ORIGINAL ARTICLE

TYPIFICATION OF THE STAPHYLOCOCCAL CHROMOSOME CASSETTE OF METHICILLIN-RESISTANT *Staphylococcus aureus* IN THE STATE OF ARAGUA, VENEZUELA

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ABSTRACT

Objective: Typify the SCC*mec* cassette in methicillin-resistant strains of *Staphylococcus aureus* in clinical isolates from health centers in the State of Aragua-Venezuela and compare the presence of SCC*mec* genotypes among the state health centers and according to the type of infection. **Materials and methods:** 81 MRSA strains from four health centers of the Aragua-Venezuela State were studied. Methicillin resistance was performed with the Kirby-Bauer method with oxacillin (1 µg) and cefoxitin (30 µg) disks. The *mec*A gene and SCC*mec* were analyzed by the multiple PCR technique. **Results:** Only 55 isolates (67.9%) amplified the *mec*A gene, and 24 strains (43.6%) amplified SCC*mec*. SCC*mec* type I was the most frequency, followed by SCC*mec* IV and SCC*mec* III, representing 62.5%, 25% and 12.5%, respectively. SCC*mec* I was predominant in health center A (80%), while in B and C 60% and 100% respectively were SCC*mec* IV. At health center D, 50% turned out to be SCC*mec* I and 50% SCC*mec* IVd. A relationship was found between the SCC*mec* and the health center with statistical significance. SCC*mec* I predominated in skin and soft tissue and respiratory infections with 63.2% and 50%, respectively. There was no association between genotype and type of infection with a p value greater than 0.05. **Conclusions:** The prevalence of SCC*mec* I and IV will allow establishing new measures in the use of antibiotics and epidemiological control.

Keywords: *Staphylococcus aureus*; SCC*mec*; *mec*A gene; Molecular Epidemiology; *Staphylococcus aureus* methicilin resistent (source: MeSH NLM).

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a global public health problem, causing serious infections in hospitals and the community. In 2018, the World Health Organization (WHO) estimated that patients with MRSA infections are 64% more likely to die than patients with non-resistant infections [(1)]. In addition, by 2017 the WHO included MRSA in the list of the twelve most dangerous pathogens to human health because of its resistance to antibiotics [(2)].

Resistance to methicillin is caused by the fact that the bacteria synthesizes a penicillin-binding protein known as PBP2a, which has a low affinity for methicillin and the rest of the beta-lactam antibiotics, preventing the entry of this type of antibiotic into the bacterial cell to exert its antimicrobial effect. PBP2a is encoded by the *mec*A gene, which is found within a mobile chromosomal element, called the staphylococcal chromosome cassette (SCC*mec*). The *mec*A gene is distributed in both S. aureus and other methicillin-resistant coagulase-negative staphylococcus species [(3,4)].

The SCC*mec* can measure between 21 and 67 Kb, and has a set of genes such as the ccr (*ccr*AB and *ccr*C) that encode recombinases, in addition to the *mec* complex that contains the *mec*A gene, its regulatory genes (*mecI, mecR*), the acquired genetic determinants, which are produced as a result of the integration of plasmids and transposons, and finally, the sequence of the J region [(5)]. It is important to know the constitution of the SCC*mec* because according to recombination events between the *ccr* and *mec*A genes, a variety of SCC*mec* are generated, and these, allow the classification of MRSA according to the SCC*mec* it possesses. Initially, five types

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of SCC*mec* (I-V) and a number of variants or subtypes [5,6] were described; however, new types were recently published as SCC*mec* VI-XI [7].

Furthermore, types of SCC*mec* differ from each other because of their resistance determinants. Therefore, SCC*mec* I, IV, V, VI and VII code for resistance to beta-lactam antibiotics only, whereas SCC*mec* II, III and VIII have additional genes for resistance to multiple classes of antibiotics other than beta-lactam antibiotics [5,8].

On the other hand, MRSA strains can be contracted from the hospital environment (MRSAH) or in the community (MRSEC). MRSAC is characterized by sensitivity to multiple antibiotics and is usually resistant to beta-lactam antibiotics only. It can cause skin and soft tissue infections, including severe cases of necrotizing pneumonia, necrotizing fasciitis, septic thrombophlebitis, and sepsis [9,10]. MRSAH is resistant to several groups of antibiotics, in addition to beta-lactams, and is associated with patients with risk factors such as high antibiotic consumption, prolonged hospital stays, invasive procedures (intravenous catheters, urinary catheters, tracheotomy), bedsores, severe illness, and contact with MRSA colonized patients [9,10]. In addition, MRSAC carries SCC*mec* IV and V [5], while MRSAH strains have SCC*mec* I, II or III [11].

The molecular typification of SCC*mec* is performed by polymerase chain reaction (PCR), using the multiple PCR technique, which allows different types of SCC*mec* to be determined simultaneously, which is very useful in epidemiological studies [12,13]. Acuña *et al* [14] applied the multiple PCR technique to typify SCC*mec* in 21 MRSA strains isolated in the bacteriological laboratory of a hospital in Cumaná, state of Sucre, where they found SCC*mec* I and IV in outpatients and in adult emergency patients. The presence of SCCmec IV genotypes indicated that the bacteria isolated came from the community and were spreading to hospital services, producing nosocomial infections. In the state of Zulia, González *et al* [15] characterized the SCC*mec* of 54 MRSA strains by multiple PCR and showed that 54% had SCC*mec* IV; 40%, SCC*mec* I; while 4% and 2%, SCC*mec* IA and SCC*mec* IIIB, respectively.

In the state of Aragua, no previous studies have determined the type of SCC*mec* circulating in the health centers of the region. This is the reason why the objective of the present investigation was to typify SCC*mec* in MRSA strains isolated from health centers in Aragua.

KEY MESSAGES

Motivation for the study: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a public health problem. Antibiotic resistance is caused by the *mec*A gene located in the SCC*mec* chromosome cassette. The SCCmec type differentiates between hospital- and community-acquired MRSA and predicts possible antibiotic resistance genes other than beta-lactams. Few studies have been done in Venezuela, and in the state of Aragua it is the first research to be carried out in four hospitals.

Main findings: The finding was the high frequency of MRSA with SCC*mec* I of hospital origin.

Implications: The research will contribute to establish measures for epidemiological control and the use of antibiotic therapy in four health centers in the state of Aragua, Venezuela.

MATERIALS AND METHODS

Study design

Cross-sectional descriptive study conducted between January and August 2015 in patients who attended four health centers in the state of Aragua, Venezuela, called A, B, C and D in this research. Health center A is a private hospital with 72 beds, outpatient consultation and hospitalization. Health center B is a public care facility for patients with diabetic foot complications, which receives an average of 70 people per day and has no hospitalization. Health center C is a public, preventive care facility for adult and pediatric patients with a capacity for 2000 people, while health center D is a public facility and the largest in the region, with a capacity for 400,000 people and 551 hospitalization beds.

S*taphylococcus strains* were isolated from samples of skin, soft tissue, catheters, auricular, ocular, and respiratory secretions, as well as other infections. It was identified whether the samples were from inpatients or outpatients.

These samples were inoculated into blood agar plates and incubated at 35±2 °C in aerobic conditions for 16-18 hours. The standard procedure described in the literature was used for bacterial identification [16]. Finally, of a total of 404 staphylococcus-positive cultures, *S. aureus* was isolated in 324. The strains were preserved at -20 °C in glycerol until the time of the study.

Antimicrobial susceptibility test

Following the guidelines of the Institute for Standardization of Clinical Laboratories for the identification of MRSA, the agar disk diffusion or Kirby-Bauer method was applied [17]. The cefoxitin disc of 30 µg (BD) and the oxacillin disc of 1 µg (BD) were used. The control strain used was *S. aureus* ATCC 25923.

DNA extraction

It was performed on a MRSA pure culture on blood agar after 18-24 hours of incubation. A suspension was prepared in an Eppendorf tube by taking 1 to 5 colonies of the mi-

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croorganism and placing them in 50 µL of sterile distilled water and then boiled at 99 °C for 10 min. Finally, it was centrifuged at 30,000 g for 1 min and the supernatant was transferred to a new Eppendorf tube. The concentrated DNA was preserved at -20 °C until the time of testing [13].

Detection of the *mec*A gene and SCC*mec* genotypes of MRSA strains

The multiple PCR test was performed to identify the SCC*mec* cassette type and the conditions for amplification according to the methodology previously described by Zhang *et al* [13]. This methodology consisted in using 9 pairs of primers, including the specific primers for SCC*mec* I, II, III, IVa, IVb, IVc, IVd and V types and subtypes and the primers for the *mec*A gene. Eight different loci were amplified based on the sequences presented in Table 1. The following conditions were used for the PCR reaction: for the Master Mix, 50 mM KCl, 20 mM Tris-HCl (pH 8.4); 2.5 mM MgCl2, 0.2 mM of each deoxynucleotide triphosphate (dATP, dUTP, dGTP and dCTP). The primers concentrations are shown in Table 1. Additionally, a Go Taq Flexi DNA Polymerase® unit (Promega Corp., USA) was used.

For controlling quality of the molecular typification tests, the *S. aureus* ATCC 259233 (methicillin-sensitive) strain was used as a negative control and the *S. aureus* ATCC 43300 (methicillin-resistant) strain was used as a positive control for the *mec*A gene. The amplification product was subject to electrophoretic migration in 2% agarose gels at 100 v for 30 min. A 100 bp molecular size marker (New England Biolabs, Inc) was used. Finally, the length of the amplicon was compared with the molecular size values recorded in Table 1 for the *mec*A gene and the SCC*mec* types and subtypes.

Statistical analysis

The provenance data of MRSA strains was collected in a Windows XP Excel 2007 database. Statistical analysis of the data was performed with EpiInfo 3.5.1. Descriptive analyses were performed using frequencies and percentages. The Chi-square test with a significance level of $p < 0.05$ was used to identify differences regarding some characteristics in the strain's origin.

Ethical aspects

This study was evaluated and approved by the Bioethics Committee of the Teaching and Research Department of the Servicio Autónomo Hospital Central de Maracay. Additionally, patients who participated in the study signed an informed consent form.

RESULTS

During the study period a total of 404 staphylococcus-positive cultures from the four health centers in the state of Aragua were analyzed, 80 strains (19.8%) were coagulase-negative staphylococci (CSN) and 324 (80.2%) were *S. aureus*, of the strains analyzed 81 (25%) were MRSA.

Detection of the *mec*A gene showed that from the 81 MRSA isolates, 55 (67.9%) amplified the *mec*A gene and 26

Table 1. Sequences of the primers that amplify each of the loci of the Staphylococcal Chromosome Cassette (SCC*mec*)

Primers	5'-3' sequence	Concentration (uM)	Amplified size	Type SCC*mec*
Type I-F Type I-R	GCTTTAAAGAGTGTCGTTACAGGGTTCTCTCATAGTATGACGTCC	0.048	613 pb	SCC*mec* I
Type II-F Type II-R	CGTTGAAGATGATGAAGCGCGAAATCAATGGTTAATGGACC	0.032	398 pb	SCC*mec* II
Type III-F Type III-R	CCATATTGTGTACGATGCGCCTTAGTTGTCGTAACAGATCG	0.042	80 pb	SCC*mec* III
Type IVa- F Type IVa-R	GCCTTATTCGAAGAAACCGCTACTCTTCTGAAAAGCGTCG	0,104	776 pb	SCC*mec* Iva
Type IVb-F Type IVb-R	TCTGGAATTACTTCAGCTGCAAACAATATTGCTCTCCCTC	0.092	493 pb	SCC*mec* IVb
Type IVc-F Type IVc-R	ACAATATTTGTATTATCGGAGAGCTTGGTATGAGGTATTGCTGG	0.078	200 pb	SCC*mec* IVc
Type IVd-F5 Type IVd-R6	CTCAAAATACGGACCCCAATACATGCTCCAGTAATTGCTAAAG	0.28	881 pb	SCC*mec* IVd
Type V-F Type V-R	GAACATTGTTACTTAAATGAGCGTGAAAGTTGTACCCTTGACACC	0.06	325 pb	SCC*mec* V
MecA147-F MecA147-R	GTG AAG ATA TAC CAA GTG ATTATG CGC TAT AGA TTG AAA GGA T	0.046	147 pb	*mecA*

Source: Zhang *et al* [13]

(32.1%) did not, with a confidence interval of 56.6% to 77.8% and a 95% confidence level. Among the 55 isolates that tested positive for the *mec*A gene, only 24 (43.6%) amplified some type of SCC*mec*, while in 31 isolates (56.4%) no amplification was obtained with confidence intervals between 30.3%-57.7% and 42.3%-68.7%, respectively (Figure 1A, B and C).

From the 24 MRSA strains that amplified SCC*mec*, the most predominant cassette among the isolates was found to be the SCC*mec* type I, followed by SCC*mec* IV (subtypes IVb and IVd) and SCC*mec* III represented by 62.5%, 25% and 12.5%, respectively. SCC*mec* II and SCC*mec* V were not found (Table 2).

The largest number of MRSA strains with some type of SCC*mec* amplification was found in health center A (15 strains), where SCC*mec* I was found to be predominant (80%), followed by SCC*mec* III (20%). In the case of health center B, a total of 5 strains amplified SCC*mec*, where it was found that three of them (60%) turned out to be SCC*mec* IV (subtypes IVb and IVd) and 40%, SCC*mec* I. In health center C, two MRSA strains were obtained that amplified SCC*mec* IV, one amplified subtype IVb and the other subtype IVd. In health center D, only two MRSA isolates amplified SCC*mec*, one of which was SCC*mec* I and the other SCC*mec* IVd. A correlation was found between the genotype isolated and the health center (p = 0.032) (Table 2).

Of the 24 strains analyzed, 19 were isolated in skin and soft tissues, 4 in secretions from respiratory infections and 1 in blood culture. Of the 19 isolates from skin and soft tissues, SCC*mec* I was the most predominant (63.2%), followed by SCC*mec* IV (26.3%), where 15.8% were subtype IVb, and 10.5% subtype IVd. Four strains were obtained in respiratory samples that amplified SCC*mec*, from which two (50%) carried SCC*mec* I, while one was SCC*mec* III and one was SCC*mec* IVd. No relationship was found between genotype and type of infection (p = 0.870) (Table 2).

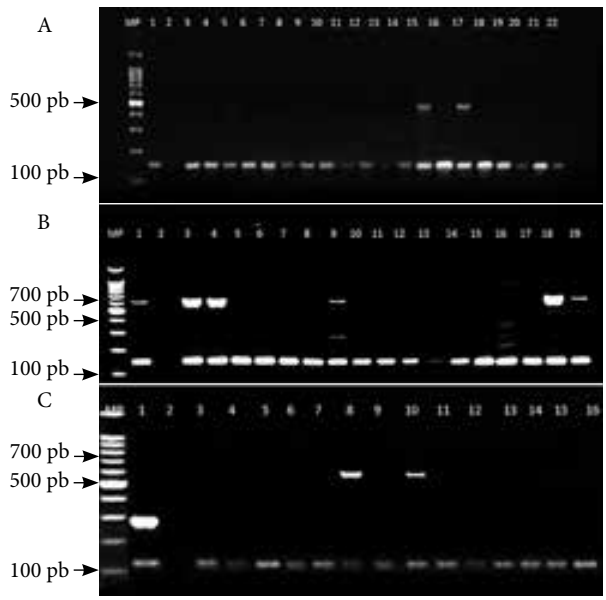

Figure 1. Electrophoresis in 2% agarose gels of the PCR amplification products of SCC*mec* genotypes and the *mec*A gene. A) MP: 100 bp molecular weight marker; lane 1: positive control; lane 2: negative control; lanes 3 to 22 MRSA isolates where 147 bp band corresponding to the *mec*A gene is observed; lanes 15 and 17 have additional 493 bp band corresponding to SCC*mec* IVb. B) MP: molecular weight marker of 100 bp; lane 1: positive control; lane 2: negative control; lanes 3 to 19 MRSA isolates where a 147 bp band corresponding to the *mec*A gene is observed; the 881 bp band is SCC*mec* IVd, 613 bp is SCC*mec* I, 395 bp corresponds to SCC*mec* II, 325 bp is SCC*mec* V and 200 bp IVc; lanes 1, 3, 4, 9, 18 and 19: SCC*mec* I genotype strains; lanes 5 to 8, 10 to 17: there was no amplification with the SCC*mec* included in the study C) MP: 100 bp molecular weight marker; lane 1: positive control; lane 2: negative control; lanes 3 to 14 MRSA isolates showing 147 bp band corresponding to the *mec*A gene; lanes 8 and 10: SCC*mec* I genotypes strains.

DISCUSSION

In this study, 25% of MRSA data was similar to the data obtained by Dorante *et al* [18], who found that out of 117 *S. aureus* isolates in a hospital in the state of Aragua, 24.7% were MRSA. Likewise, Chavez *et al* [19] reported that, in a hospital in Medellín (Colombia), out of 35 isolates, 28.6% were MRSA. However, Guillen *et al* [20] found in Paraguay that, from 77 strains, 18.7% were MRSA, slightly lower than the percentage reported in this study.

On the other hand, detection of MRSA using *mec*A gene identification by PCR, showed discrepancies with the results obtained with the use of oxacillin and cefoxitin discs because from the 81 MRSA isolates, only 55 amplified the *mec*A gene. Other studies have reported similar results, such as Acuña *et al* [14], who observed that from 21 MRSA strains, only 19 amplified the *mec*A gene. In contrast, the research by Chavez *et al* [19] and Guillen *et al* [20] reported that all of the MRSA strains they studied possessed the *mec*A gene. In this study, the strains that did not amplify the *mec*A gene had their identification and their phenotypic methicillin resistance profiles confirmed.

According to the results obtained, it is possible that resistance is related to some mechanism other than the expression of PBP2a. One of them may be the hyperproduction of β-lactamases by *S. aureus* strains, known as BORSA (Borderline oxacillin-resistant *S. aureus*) [21]. In addition, it would be possible to imply that the lack of amplification of the *mec*A gene may be due to the fact that the strain carries the *mec*C gene, which is not detectable by conventional methods, and is responsible for 2% of MRSA infections in humans. The *mec*C gene is 70% homologous to the *mcc*A gene and synthesizes a transpeptidase that is 60% homologous with PBP2a [22].

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Table 2. Genotype frequency of the staphylococcal chromosome cassette and its distribution according to health centers and sample type.

Characteristic	Type of staphylococcal chromosome cassette (SCC*mec*)						
	I	II	III	IVb	IVd	V	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Number of isolates	15 (62,5)	0 (0)	3 (12,5)	3 (12,5)	3 (12,5)	0 (0)	24 (100)
Health centers							
A	12 (80)	0 (0)	3 (20)	0 (0)	0 (0)	0 (0)	15 (100)
B	2 (40)	0 (0)	0 (0)	2 (40)	1 (20)	0 (0)	5 (100)
C	0 (0)	0 (0)	0 (0)	1 (50)	1 (50)	0 (0)	2 (100)
D	1 (50)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	2 (100)
Sample type							
Hemoculture	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)
Skin and soft tissue	12 (63)	0 (0)	2 (10)	3 (16)	2 (10)	0 (0)	19 (100)
Respiratory	2 (50)	0 (0)	1 (25)	0 (0)	1 (25)	0 (0)	4 (100)

Regarding the typification of SCC*mec*, 24 strains amplified some type of SCC*mec*, with SCC*mec* I being the most predominant, followed by SCC*mec* IV (subtypes IVb and IVd) and SCC*mec* III in a smaller proportion. Distribution of SCC*mec* genotypes was different in health centers A, B, C and D, health center A had the highest number of strains found, with a clear predominance of SCC*mec* I and, in less frequency, type III, which confirms its in-hospital origin and demonstrates that those isolates carrying type III, must possess resistance to a wide variety of antibiotics other than β-lactams [5,8]. Center B occupied the second position according to the total number of isolates, with predominance of SCC*mec* type IV (subtypes IVb and IVd), followed by type I. The presence of SCC*mec* IV in health center B, an outpatient center for diabetic foot complications, seems to indicate its origin in the community [5,8]. However, in the same center, the finding of SCC*mec* I is related to MRSA-H strains [5,8], which could predict the possible dissemination of strains acquired in the hospital environment at this health center.

In addition, SCC*mec* type IV was mainly found in health centers C and D. However, the number of MRSA strains associated to some type of SCC*mec* was low, which could be explained by the low resources from most bacteriology laboratories and this affects detection and identification of MRSA strains. The low number of detected MRSA strains and SCC*mec* is considered a limitation to interpret the results of this study. Therefore, further research will be necessary to deepen the data about distribution of SCC*mec* in both health centers, being health center D the largest and most important one in the region.

Results related to the high frequency of SCC*mec* I are similar to those reported in other regions of Venezuela and in other cities of Latin America. In fact, Acuña *et al* [14] found in a hospital in Cumaná (Venezuela) the predominance of SCC*mec* I (14 of 19 MRSA isolates), followed by SCC*mec* IV (3 of 19 MRSA strains). In Valdivia, Chile, SCC*mec* I was identified, followed by SCC*mec* IV [23]. The cited investigations also coincide with the predominance of SCCmec I found in health center A. On the other hand, the presence of SCCmec IV in health centers B and C, coincides with that proposed by Romero *et al* [24] and Castellano *et al* [25] in hospitals in the state of Zulia, as well as that referred to by Sanchez *et al* [26] in hospitals in Medellín (Colombia). A study carried out in a hospital in Cali (Colombia) [19] reported that 26.6% of MRSA strains carried SCC*mec* II, unlike what was observed in this study in the four health centers investigated. This result could be due to differences in the predominance and distribution of SCC*mec* between hospitals and the geographical area of reference. In fact, the studies published to date in other regions of Venezuela have not identified SCC*mec* II [14,15,24,25].

Identifying the presence of SCC*mec* I in health centers in the state of Aragua could improve therapeutic options for the treatment of MRSA infections [5,8]. However, the presence of SCC*mec* IV also gains relevance, because of its exclusive resistance to beta-lactam antibiotics and because it is related to MRSA-C [5,8]. MRSA-C isolates with SCC*mec* IV, and to a lesser extent type V, also carry the genes for Panton-Valentine Leukocidin toxin (PVL) [5], while MRSA strains acquired in the hospital environment, have SCC*mec* II or III, and in very few cases LPV has been found [5,8]. That is why it has been proposed that the identification of PVL in hospital strains carrying SCC*mec* IV allows to corroborate its origin and to clarify the epidemiological panorama [5,8].

In relation to the infection localization, most of the typified strains came from skin and soft tissues, with predominance of SCC*mec* I, confirming the hospital origin of the infections and orienting antibiotic therapy, since type I is a carrier of resistance to beta-lactam antibiotics [5,8]. Results from this study differ from those found by Romero *et al.* [24], who found a high percentage of SCC*mec* IV isolates in skin and soft tissue samples.

From the 55 MRSA isolates that amplified the *mec*A gene, 56.4% did not amplify the SCC*mec*, and it is possible to assume that primers were used to detect SCC*mec* I to V and their subtypes, which was proposed by Zhang *et al* [13]. This was another limitation for this study. The possible existence of other SCC*mecs* would indicate the presence of other genotypes with new antibiotic resistance determinants.

In conclusion, the identification of MRSA by SCC*mec typification* showed evidence of the predominance of SCC*mec* I and III related to MRSA acquired in the hospital environment and SCC*mec* type IV associated to the community. It was shown that there is a correlation between the isolated genotype and the health center. In skin and soft tissue samples, SCC*mec* I predominated; however, no correlation was found between SCC*mec* and the type of infection.

It is recommended to carry out prospective studies regarding the detection of SCC*mec*, including the main health centers in the region, the use of alternative methods to verify methicillin resistance, and the introduction of new primers to identify the existence of other SCC*mec*. In addition, it is recommended to include the assessment of hyperproduction of beta-lactamases and the determination of minimum inhibitory concentrations of oxacillin for those MRSA strains that do not amplify the *mec*A gene.

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Conflicts of interest: The authors declare that there is no conflict of interest regarding the publication of this article.

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