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Full Length Research Paper

Determinants of nasal carriage of MRSA in Bukavu city

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Staphylococcus aureus **infections in their community form, is an important public health problem. Carrier screening for *S. aureus* especially those resistant to methicillin (MRSA) is an important factor in the control and spread of *S.aureus* infection. However, the extent of this carriage remains unknown in Bukavu. Our aim was to determine the prevalence and determinants of nasal carriage of *S. aureus* and MRSA. An analytical cross-sectional study community-based was carried out in Bukavu from October 2015 to February 2016. A sampling nasal swab was performed for all persons included in the study. Cultures and identification of *S. aureus* were made by conventional methods. Any resistance to cefoxitin 30ug was regarded as the expression of the *mecA* gene (methicillin resistance). Samples of 312 persons enrolled in this study have been subjected to culture and research of the expression of *mecA* gene. 45 isolates were identified as *S. aureus*, giving a carriage prevalence of 14,4% [IC 95%: 10.52-18.32%], the median age was 24 years (1-71 years); the nasal carriage prevalence of MRSA was 5,1% [IC 95%: 2.68-7.58%] (16/312). The carriage of *S. aureus* and MRSA was independent of age, gender, origin, profession, concept of diabetes, chronic wound and even level of study (p> 0.05). This study shows a high prevalence of nasal carriage of MRSA in the community of Bukavu, other risk factors should be sought later to understand this phenomenon.**

Keywords: Bukavu, determinants, MRSA, nasal carriage, *Staphylococcus aureus*

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is the basis of an endemic hospitalization in the world. MRSA has a great significance in terms of mortality and morbidity with an important economic consequence [1,2]. *Staphylococcus aureus* (SA) is a ubiquitous bacterium that colonizes 20-30% of the human population, often asymptomatic, causing widespread lesions of infection found in skin, soft tissue, bone and joint, implants, pneumonia, sepsis and a variety of toxicosis conditions such as toxic shock syndrome [3].

Nasal colonization of *S. aureus* is an important risk factor for invasive disease; However, in some studies, almost half of patients colonized were also extra-nasal (skin, groin fold, armpit, intestine) [4].

Approximately 20-30% of healthy people are persistent carriers of *S. aureus* and 60% are intermittent carriers with high rates of colonization among disadvantaged groups including inpatients, children and prison inmates [5,6]. Studies of the prevalence related to asymptomatic carriage of *S. aureus* and MRSA are much more reported in developed countries. In sub-Saharan Africa, data reporting this issue is very limited [7].

In the Democratic Republic of Congo (DRC), especially in Kinshasa, Phaku et al, carried out a study

in the community in 2014 with 753 samples. They found an asymptomatic *S. aureus* nasal carriage of 13.6% (100/753), with a prevalence of 34.6% of MRSA [8]. This raised serious worries about the possibility of MRSA transmission outside of the health system. If MRSA becomes the most common form of *S. aureus* in a community, that will make treatment of community infections much more difficult [9].

The lack of population studies on MRSA nasal carriage in the eastern region of DRC, the implications of its control and the prevention of infections in asymptomatic carriers, both on the risk of transmission in non-carriers or to maintain an epidemic, urges us to conduct this study in Bukavu, in South Kivu.

METHODOLOGY

Study design.

This is a descriptive, cross-sectional analytical study, which took place in Bukavu from September 2015 to February 2016.

Study site

The city of Bukavu, the chief town of the South Kivu Province, is located in the east of the DRC between latitude 2 ° 30'55 '' and longitude 28 ° 50'42 '' precisely in the Great Lakes region called Eastern Valley Grabben. Bukavu covers an area of 44.9 km² and is divided into three municipalities: Bagira, Ibanda and Kadutu. These 3 municipalities are subdivided into 32 health areas. Bukavu has a climate approaching the subequatorial or tropical humid climate (of short duration) with the influence of the climate of altitude of 1,500 to 2,000m. It has two seasons, namely the rainy season (8 months from mid-September to mid-May) and the dry season (from mid-May to mid-September). The average annual temperature is 19 ° C in Bukavu [10,11].

Study population and sampling.

Our study population consisted of healthy subjects regardless of age or sex, volunteers, living in Bukavu during the study period. The sample size was proportional to the population size of each health area. The interviewers visited the randomly selected households in each health area. Participants in the study were randomly selected from these households that met the inclusion criteria that were to be any supposedly healthy subjects, consenting, without the notion of hospitalization, or taking antibiotics or antituberculosis drugs in the 4 weeks preceding the survey. Using Schwartz formula and a reported prevalence of nasal carriage of MRSA of 5 % at Kinshasa City, by Phaku et al. [8], the sample size was obtained by multiplying it with the cluster effect which was 2 ; a minimum sample size of 279 was targeted, 312 volunteers met these conditions and were included in this study.

Data collection

For each participant included in the study, we collected sociodemographic data (age, sex, occupation, origin, level of study) and information on the risk factors of the study Nasal carriage of *S. aureus* using a pre-established form (history of diabetes, presence of a medical agent in the household, alcohol and tobacco use, history of chronic wound and hospitalization). Simultaneously with the collection of socio-demographic information, a nasal swab was taken using a sterile swab and then sent directly to the Bacteriology laboratory of the Provincial Hospital of Bukavu.

Laboratory analyzes

On the first day (day 1), isolation of *S. aureus* was carried out by plating swabs on human blood agar (or sheep blood if available), and incubated at 37 ° C for 18 to 24 hours. For each sample (day 2), all morphological and suspected colonies indicatives for *S. aureus* (beta-hemolytic colonies, golden yellow); were selected and phenotypically processed as follow: one colony was put in contact with the latex of Pastorex Staph Plus (Bio-Rad) a rapid test for the simultaneous detection of affinity antigen for fibrinogen, protein A and capsular polysaccharides of *S. aureus*. An isolated suspect colony was subcultured on MSA (Mannitol Salt Agar) and on nutrient agar incubated for 18-24 hours at 37 ° C in order to obtain the fermentative character of Mannitol on MSA and for catalase testing on nutrient agar. In order to confirm the identification of *S. aureus* from suspect colonies, we performed a coagulase tube test using human plasma. It consisted in taking 400 µl of human plasma in a sterile plastic test tube, in which we added a suspect colony of *S. aureus*. Using a vortex, we mixed the plasma and the colony deposited in the tube until a homogeneous mixture was obtained. Then the tube will be incubated at 37 ° C for 4 hours. The presence of the coagulum after incubation confirms the isolation of a *S. aureus*. In order to investigate the methicillin resistance of *S. aureus* strains, the cefoxitin 30 µg disc (Liofilchem, Italy) was used by Kirby-Bauer disc diffusion method. Any inhibition diameter ≤ 21 cm was considered as an isolate of *S. aureus* resistant to Methicillin (MRSA) [12].

Data analysis

The data were entered into an Excel database and exported for analysis with SPSS 21. The usual

descriptive statistics: proportion, mean and standard or median deviations and interquartile differences (P25-P75) depending on whether the continuous variables will follow the normal distribution or not have been used in the presentation tables. The exact Pearson or Fisher chi-square test was applied to compare the proportions. The associations between determinants and prevalence were made by Odd ratio (OR) calculation. A p-value <0.05 was considered statistically significant.

Ethical considerations

The study was approved by the Ethics Committee of the Catholic University of Bukavu under number UCB/CIE/NC/11/2015. Informed consent was obtained for all participants. The nasal swab samples were carried out by persons qualified and entrained. The anonymity of participants and the confidentiality of the results were scrupulously respected.

RESULTS

General characteristics of participants

From October 2015 to February 2016, 312 volunteers meeting the criteria mentioned above were included in this study. There are more female than male with a sex ratio of 0.8. Their median age was 24 years with a minimum of 1 month and a maximum of 71 years. Nearly 70% are unemployed and the majority of volunteers come from the municipality of IBANDA (Table 1).

Table 1: General characteristics of participants

	N	%
Gender		
F	163	52.2
M	149	47.8
Age (in years), median (min-max)*		24 (1-71)
≤15	41	13.2
16-25	150	48.2
26-35	76	24.4
36-45	24	7.7
>45	20	6.4
Profession		
Unemployed	223	71.5
Private employee	41	13.1
State employee	26	8.3
Trade	22	7.1
Origin		
BAGIRA	43	13.8
IBANDA	151	48.4
KADUTU	118	37.8
Level of study		
Illiterate	15	4.8
Primary	28	9.0
Secondary	92	29.5
University	177	56.7

* min-max: minimum and maximum

The history of participants

In table 2, of the 312 volunteers in our study, only 7.1% had a personal history of diabetes, 29.8% were or lived with a medical agent in the household, 33.3% were alcohol consumers while only 2.6% smoke, 1.5% have a chronic wound and 9.6% had been hospitalized in the three months preceding our survey.

Table 2: The history of participants.

	n	%
History of diabetes mellitus		
No	290	92.9
Yes	22	7.1
Healthcare worker in household		
No	219	70.2
Yes	93	29.8
Taking Alcohol		
No	208	66.6
Yes	104	33.3
Taking Tobacco		
No	304	97.4
Yes	8	2.6
History of chronic wound		
No	307	98.4
Yes	5	1.6
History of hospitalization		
No	282	90.4
Yes	30	9.6

In figure 1, of the 312 samples submitted for bacteriological study, 45 *S. aureus* were isolated, giving a prevalence of nasal carriage of 14.4% [95% CI: 10.52 - 18.32%].

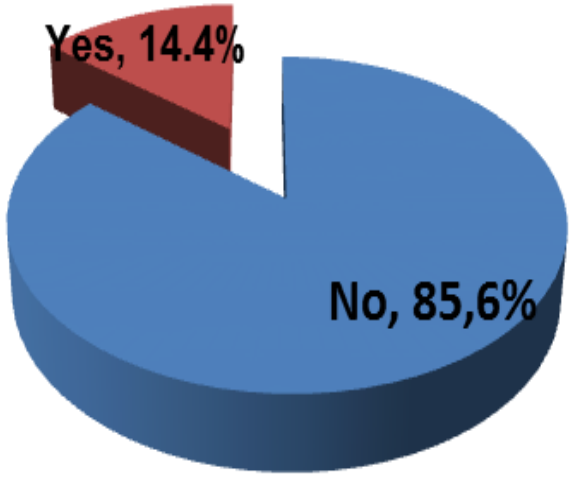

Figure 1: Nasal carriage of SA

General determinants of nasal carriage of *Staphylococcus aureus*.

When we considered sex, we noted that the prevalence was 14.1% in female and 14.8% in male without statistical difference p = 0.86. We also noted that it was 19.5% in the under 15 years and 16.2% in the 26-35 age group. The unemployed are 15.7% and traders are 13.6%. The municipality of Kadutu was the most concerned with 17.8%. High-level individuals were more likely to have nasal carriage of Staphylococcus aureus. We did not find a statistically significant association (Table 3).

Table 3: General determinants of nasal carriage of *Staphylococcus aureus*.

	Carriage of *Staphylococcus aureus*			
	YES	NO	OR (95% CI)	P
Gender				
F	23 (14.1)	140 (85.9)	0.9 (0.5-1.8)	0.86
M	22 (14.8)	127 (85.2)	1	
Age (in years)				
≤15	8 (19,5)	33 (80,5)	1,4 (0,3-7,1)	0,96
16-25	23 (15,3)	127(84,7)	1,0 (0,3-4,7)	0,99*
26-35	10 (13,2)	66 (86,8)	0,9 (0,2-4,3)	0,99*
36-45	1 (4,2)	23 (95,8)	0,3 (0,0-2,5)	0,47*
> 45	3 (4,2)	17 (85,5)	1	
Profession				
Unemployed	35 (15,7)	188 (84,3)	1	
Private employee	5 (12,2)	36 (87,8)	0,7 (0,2-1,9)	0,56
State employee	2 (7,7)	24 (92,3)	0,4 (0,1-1,7)	0,44*
Trade	3 (13,6)	19 (86,4)	0,8 (0,2-2,8)	0,99*
Origin				
BAGIRA	6 (14,0)	37 (86,0)	0,8 (0,3-1,9)	0,56
IBANDA	18 (11,9)	133 (88,1)	0,6 (0,3-1,2)	0,17
KADUTU	21(17,8)	97(82,2)	1	
Level of study				
Illiterate	1 (6,7)	14 (93,3)	1	
Primary	7 (25,0)	21 (75,0)	4,5 (0,6-113,0)	0,28*
Secondary	10 (10,9)	82 (89,1)	1,7 (0,2-39,9)	0,99*
University	27 (15,3)	150 (84,7)	2,5 (0,4-55,7)	0,64*

*Fisher exact

Association between the nasal carriage of *Staphylococcus aureus* and history of participants.

In table 4, the 45 participants with nasal carriage of *Staphylococcus aureus*, only 4.5% had a history of diabetes, 14% reported being or living with healthcare worker, 10% were alcohol consumers and 25% were smokers, 20% had chronic wounds while 10% had been hospitalized three months prior to data collection. There was no statistical difference between the frequency of nasal carriage of *S aureus* according to the different variables.

In figure 2, of the 45 *S. aureus* isolates out of the 312 swabs taken, 16 of them had resistance to cefoxitin, thus giving a prevalence of 5.13% (16/312) MRSA nasal carriage [95% CI 2.68-7.58%].

Table 4: Association between the nasal carriage of *Staphylococcus aureus* and history of participants.

	Nasal carriage of *Staphylococcus aureus*			
	YES	NO	OR (95% CI)	p
History of diabetes mellitus				0.28*
No	44(15.2)	246(84.8)	1	
Yes	1(4.5)	21(95.5)	0.3 (0.0-1.5)	
Healthcare worker in household				0.88*
No	32(14.6)	187(85.4)	1	
Yes	13(14.0)	80(86.0)	0.9 (0.5-1.9)	
Taking Alcohol				0.17
No	34(16.3)	174(83.7)	1	
Yes	11(10.6)	93(89.4)	0,6 (0.3-1.2)	
Taking Tobacco				0.75*
No	43(14.1)	261(85.9)	1	
Yes	2(25.0)	6(75.0)	1.7 (0.2-8.1)	
History of chronic wound				0.99*
No	44(14.3)	263(85.7)	1	
Yes	1(20.0)	4(80.0)	1.5 (0.1-12.1)	
History of hospitalization				0.68*
No	42(14.9)	240(85.1)	1	
Yes	3(10.0)	27(90.0)	0.6 (0.1-2.0)	

*Fisher exact

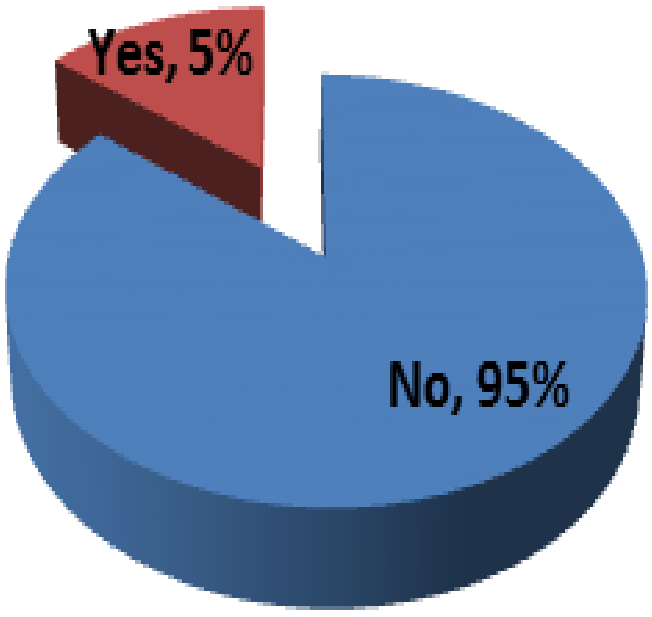

Figure 2: Nasal carriage of MR*SA*

Association between MRSA nasal carriage and general characteristics of participants

From table 5, carriage affects both male 5.4% and female 4.9%, the age range of 16-35 is more concerned at 11%. We noted an asymptomatic carriage of 7.6% among the State employees with the municipality of IBANDA which remains predominant to 5.3% of the carriage. Furthermore, being illiterate seemed to be a protective factor in MRSA nasal carriage (0%).

Table 5: Association between MRSA nasal carriage and general characteristics of participants

	MRSA		OR (95% CI)	P
	YES (n=16)	NO (n=296)		
Gender				0.854
F	8 (4.91)	155 (95.09)	1.09 (0.40-3.00)	
M	8 (5.37)	141 (94.63)	1.00	
Age (in years)				
≤15	1 (2.38)	41 (97.62)	1.00	
16-25	9 (6.00)	141 (94.00)	2.60 (0.34-117.46)	0.693*
26-35	4 (5.26)	72 (94.74)	2.26 (0.27-114.86)	0.654*
36-45	0 (0.00)	24 (100.00)	0.00 (0.00-68.25)	1.000*
>45	2 (10.00)	18 (90.00)	4.43 (0.21-275.14)	0.241*
Mean ± SD	28.6±14.2	25.7±12.1		0.438**
Profession				
Unemployed	12 (5.38)	211(94.62)	1.10 (0.23-10.58)	1.000*
Private employee	2 (4.88)	39 (95.12)		
State employee	2(7.69)	24(92.31)	1.61 (0.11-23.61)	0.638*
Trade	0(0.0)	22(100.0)	0.00 (0.00-9.97)	0.538*
Origin				
BAGIRA	2(4.65)	41(95.35)	1.00	
IBANDA	8(5.30)	143(94.70)	1.14 (0.21-11.49)	1.000*
KADUTU	6(5.08)	112(94.92)	1.09 (0.18-11.54)	1.000*
Level of study				
Illiterate	0(0.0)	15(100.0)	0.00 (0.00-33.40)	1.000*
Primary	2(7.14)	26(92.86)	3.41 (0.23-49.26)	0.231*
Secondary	2(2.17)	90(97.83)	1.00	
University	12(6.78)	165(93.22)	3.26 (0.70-30.64)	0.149*

*: Fisher Exact; **: Student's test

Association between the MRSA nasal carriage and history of participants

From table 6,of the 16 isolates of methicillin-resistant *Staphylococcus aureus* (MRSA), when we considered the participants' history, 4.5% had a history of diabetes in their family, 20% had a history of chronic wound, and 4.3 % reported being or living with a healthcare worker in household, 3.8% were alcohol consumers and 12.5% were smokers. Based on the concept of hospitalization, 6.7% were in the last three months preceding collection. Of these evaluated parameters, there was no statistically significant value.

Table 6: Association between the MRSA nasal carriage and history of participants

	MRSA		OR (95% CI)	p
	YES (n=16)	NO (n=296)		
History of diabetes mellitus				
No	15 (5.17)	275 (94.83)	1.14 (0.16-50.48)	1.000*
Yes	1 (4.55)	21 (95.45)	1.00	
Healthcare worker in household				
No	12 (5.48)	207 (94.52)	1.28 (0.37-5.63)	0.784*
Yes	4 (4.30)	89 (95.70)	1.00	
Taking Alcohol				
No	12 (5.77)	196 (94.23)	1.52 (0.44-6.67)	0.591*
Yes	4 (3.85)	100 (96.15)	1.00	
Taking Tobacco				
No	15 (4.93)	289 (95.07)	1.00	0.347*
Yes	1 (12.50)	7 (87.50)	0.5 (0.0-21.5)	
History of chronic wound				
No	15 (4.89)	292 (95.11)	1.00	0.344*
Yes	1 (20.00)	4 (80.00)	0.36 (0.04-17.27)	
History of hospitalization				
No	14 (4.96)	268 (95.04)	1.00	0.658*
Yes	2 (6.67)	28 (93.33)	0.73 (0.15-6.96)	

*: Fisher Exact

DISCUSSION

Nasal carriage of MRSA is a risk factor for serious bacterial infections both into hospital and community. However, its prevalence in the community is not known in Bukavu, South Kivu. Our objective was to determine the prevalence of nasal carriage of *S. aureus*, MRSA and their determinants. Among 312 participants included, 45 of whom were carriers of *S. aureus* (14.4%, 45/312) -16 (5.1%) were carriers of MRSA nasal carriage. Of these 45 *S. aureus* isolates, 16 (35%) were resistant to cefoxitin. The carriage of *Staphylococcus aureus* and MRSA did not depend on age, gender, origin, profession and even the level of study (p-value> 0.05).

Age, gender and prevalence of nasal carriage of *Staphylococcus aureus*.

The prevalence of nasal carriage of *S. aureus* was 14.4% in our study. Our prevalence was lower than recognized in the overall population (20-30%) [3.5] and in those described in Africa and the World: 31.0% in Nepal [19], 29% in Gabon [13, 14], 21% in Ghana [15], 33.3% in Nigeria [16] and 19.1% in Spain [17]. We believe this low rate would be due to the different approaches used. Indeed, in most of these studies, the swabs were taken at different sites of the human organism associated with the nasal site (armpit, skin, perineum, etc.) while we have made only one nasal swab by voluntary participant.

In our study, we found that male were involved (14.8%) as much as female (14.1%) (p = 0.86). Kuehnert M et al. found a higher rate of carriage of *S. aureus* in male (37%) versus female (28%) [18]. Respectively, the age groups ≤ 15 and 16 - 25 years had higher *S.aureus* nasal carriage rates of 19.5% and 15.3%. A study conducted in the USA in 2006 reported a prevalence of 36.9% in subjects aged 1 to 19 years [18]. The latter rate appears to be more important when in reality this is not the case. Indeed, the distribution of participants by age groups is not identical. Rijal KR., N.Pahari et al found a rate higher than ours among school children ≤ 15 years of age in a school in Pokhara, Nepal, in 2008 [19].

Determinants of nasal carriage of *Staphylococcus aureus*.

In our study, we found that carriage of *S. aureus* remained predominant among unemployed (15.7%),

disadvantaged groups (17.8%) and primary level (25%). The association was not statistically significant. Tekaligukejele Ketema Bacha and found a high rate among primary school children and prisoners in Jimma in Ethiopia in 2009 [20]. Indeed, promiscuity and community life favor the carriage. Matheson et al. In USA found in their study that people who drank much more hot tea and coffee seemed to be protected from the nasal carriage of *S. aureus*[21].

Prevalence and determinants of MRSA nasal carriage.

In this study, we reported a prevalence of 5% of MRSA nasal carriage. The same prevalence was found in Greece in 2008 (5.5%) by Sdougkos et al. in children ≤ 15 years of age; Bilavsky et al. in 2012, found in its multi-center study in France, Spain, Israel and Italy a slightly higher prevalence of 8.7% in different rehabilitation centers [22,23]. However, in Kinshasa, Phaku et al. in 2014 found a prevalence of 4.3% in the community [8]; other authors in the United States and Nigeria found, respectively, a prevalence of 25% and 27.5%, 48% in Ethiopia and 56.1% in Pokhara [19,20,24]. This difference could be explained by the fact that not all of these studies were carried out on the same type of population (some in the under-15s, others in the senior citizens and in the urban as well as the rural community).

The present study shows that nasal carriage of MRSA is predominant in participants aged 16 to 35 years at 11%, from disadvantaged areas at 9.73%, primary and university level respectively at 7.14% and 6.78 %, carrier of tares (diabetes, chronic wound) respectively 4.55% and 20%; smoking at 12.5% with a history of hospitalization in the last three months preceding the swab at 6.67%and the presence of a healthcare worker in the household at 4.30%. However the association was not statistically significant with any of the aforementioned determinants p> 0.05). Egyir et al. in Ghana in 2014 did not find an association between MRSA nasal carriage and certain determinants including age, history of hospitalization, and presence of a healthcare worker in the household [15]. Lamaro-cardoso et al. in Brazil in 2009 found that the mother's level of education was a protective factor for MRSA nasal carriage in a study of 1192 children under the age of 5 years [25]. There is a disparity in the determinants of MRSA nasal carriage in different regions and in the type of population on which they were sought. This may be due to the difference in the interaction between *Staphylococcus aureus* and Immune system during its contact with the nasal mucosa of its host and certain practices that promote this contact.

CONCLUSION

The nasal carriage of *S. aureus* and MRSA is a real public health problem in Bukavu. The prevalence is almost similar to that described in Kinshasa but different from that described in some other regions of the world. The search for determinants of this carriage has not been conclusive to date (lack of statistical significance), whence the extension to other determinants not taken into account in this study (family contact, contact sport, drug addiction, belonging to a rural population, etc.) may be the subject of other studies to fully understand this phenomenon of health in Bukavu.

Otherwise, this study is the first to touch on this issue of community-based MRSA nasal prevalence, laying the foundation for future research on MRSA infections in the city of Bukavu

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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