Original Article

Clonal Lineage Diversity, Antibiotic Resistance, and Virulence Determinants Among Methicillin-Resistant and Methicillin-Susceptible *Staphylococcus aureus* Isolated from Nurses at a Teaching Hospital in Ilam, Iran: Successful Nares Decolonization by Mupirocin

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Abstract

Background: *Staphylococcus aureus* is known to be responsible for nosocomial infections, and the typing method was useful in managing the reservoir of bacteria. The main aim of this study was to determine the prevalence of *S. aureus* in the nares and hands of nurses working in Imam Khomeini hospital, Ilam, Iran, as well as to determine the clonal relatedness, antimicrobial susceptibility profiles, different virulence, and resistance determinants among these isolates. The evolution of mupirocin activity in the eradication of methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) colonization in the nares of the healthcare workers in Ilam, Iran, was also determined in this study. **Materials and Methods:** In this cross-sectional study, 80 nurses, auxiliary nurses, and service workers from Imam Khomeini Hospital were enrolled. MRSA, antibiotic susceptibility, and virulence determinants were evaluated. Then, the isolates were subjected to pulsed field gel electrophoresis (PFGE) and Staphylococcal cassette chromosome mec typing. **Results:** Our results demonstrated that 23% of isolates were MRSA. PFGE results demonstrated that pulsotypes A (3 out of 30; 10%) and J (3 out of 30; 10%), pulsotypes E (2 out of 30; 6.7%), M (2 out of 30; 6.7%), *P* (2 out of 30; 6.7%), and V (2 out of 30; 6.7%) were the most predominant pulsotypes, respectively. **Conclusion:** We cannot give conclusive suggestions about the correlation between nasal carriage and infections, but we suggest the monitoring of all healthcare workers annually, decontamination of their noses by using mupirocin and other antistaphylococcal agents, and also the washing of their hands at least every 2 h.

Keywords: Ilam, nasal carriage, pulsed field gel electrophoresis, *Staphylococcus aureus,* Staphylococcal cassette chromosome mec

Introduction

Recently, the rate of nosocomial infections due to *Staphylococcus aureus* has significantly increased, probably due to the increasing use of invasive methods in diagnosis and treatment and improper implementation of infection control procedures. *S. aureus* is a leading cause of bacteremia, septicemia, surgical wound infections, community-onset skin and soft tissue infections, right-sided endocarditis, osteomyelitis, and septic arthritis, and it is considered to be the second leading cause of nosocomial infections.[1-3] The colonization of *S. aureus* in the nasopharynx, body surface, and vagina of a human body appears to play a key role in causing most of other subsequent infections in different sites

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of the body.[4,5] The prevalence of persistent nasal carriage of *S. aureus* varies from 16 to 90% according to the population studied.[6-8]

Unfortunately, antibiotic resistance among *S. aureus* is rising, especially in hospital settings, and now the emergence of methicillin-resistant *S. aureus* (MRSA) is becoming a global problem.[8-10]

The most effective regimen for the eradication of *S. aureus* is the mupirocin ointment. Although there are some differences in the reports on the eradication rates of intranasal mupirocin ointments, there are insufficient data from Iran.[11,12]

The main aim of this study was to determine the prevalence of *S. aureus* in the nares and hands of nurses resident in Imam Khomeini hospital of Ilam, as well as to determine the clonal relatedness, antimicrobial susceptibility profiles, different virulence, and resistance determinants among these isolates.

Materials and Methods

Study population

During a 6-month period, between May and July 2015, 80 nurses and nurse assistants were resident in all the wards of the 200-bed Imam Khomeini Hospital, Ilam, Iran. All nurses that received antibiotics, intranasal corticosteroids, and those with recent upper respiratory tract infections were excluded.

Sampling and microbiological analysis

Sterile cotton wool swabs moistened with sterile normal saline were used to collect specimens from the anterior nares. Similar swabs of the palms and the web spaces of the hands were also taken at least 2 h after the hands were washed. Mannitol salt agar (MSA) plates were incubated at 35°C for up to 48 h. After incubation, yellow colonies that were subcultured on 5% of sheep blood agar were identified as *S. aureus* based on the colony morphology, gram stain, catalase and coagulase tests, and MSA fermentation. The cases in which nasal cultures yielded more than three *S. aureus* colonies were accepted as carriers.

Eradication of nasal carriage

For the nurses colonized with both MRSA and methicillin-susceptible *S. aureus* (MSSA), decolonization procedures were administrated with topical 2% mupirocin ointment twice for seven consecutive days (a week), using a cotton swab. Follow-up blood cultures were then obtained 1 week later, repeated once weekly and were discontinued if two consecutive blood cultures were negative.

Methicillin-resistant *Staphylococcus aureus* detection

Methicillin resistance was tested by disc diffusion with cefoxitin 30 µg disk and also by oxacillin agar screening with Mueller-Hinton agar containing 4% NaCl and 6 µg/ml oxacillin according to the Clinical Laboratory Standard Institute (CLSI) guidelines.[13]

Antimicrobial susceptibility testing

All the isolates were tested for their susceptibility to penicillin, vancomycin, imipenem, tetracycline, erythromycin, clindamycin, tigecyline, synercid, mupirocin, and linezolid by disk agar diffusion method according to the CLSI guidelines.[13] Inducible resistance to clindamycin and susceptibility to vancomycin were also detected according to the CLSI guidelines.[13] *S. aureus* ATCC 25923 was used as a quality control throughout the study.

DNA extraction

The total DNA of the isolates was extracted by phenol-chloroform method according to the previous reports.[14]

Detection of *mec*A and *nuc*A genes

All the isolates phenotypically detected as *S. aureus* were genotypically confirmed by the detection of the *nucA* gene with primers designed in this study to amplify a165 bp product. The primer sequences were as follows: *Nuc*A-F (5'-CGGGTCCTTTCAAAAAGGGGA-3') and *nuc*A-R (TCACCGTTTCTGGCGTATCA). All the phenotypically resistant strains were also subjected to a confirmatory polymerase chain reaction (PCR) for the detection of 297 bp *mec*A gene with primers designed in this study including *mec*A-F(5'-TGGCTCAGGTACTGCTATCCA-3') and *mec*A-R(5'-ACGTTGTAACCACCCCAAGAT-3'). PCR was carried out in a final volume of 25 µl containing 1X PCR buffer, 2 mM MgCl2, 0.2 mM dNTPs, 10pmol of primers, and 50 ng of DNA template. The PCR condition was as follows: a primary denaturation at 94°C for 5 min 30 cycles including 94°C for 45s, 58°C for 45s and 72°C for 45s and a final extension at 72°C for 5 min.

Detection of macrolide-resistant genes

Specific primers were used to amplify the resistance genes *erm*A, *erm*B, *erm*C, *lin*A, and *msr*A according to previously designed primers and protocols.[14,15]

Detection of aminoglycoside resistance genes

All the gentamicin resistance *S. aureus* isolates were examined for the existence of aminoglycoside resistance genes using the specific primers designed previously.[16,17]

Detection of enterotoxins, hemolysins, and exotoxins

For the detection of the five common enterotoxins (sea, seb, sec, sed, see), two exfoliative toxins (*et*A and *et*B) and Toxin-1-sendrom shock toxic (*tst*) were used according to the procedure described by Mehrotra *et al*.[18]

Pulsed field gel electrophoresis typing

The total DNA was extracted and pulsed field gel electrophoresis (PFGE) was performed according to the previous reports.[19-21] To identify PFGE polymorphism, all the samples were also analyzed by Gel Compare II version 6.6. The grouping method was used to deduce dendrogram from the matrix by the unweight pair group method using the arithmetic averages clustering technique after the calculation of similarities using the Dice correlation coefficient between each pair of

organisms; the PFGE patterns were distinguished at the 80% similarity level.

Staphylococcal cassette chromosome mec typing

Staphylococcal cassette chromosome mec (SCCmec*)* typing of MRSA isolates was performed as described previously.[22,23] MRSA reference strains COL (SCCmec Type I, *ccr*1), XU642 (EMRSA-16, SCCmec Type II, *ccr*2), WBG525 (EMRSA-1, SCCmec Type III, *ccr*3), WBG9465 (EMRSA-15, SCCmec Type IV, *ccr*2), and WIS (SCCmec Type V, *ccr*C) were used as controls.

Statistical analysis

Data were analyzed using SPSS 21 software. Qualitative variables were compared using the Chi-square or Fisher's exact test. All *P* values were two-sided with $P < 0.05$ and was considered as significant.

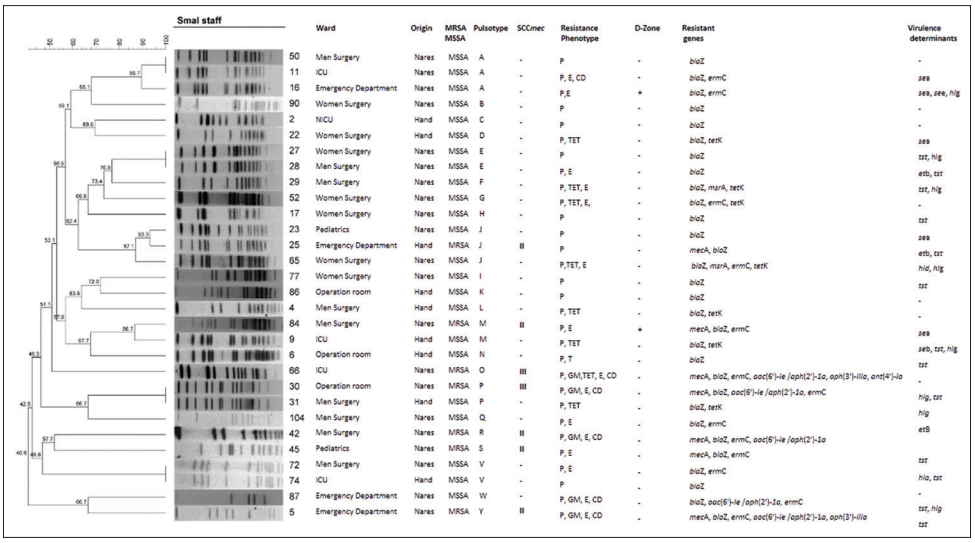
Results

In total, 80 persons were evaluated in this study among which 37% (30 out of 80) harbored S. *aureus* in their nares (21 out of 30; 70%) and hands (9 out of 30; 30%). The distribution of MRSA among these isolates was 23.3% (7 out of 30), among which 71.4% (5 out of 7) and 28.6% (2 out of 7) belonged to the SCCmec Types II and III, respectively. Men surgery ward having the isolation rate of 30% (9 out of 30) contained the highest rate of *S. aureus* isolated from the staff [Table 1]. The prevalence of *S. aureus* in the nares and hands among the nursing staff at Imam Khomeini Hospital according to the different wards, sex, occupation, and number of working years is shown in Table 1. PFGE analysis showed that 22 different PFGE pulsotypes were distributed among the isolates and pulsotypes A (3 out of 30; 10%) and J (3 out of 30; 10%). Pulsotypes E (2 out of 30; 6.7%), M (2 out of 30; 6.7%), *P* (2 out of 30; 6.7%), and V (2 out of 30; 6.7%) were the most predominant pulsotypes, respectively [Dendrogram 1]. All the isolates were sensitive to the mupirocin, linezolid, tigecycline, imipenem, vancomycin, and synercid, and all the isolates were resistant to penicillin. The rate of resistance to erythromycin, tetracycline, cefepime, oxacillin, clindamycin, and gentamycin was 46.6% (14 out of 30), 30% (9 out of 30), 23.3% (7 out of 30), 23.3% (7 out of 30), 20% (6 out of 30), and 16.6% (5 out of 30), respectively. Among the 9 isolates (9 out of 30; 30%) that were resistant to erythromycin and susceptible to clindamycin, only 2 (2 out of 9; 22.2%) showed D-zone phenomenon and were considered as inducible macrolide resistant. All the MRSA isolates showed resistance to penicillin and cefepime. Resistance rate of MRSA isolates to erythromycin, clindamycin, gentamicin, and tetracycline was 85.7% (6 out of 7), 57.1% (4 out of 7), 57.1% (4 out of 7), and 14.3% (1 out of 7), respectively. Three (42.9%) MRSA isolates showed coexistence resistance to penicillin, cefepime, gentamicin, erythromycin, and clindamycin. All the erythromycin- and clindamycin-resistant isolates contained the *erm*C (12 out of 14; 85.7%) and *msr*A (2 out of 14; 14.3%) genes. We could not detect any *erm*A or *erm*B gene among these resistant isolates. Our result also showed that all the tetracycline-resistant isolates harbored *tet*K gene. The distribution of the genes encoding aminoglycoside-modified enzymes among gentamicin-resistant isolates was *aac (6')-Ie/aph (2")-Ia* (5 out of 5; 100%), *aph (3')-IIIa* (2 out of 5; 40%), and *ant (4')-Ia* (1 out of 5; 20%). Distribution of the most prevalent virulence determinants among *S. aureus* isolates was *tst* (13 out of 30; 43.3%), *hlg* (7 out of 30; 23.3%), *sea* (5 out of 30; 16.6%), and *etB* (3 out of 30; 10%), respectively. All the staff that harbored *S. aureus* in their nares was checked after mupirocin treatment, and subsequently, the results showed that all the *S. aureus* isolates were eradicated in all the carriers. Distribution of different PFGE pulsotypes, resistance genes, and virulence determinants according to different wards is shown in Dendrogram 1.

Table 1: Prevalence of nasal and hand carriers among the nursing staff at Imam Khomeini Hospital

Carriers	Nasal and hand carrier		Noncarrier, *n* (%)	Total, *n* (%)
	MSSA, *n* (%)	MRSA, *n* (%)		
Ward				
Men surgery	7 (8.7)	2 (2.5)	6 (40)	15 (18.7)
Women surgery	7 (8.7)	0	8 (10)	15 (18.7)
ICU	3 (3.7)	1 (1.2)	5 (6.2)	9 (11.2)
Emergency department	2 (2.5)	2 (28.6)	10 (71.4)	14 (17.5)
Operation room	2 (6.1)	1 (14.3)	9 (75)	12 (15)
Pediatric	1 (3.0)	1 (14.3)	6 (75)	8 (10)
NICU	1 (3.0)	0	6 (85.7)	7 (8.7)
Total	33 (76.7)	7 (23.3)	50 (62.5)	80 (100)
Sex				
Men	11 (13.7)	2 (2.5)	22 (27.5)	35 (43.7)
Women	17 (21.2)	5 (6.2)	23 (28.7)	45 (56.3)
Total	28 (35)	7 (8.7)	45 (56.2)	80 (100)
Occupation				
Nurse	15 (18.7)	7 (8.7)	16 (20)	40 (50)
Auxiliary nurse	12 (15)	0	18 (22.5)	30 (37.5)
Service workers	1 (1.2)	0	9 (11.2)	10 (12.5)
Total	28 (35)	7 (8.7)	45 (56.2)	80 (100)
Age				
<30	15 (18.7)	4 (5)	19 (23.7)	38 (47.5)
30-40	8 (10)	3 (3.7)	17 (21.2)	28 (35)
>40	5 (6.2)	0	9 (11.2)	14 (17.5)
Total	28 (35)	7 (8.7)	45 (56.2)	80 (100)
Year of working				
1-3	7 (8.7)	2 (2.5)	11 (13.7)	20 (25)
3-5	13 (16.2)	4 (5)	8 (10)	25 (31.2)
>5	8 (10)	1 (1.2)	26 (32.5)	35 (45.8)
Total	28 (35)	7 (8.7)	45 (56.2)	80 (100)

MSSA: Methicillin-susceptible *Staphylococcus aureus*, MRSA: Methicillin-resistant *Staphylococcus aureus*, ICU: Intensive Care Unit, NICU: Neonatal Intensive Care Unit



Dendrogram 1: Pulsed-field gel electrophoresis dendrogram and Staphylococcal cassette chromosome mec types with molecular characterization of resistant and virulence determinants for 21 nasal and 9 hand carriers. Pulsed-field gel electrophoresis cluster was assigned to isolates having 80% or greater similarity from the dendrograms (A similarity cutoff of 80%)

Discussion

The nares that provided the good conditions for a long period of maintenance and multiplication of *S. aureus* are considered as the main sources of *S. aureus* among healthy people and it can spread to other sites of the body such as the hands.[24,25]

Healthcare workers (HCWs) are probably important in the transmission of *S. aureus*, but more frequently, they act as vectors rather than being the main source of *S. aureus* transmission.[25] There is an increasing concern on the spread of MRSA among healthcare workers, especially among those who work in wards with hospitalized patients. MRSA is now introduced as one of the most important agents of nosocomial infections, and therefore, various studies are necessary.[26]

The results of our study showed that 28.5% of the nursing staff harbored *S. aureus*. Similar reports from Iran and other parts of the world showed that the carrier rates ranged from 19.8%–36% to 6%–50%, respectively.[25,27] In contrast with the present study, Silvia *et al*. in Brazil and Panta *et al*. in Nepal have reported that more than 40% of the staff harbored *S. aureus* in their nares.[28,29] However, the result of Shakya *et al*. from Ethiopia was slightly closer to that of this study and reported that 25% of the staff carried *S. aureus* in their nares.[27]

Similar to the present study, most reports have not reported any significant correlation between the carrier rate and age, sex, or work experience.[30]

One of the most common infections in the surgery department is the skin infection after surgery procedures. Infections are mostly transferred to patients through hospital staffs who are the main carriers of the infection.[2] Nares and hands of these staff act as potential reservoir for the transmission of *S. aureus* to patients. *Staphylococcus aureus* colonization in the nares, in combination with inadequate implementation of hygiene principles, especially the regular washing of hands by staff is considered as the potential source of nosocomial infections.[2] In this study, the highest rate of *S. aureus* carrier was seen among nurses at the men surgery department and the result was higher than the reports from Sweden (19.5%) and Ethiopia (33.2%).[2]

The differences in the frequency of *S. aureus* carrier rate in the present study compared to others may be due to their variability in sample sizes, places, and healthcare control levels.[6,8] On the other hand, inadequate practice of hands' hygiene, increase in the number of admitted patients, prolongation of hospital stay, and trivial visits to the surgery ward may be considered as important causes of *S. aureus* colonization among the staffs in the surgery department.[4,11] Given the state of patients hospitalized in this department, a study of the *S. aureus* carriers is very important because the carrier state can be eradicated by applying simple methods such as frequently washing of hands with soaps and antiseptis and also by applying intranasal mupirocin ointments and other antistaphylococcal agents.

MRSA is nowadays a big problem in the medical setting. In agreement with several studies, the MRSA nasal carriage rate in the present study was 8.7%. The ranges of MRSA in our country and worldwide have been reported to be between 16%–83% and 4%–16%, respectively. It seems that our results are more similar to the reports from other countries other than Iran.[2,4-6,8,25,29] These differences may be due to differences in the sizes and qualities of the samples, techniques, and interoperation guidelines.[8]

Some studies have reported the increasing risk of infection among nursing staff significantly, and it also has been shown that MRSA multiply in the nares of nurses, at least two to three times higher than other healthcare staffs.[31]

We did not identify any significant correlation between the existence of MRSA and the sex of the health care staff, which

was comparable with other reports from Nigeria and USA.[32] We also could not find any significant correlation between *S. aureus* colonization and age, but this is in agreement with Khalili *et al*., the prevalence of MRSA was higher among the staff within the age group of 30–40 years.[30]

The control of *S. aureus* colonization among healthcare staff is very important because of the vulnerable patients in the hospital, and the staff should be sensitized about nosocomial infections. In addition to the administration of appropriate treatments, physicians should continuously investigate the carriers of resistant infections in the hospital environment and the known risk factors involved in *S. aureus* colonization. Thus, physicians and infection control committees should use the proper infection-control protocols to adequately reduce the transmission of bacteria inside the hospital.

In the present study, all the isolates were sensitive to mupirocin, linezolid, tigecycline, imipenem, vancomycin, and synercid. The susceptibility to these antibiotics showed that they were very effective against both MRSA and MSSA isolates in our hospital. There are different reports about susceptibility and resistance to these antibiotics in our country.[8,10,20] Till date, there is no report about resistance to mupirocin among *S. aureus* isolates in Ilam. However, some reports about resistance to mupirocin worldwide were released in recent years.[11] Nevertheless, after the application of mupirocin ointment, all the carriers were cured in the present study showing the effectiveness of mupirocin as an antistaphylococcal agent in our hospital.

In agreement with other reports, most of the MRSA isolates were resistant to gentamicin, erythromycin, and clindamycin.[9,14] There are some reports about the correlation between resistance to methicillin and resistance to other antibiotics such as macrolide and aminoglycoside, which raise a major problem in the treatment of MRSA isolates.[19,20] Unfortunately, more than 50% of the MRSA isolates in the present study were resistant to erythromycin, clindamycin, and gentamicin and harbored *erm*C, *msr*A and *aac* (6')-Ie/*aph* (2')-1a, *aph* (3')-IIIa, *ant* (4')-Ia. It seems that the application of these antibiotics in the treatment of MRSA isolates in our hospital is disputed, and therefore, it is essential to perform the antimicrobial susceptibility test. *Tet*K and *erm*C and *msr*A were distributed among all tetracycline and erythromycin and clindamycin-resistant isolates, which confirmed the significant role of these resistance determinates on the induced resistance to the above antibiotics.[33,34] Similar to other reports, we could not find any *erm*A and or *erm*B genes among macrolide-resistant isolates, which confirms the hypothesis that there may be a shift of *erm*A to *erm*C among macrolide-resistant *S. aureus* isolates worldwide.[33]

The role of aminoglycoside-modifying enzymes in encoding resistance to aminoglycoside was confirmed previously.[18,17] All the *S. aureus* isolates that were resistant to gentamycin harbored at least one aminoglycoside-modifying resistant gene. It seems that this gene still plays an important role in the resistance of *S. aureus* isolates to gentamicin in Iran.[9,17,19,33]

There are many reports about the distribution of different staphylococcal toxins worldwide. In the present study, the most prevalent enterotoxin was the *sea* (16.6%). This result was in agreement with a report from China but was inconsistent with some reports from Iran.[14,35]

The prevalence of exfoliative toxin was very low, but 40% of the isolates contained the *tst* gene. We just found two *etb*-positive MSSAs. These results were lower than other reports throughout the world.[14,36]

Although *hlg* was shown to be a prevalent hemolysin gene among *S. aureus* isolates and nearly all *S. aureus* isolates contain this gene, the results of the present study showed that only 26.6% of the isolates harbored this gene. Nevertheless, in comparison with other hemolysin genes, *hlg* still remained a more prevalent hemolysin among these isolates.[37-39]

In total, the virulence gene was mostly distributed among MSSA than MRSA. Similar studies showed that the acquisition of resistant genes alters secretion of virulence determinants, that means the expression of antibiotic-resistant genes reduces the expression of toxins.[37-39]

The results of the present study showed that 22 different pulsotypes were distributed among the *S. aureus* isolates, so we could not find any epidemic correlation between the isolates. These pulsotypes had different resistant genes and virulence determinants. We also found that three persons that worked at three distinct wards of men surgery, ICU, and emergency had the same pulsotype A and were MSSA but another pulsotype J that was identified in three persons working at the distinct wards of pediatrics, men surgery, and women surgery. One of them harbored MRSA on his hand. It seems that this person acquired this isolate from a surface or contact with another person because the hand is the main vector of transmission of *S. aureus* from a surface to the nose. The MRSA isolates pulsotype O-SCCmec Type III that was recovered from staff workers in ICU was very important because it showed resistance to gentamicin, tetracycline, erythromycin, and clindamycin and harbored different resistant genes. Considering the conditions of patients admitted to ICU, the presence of nurses who carry the MRSA in their noses calls for concern. Fortunately, by the application of 2% mupirocin ointment for 2 weeks, we successfully eradicated these carrier statuses, but annual screening and eradication of carrier statuses among health care workers are very essential. All the MRSA isolates had SCCmec Types II and III, which was in agreement with other reports that showed these SCCmec types occurred more frequently among healthcare staffs infected with MRSA.[8,14,40]

Monitoring, detecting, and disconnecting the circulation of these resistant clones in the hospital environment are very important and may reduce the chance of transmission of bacteria to patients and to other staffs.

Although we cannot give conclusive remarks about the correlation between nasal carriage and infection, we suggest annual monitoring of all healthcare workers and decontamination of their noses by using mupirocin and other

antistaphylococcal agents and also washing their hands at least every 2 h.

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Conflicts of interest

There are no conflicts of interest.

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