	19 (37.3%)	79 (56.8%)	0.008*
Cefuroxime	17 (33.3%)	48 (34.5%)	0.766
Ceftazidime	14 (27.4%)	52 (37.4%)	0.196
Ceftriaxone	18 (35.3%)	71 (51.1%)	0.032*
Cefepime	20 (39.2%)	59 (42.4%)	0.508
Cefotan	8 (15.7%)	37 (26.6%)	0.093
Aztreonam	15 (29.4%)	70 (50.4%)	0.005*
Ertapenem	8 (15.7%)	49 (35.2%)	0.006*
Meropenem	7 (13.7%)	45 (32.4%)	0.007*
Imipenem	9 (17.6%)	47 (33.8%)	0.022*
Tobramycin	10 (19.6%)	35 (25.2%)	0.363
Amikacin	7 (13.7%)	33 (23.7%)	0.110
Gentamicin	13 (25.5%)	48 (34.5%)	0.186
Levofloxacin	8 (15.7%)	47 (33.8%)	0.010*
Ciprofloxacin	8 (15.7%)	52 (37.4%)	0.003*
Trimethoprim-sulfamethoxazole	11 (21.6%)	48 (34.5%)	0.065
Furantoin	13 (26.0%)	62 (47.0%)	0.010*

*A *P* value < 0.05 was considered to be statistically significant.

3.2 | Antimicrobial resistance of *pks*$^+$ and *pks*$^-$ *K pneumoniae* bloodstream isolates

Overall, the *pks*$^+$ *K pneumoniae* isolates displayed lower resistance to all tested antimicrobial agents than the *pks*$^-$ strains. In detail, the *pks*$^+$ *K pneumoniae* isolates were significantly more susceptible to piperacillin-tazobactam, cefoperazone-sulbactam, cefazolin, ceftriaxone, aztreonam, ertapenem, meropenem, imipenem, levofloxacin, ciprofloxacin, and furantoin ($P < 0.05$). A summary of the results is shown in Table 3.

3.3 | Clinical characteristics of *pks*$^+$ and *pks*$^-$ *K pneumoniae* bloodstream isolates

The clinical characteristics of the *pks*$^+$ and the *pks*$^-$ isolates are shown in Table 4. There was no significant difference in age and sex between the two groups. More *pks*$^+$ isolates (60.8%, 31/51) than *pks*$^-$ isolates (42.4%, 59/139) were community-acquired. Individuals with diabetes mellitus and hypertension are more susceptible to the *pks*$^+$ isolates than the *pks*$^-$ isolates ($P < 0.05$). There was a trend of more *pks*$^+$ bloodstream isolates originated from liver abscess, but the difference was not significant. Notably, the lymphocyte counts were significantly lower in the *pks*$^+$ group than in the *pks*$^-$group ($P < 0.05$). Multivariate regression analysis found that diabetes mellitus (OR 2.637, 95% CI: 1.001-6.948) and the carriage of K1 and K20 (OR 4.581, 95% CI: 1.271-16.521 and OR 11.716, 95% CI: 2.301-59.643) capsular types were independent risk factors for *pks*$^+$ *K pneumoniae*-induced BSIs.

4 | DISCUSSION

This retrospective study was conducted in 190 patients with *K pneumoniae*-induced BSIs during a 24-month period from March 2016 to March 2018. It was the first systematic study focusing on the *pks* prevalence of *K pneumoniae* bloodstream isolates. Meanwhile, the clinical and microbiological characteristics were also analyzed in this study.

Currently, there is no absolute definition of hvKP. But it is clear that hypermucoviscosity and iron acquisition systems contributed to the virulence of *K pneumoniae*.[3,17] Hence, strains positive for *p-rmpA* and *iucA* were defined as hvKP in the present study. Our

TABLE 4 Clinical characteristics of *pks*-positive and *pks*-negative *K pneumoniae*-induced bloodstream infections

Characteristics	*pks*-positive isolates (n = 51)	*pks*-negative isolates (n = 139)	*P* value
Age	54.3 ± 19.8	37.7 ± 26.5	0.099
Female	14 (27.5%)	22 (15.8%)	0.841
Acquisition			
Community-acquired	31 (60.8%)	59 (42.4%)	0.000*
Hospital-acquired	20 (39.2%)	80 (57.6%)	0.000*
Underlying condition			
Diabetes mellitus	15 (29.4%)	19 (13.6%)	0.012*
Hypertension	17 (33.3%)	19 (13.7%)	0.002*
Biliary tract disease	3 (5.9%)	17 (12.2%)	0.206
Liver cirrhosis	4 (7.8%)	4 (2.9%)	0.131
Pulmonary infection	7 (13.7%)	13 (9.3%)	0.384
Hematologic diseases	7 (13.7%)	17 (12.2%)	0.783
Cancer	8 (15.7%)	23 (16.5%)	0.846
Surgery within 30 d	19 (37.3%)	44 (31.7%)	0.467
Chemotherapy within 7 d	8 (15.7%)	21 (15.1%)	0.704
Primary site			
Biliary tract	2 (3.9%)	10 (7.2%)	0.411
Respiratory tract	29 (56.9%)	93 (65.5%)	0.276
Urinary tract	5 (9.8%)	10 (7.2%)	0.554
Intra-abdomen	5 (9.8%)	13 (9.4%)	0.925
Brain	2 (3.9%)	3 (2.2%)	0.182
Liver abscess	3 (5.9%)	0	0.573
Laboratory data (mean ± SD)			
WBC count, $\times10^9$/L	8.7 ± 6.6	10.9 ± 8.9	0.746
RBC count, $\times10^{12}$/L	3.2 ± 0.9	3.1 ± 0.8	0.051
HB, g/L	95.4 ± 28.5	97.4 ± 26.2	0.272
PLT, $\times10^9$/L	119.4 ± 97.4	105.0 ± 92.4	0.876
NEUT count, $\times10^9$/L	7.5 ± 6.3	7.9 ± 7.6	0.952
LY count, $\times10^9$/L	0.6 ± 0.6	1.6 ± 1.5	0.016*

HB, hemoglobin; LY, lymphocyte; NEUT, neutrophile granulocyte; PLT, platelet; RBC, red blood cell; WBC, white blood cell.
*A *P* value <0.05 was considered to be statistically significant.

investigation indicated that HvKP accounted for 21.6% of *K pneumoniae*-induced BSIs. In two previous studies conducted in China, the prevalence of hvKP among *K pneumoniae* bloodstream isolates was 31.4% and 36.8%, respectively.[10,18]

In this study, the prevalence of *pks* gene cluster among *K pneumoniae* bloodstream isolates was 26.8%. To date, there have been few epidemic reports on emerging *pks*$^+$ *K pneumoniae* in mainland China. In two previous studies conducted in Taiwan, the positive rates of *pks* among *K pneumoniae* isolated from various body sites were reported 25.6% and 16.7%, respectively.[16,19] In *E coli*, the prevalence of *pks* gene was high, ranging from 31.5% to 58%, and reported to be significantly associated with bacteremia.[5] Our results revealed that the rates of *pks*$^+$ among *K pneumoniae* isolates collected from blood were higher than the overall *pks*$^+$ rate in Taiwan and lower than that in *E coli*.

The capsule is an important virulence factor of *K pneumoniae*. Some capsular serotypes, especially K1, K2, K5, K20, K54, and K57, are recognized as hypervirulent variants of *K pneumoniae*.[3] The above six capsular serotypes were detected by the PCR, and K2 was the most frequently identified serotypes of *K pneumoniae* bloodstream isolates in this study. The analysis of distribution showed that K1, K2, K5, K20, K54, and K57 were all present among *pks*$^+$ isolates while the serotypes of *pks*$^-$ isolates were less diverse. Statistical analysis revealed that compared with *pks*$^-$ strains, the rates of K1 and K57 in *pks*$^+$ strains were significantly higher. In addition, the K1 strains appeared to be associated with the *pks* genes, as all the K1 strains were positive for *pks*. In a word, these results suggested the diverse serotype distribution and potential pathogenicity of *pks*$^+$ isolates.

Multiple studies emphasized a positive correlation between the presence of virulence genes and *pks*$^+$ *E coli*.[5,20,21] Similar results were found in our study. The analysis of virulence factors associated with hvKP showed that the proportion of all these virulence genes in *pks*$^+$ isolates was significantly higher than that in *pks*$^-$ isolates. The *mrkD* gene was carried by all *pks*$^+$ isolates. Besides, *rmpA*, *allS*, *ybtS*, and *iucA*, the genes involved in hypermucoviscosity, allantoin metabolism, yersiniabactin, and aerobactin production, were identified in more than half of *pks*$^+$ isolates. These findings further supported the notion that *pks* genotype may have a relationship with hypervirulent strains. Relevant experiments are needed to figure out whether *pks* gene cluster contributes to virulence directly or serve as a marker for something else involved in pathogenesis.

It is found that *pks*$^+$ isolates are associated with low antimicrobial resistance. Statistical analysis revealed that *pks*$^+$ isolates were significantly less resistant to 11 of 20 tested antimicrobial agents than *pks*$^-$ isolates. This circumstance was possibly owing to the fact that *pks*$^+$ isolates possessed high percentages of hypervirulent serotypes and virulence genes as the acquisition of virulence is usually accompanied by reduced drug resistance. Currently, the emergence of multidrug-, extremely drug-, or pan-drug-resistant cKP has already become a tough situation in clinical studies.[22,23] Nonetheless, multidrug-resistant hvKP strains producing extended spectrum β-lactamase (ESBL) or carbapenemase have also been described.[25,26] It is noteworthy that the confluence of genotoxicity and drug resistance is also a disturbing situation in future. Epidemiologic surveillance, effective infection control measures, and novel therapeutic measures targeting the virulence factors are needed to prevent insurmountable *K pneumoniae* infections.

The analysis of clinical characteristics showed that *pks*$^+$ isolates were more frequently encountered in community-acquired infection. This implied that *pks*$^+$ isolates may play an important part in community-acquired infection like hvKP, which is commonly reported as the cause of community-acquired infections in young people, particularly pyogenic liver abscesses (PLA).[27,28] The crucial information obtained from laboratory data was a remarkable decrease in lymphocytes among *pks*$^+$ isolates. In comparison with *pks*$^-$ isolates, the lymphocyte count of *pks*$^+$ isolates was significantly lower. A similar discovery that production of colibactin by *E coli* induced profound lymphopenia in a mouse model of sepsis was noted by Ingrid et al[5] We thus speculated that the colibactin generated from *pks*$^+$ *K pneumoniae* may harbor the same genotoxicity to lymphocytes as *E coli*. More data are needed to clarify the mechanism, which may enlighten the invention of therapeutic targets since the prevention of lymphopenia improved survival in sepsis. In accordant with other studies,[19] underlying disease including diabetes mellitus, and K1 and K20 capsular types were significant risk factors for *pks*$^+$ *K pneumoniae* infections. It is noticeable that all the strains originated from PLA were positive for *pks*, even though there were only three PLA cases in our study. Large number researches are required to corroborate the association between *pks*$^+$ *K pneumoniae* and PLA.

In conclusion, the *pks*$^+$ *K pneumoniae* was prevalent in individuals with bloodstream infections in mainland China. The high rates of hypervirulent determinants among *pks*$^+$ *K pneumoniae* revealed potential pathogenicity of this emerging gene cluster. Diabetes mellitus, and K1 and K20 capsular types were identified as independent risk factors associated with *pks*$^+$ *K pneumoniae* bloodstream infections. This study highlights the significance of clinical awareness and epidemic surveillance of *pks*$^+$ strains.

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DISCLOSURE

This work was original research that has not been published previously and not under consideration for publication elsewhere, in whole or in part.

CONFLICT OF INTEREST

None declared.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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