



Biochemical Characterization and Technological Potential of Lactic Acid Bacteria Strains Isolated from Dried and Salted Camel Meat (*Kadid*): A Traditionally Processed Meat Product of Southern Algeria

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ABSTRACT

Background: Camel meat is highly valued and commonly consumed in the Algerian desert. In southern Algeria, many families use fresh camel meat to produce dried and salted camel meat (*Kadid*). This study aims to evaluate the biochemical characterization and technological potential of lactic acid bacteria (LAB) isolated from *kadid*.

Methods: LAB strains were isolated from *kadid* and subjected to a series of physiological and biochemical tests, as well as evaluated for their technological potential.

Result: Fourteen LAB strains were isolated from *kadid*. Most of the LAB isolates are heat-resistant, capable of fermenting several carbohydrates and exhibit notable acidifying (2.5 to 3.6 g/L over 6 hours), lipolytic and proteolytic properties. However, they are unable to hydrolyze starch. Regarding virulence factors, they are γ -hemolytic and show resistance to at least 3, up to 14, out of 16 antibiotics tested. It can be concluded that dried and salted camel meat could be a source of lactic acid isolates with technological interest.

Key words: *Kadid*, Lactic acid bacteria, Technological potential.

INTRODUCTION

Lactic acid bacteria (LAB) are a group of microorganisms known for their role in food fermentation, contributing to the preservation, flavor and health benefits of food products (Christianah and Oyewumi, 2024). In Southern Algeria, *kadid*, a traditional dried and salted meat product made from various species, including camel meat (*Camelus dromedarius*), holds significant cultural and dietary value (Benyagoub and Bessadet, 2023; Benyagoub and Mammeri, 2023). However, despite its widespread consumption in Southern Algeria, the microbial communities in *kadid* made from camel meat remain largely unexplored, particularly with regard to LAB (Bendada *et al.*, 2022; Benyagoub *et al.*, 2022). These bacteria are believed to play a central role in the fermentation processes that influence the sensory qualities and safety of the product (Fadda *et al.*, 2010; Benyagoub *et al.*, 2024). However, their biochemical properties and potential for industrial applications have not been thoroughly investigated.

The production of *kadid* involves traditional methods that may not always ensure microbiological safety or consistency in quality (Benyagoub and Bessadet, 2023). Furthermore, the specific role and potential of LAB in enhancing the technological qualities of *kadid* have not been fully understood, which presents challenges in improving the product's preservation, flavor development and overall quality. There is also limited data on the strain diversity and biochemical characteristics of LAB isolated

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from this traditionally processed meat, which hinders the ability to fully harness their technological potential.

This study aims to characterize the technological potential of LAB strains isolated from *kadid*, providing insights into their possible future applications in the food industry.

MATERIALS AND METHODS

All experiments were carried out at Mohammed Tahri University of Bechar (Algeria), over a period of eight months, from December 2023 to July 2024.

Sampling

Kadid is a traditional dried meat product in Algeria, prepared by dehydrating meat from various animal species, including that of the one-humped camel (*Camelus dromedarius*) (Benyagoub and Mammeri, 2023; Benyagoub *et al.*, 2024). The preparation involves cutting fresh camel meat, salting it, seasoning it with a few spices and drying it at room temperature in the shade (Benyagoub and Bessadet, 2023). The *kadid* samples used in this study were sourced from families in the Bechar region (Fig 1).

Isolation of lactic acid bacteria from *kadid*

Lactic acid bacteria (LAB) were isolated following standardized protocols as described by Benyagoub (2022a, 2023a). The isolation was performed on M17 and MRS agar supplemented with 1% cycloheximide, after an enrichment step in M17 and MRS broths (Benyagoub, 2022b, Benyagoub *et al.*, 2022, Benyagoub, 2023b). Successive



Fig 1: *Kadid* product made from camel meat (Original, 2024) - Photo by E. Benyagoub.

subculturing was carried out on M17, MRS broth, followed by streaking onto M17 and MRS agars to purify the bacterial isolates. Final confirmation of the isolates was based on their macroscopic and microscopic characteristics.

Biochemical tests of LAB strains

Pure LAB isolates were identified through a series of tests, including, catalase test; Gram staining; gas production test by glucose fermentation; growth test at different temperatures (10°C, 22°C and 45°C) and growth at varying pH levels (pH 4 and 9). Additional test included growth in media with different NaCl concentrations (2%, 3%, 4% and 9%); acetoin production on Clark and Lubs medium, the citrate test and esculin hydrolysis on esculin agar medium (Damayanti *et al.*, 2014; Zaaraoui *et al.*, 2021; Benyagoub, 2022a; Benyagoub, 2023a).

Antibiotic susceptibility testing (AST)

The antibiotic susceptibility of the isolated LAB strains was tested using the Kirby-Bauer disc diffusion method (Delgado *et al.*, 2005; Benyagoub, 2023a). The results were interpreted according to the European Food Safety Authority (EFSA, 2012).

In this study, the following antibiotics were used: Amoxicillin (AMX 25 µg), sulfamethoxazole/trimethoprim (COT 25 µg), erythromycin (E 15 µg), tetracycline (TE 30 µg), penicillin (P 10 IU), chloramphenicol (C 30 µg), ciprofloxacin (CIP 5 µg), gentamicin (GEN 10 µg), ofloxacin (OF 5 µg), pefloxacin (PEF 5 µg), streptomycin (S 30 µg), amoxicillin/clavulanic acid (AUG 30µg), ampicillin (AMP 10 µg), oxacillin (OX 5 µg), vancomycin (VA 30 µg) and clindamycin (CM 2 IU). The Petri dishes were incubated anaerobically at 30°C for 24 hours.

Characterization of the technological potential of isolated LAB strains

This study focused on assessing the technological characteristics of the isolated LAB (Benyagoub, 2022a;

Table 1: Microscopic and macroscopic characteristics of isolated LAB strains.

LAB strains	Characters	Motility	Cell arrangement mode	Gram-type	Form	Macroscopic appearance
Lb. MRS A 30			Single/pairs		Bacilli	
S. MRS A 44			Chains		Cocci	
S. MRS C 30			Chains		Cocci	
Lb. MRS D 30			Single/pairs		Bacilli	
S. MRS D 44			Chains		Cocci	
Lb. MRS E 30		Non-motile bacteria	Single/pairs	Positive	Bacilli	Circular, whitish, raised and smooth colonies
Lb. MRS F 30			Single/pairs	Gram	Bacilli	
Lb. MRS G 30			Single/pairs	bacteria	Bacilli	
S. MRS H 30			Chains		Cocci	
S. MRS TS 30			Chains		Cocci	
S. VF b 30			Chains		Cocci	
S. VF J 30			Chains		Cocci	
S. M17 C 44			Chains		Cocci	
S. M17 D 44			Chains		Cocci	

LAB: Lactic acid bacteria, S: Cocci LAB strains, Lb: Bacilli LAB strains.

Table 2: Physiological and biochemical characteristics of LAB strains isolated from *kadid*

Lactic acid bacteria	Catalase test	Metabolic types	pH		T (°C)					NaCl (%)				Thermo resistance test	VP test	ADH	Hemolysis test	Citrate testing
			4	7	9	10	22	45	2	3	4	9						
Lb. MRSA 30	-	Hetero-F	+	+	+	-	+	-	+	+	+	-	+	+	-	-	γ-h	-
S. MRSA 44	-	Hetero-F	-	+	+	-	+	+	+	+	+	+	+	+	-	+	γ-h	-
S. MRSC 30	-	Hetero-F	+	+	+	-	+	+	+	+	+	+	+	-	-	-	γ-h	-
Lb. MRSD 30	-	Homo-F	-	+	+	-	+	-	+	+	+	+	+	-	-	-	γ-h	-
S. MRSD 44	-	Hetero-F	-	+	+	-	+	+	+	+	+	-	+	+	-	+	γ-h	+
Lb. MRSE 30	-	Hetero-F	-	+	+	-	+	-	+	+	+	-	+	-	-	-	γ-h	-
Lb. MRSF 30	-	Hetero-F	+	+	+	+	+	-	+	+	+	+	+	+	-	+	γ-h	+
Lb. MRS G 30	-	Hetero-F	+	+	+	-	+	+	+	+	+	-	+	+	-	-	γ-h	-
S. MRSH 30	-	Hetero-F	+	+	+	+	+	+	+	+	+	-	+	+	-	-	γ-h	-
S. MRST 30	-	Homo-F	+	+	+	+	+	+	+	+	+	-	+	-	-	+	γ-h	+
S. VF b 30	-	Hetero-F	-	+	+	+	+	+	+	+	+	-	+	+	-	+	γ-h	-
S. VF J 30	-	Hetero-F	-	+	-	+	+	+	+	-	+	-	+	-	-	+	γ-h	-
S. M17 C 44	-	Hetero-F	-	+	+	+	+	+	+	+	+	-	+	+	-	+	γ-h	-
S. M17 D 44	-	Hetero-F	+	+	+	+	+	+	+	+	+	-	+	+	-	+	γ-h	-

T (°C): Temperature in (°C), NaCl (%): The concentration of sodium chloride in %, Homo-F: Homo-fermentative, Hetero-F: Hetero-fermentative, VP test: Voges-proskauer test, ADH: Arginine dihydrolase test, γ-h: γ-hemolysis.

Benyagoub, 2023a), including heat resistance at 60°C; sugar assimilation tests; acidifying power (measured by titratable acidity); proteolytic activity; lipolytic activity and amylolytic activity.

As part of the safety tests, the hemolytic activity (α, β, or γ-hemolysis) was evaluated through a phenotypic hemolysis test on blood agar medium.

RESULTS AND DISCUSSION

Isolation and phenotypic identification of LAB strains

Through the analysis of phenotypic characteristics (both macro- and microscopic) and the negative catalase test results, 14 lactic acid bacteria strains were isolated from dried and salted camel meat (*Kadid*) (Table 1).

Physiological profile of LAB strains isolated from *kadid*

The physiological and biochemical characteristics of the LAB strains are detailed in Table 2.

All of these isolates were Gram-positive and catalase-negative bacteria, characteristics that allowed their classification as lactic acid bacteria (Joffin and Leyral, 1996). Conventional phenotypic identification of lactic acid bacteria, based on morphological, physiological and biochemical tests, revealed that all isolated LAB strains were able to grow at 22°C. Additionally, 10 out of 14 strains grew at 45°C. Seven and twelve strains were tolerant to acidic pH 4 and basic pH 9, respectively. Among the strains, 12 were heterofermentative and 2 were homofermentative.

In a hypersaline medium with varying NaCl concentration, the results showed that all LAB isolates were tolerant to media containing 2%, 3% and 4% NaCl. However, only four strains (S. MRSA 44, S. MRS C 30, Lb. MRS D 30 and Lb. MRS F 30) were able to grow in a medium with 9% NaCl.

LAB strains are the most common microbes capable of digesting lactose, converting it into lactic acid, which in turn lowers the gastrointestinal pH (Krishna *et al.*, 2023, Razni *et al.*, 2024). However, pH is a critical factor for lactic acid bacteria to function as probiotics. The ability of LAB strains to survive at different pH levels enables them to reach the small intestine and colon, where they contribute to balancing the intestinal microflora (Mahajan *et al.*, 2022).

In the hemolysis test, none of the isolated LAB strains showed a hemolytic reaction (either α or β hemolysis) when tested on sheep blood. When selecting strains for use as starter or adjunct cultures in dairy products, the lack of certain activities should be considered a critical factor. This is because it offers valuable insights into the strain's potential pathogenicity (FAO/WHO, 2002; Zaaraoui *et al.*, 2021). Additionally, Suvorov *et al.* (2019), emphasize that LAB strains free from virulence factors, toxins and biogenic amines, can be safely used as probiotics.

Technological potential of LAB strains isolated from *kadid* fermentation of sugars

Table 3 shows the fermentation results for the various sugars tested.

Table 3: Carbohydrate metabolism of LAB strains isolated from *Kadid*.

Fermentation of sugars LAB	Galactose	Raffinose	Maltose	Cellobiose	Sorbose	Melibiose	Starch	Gellulose	Xylose	Fructose	Mannitol	Esculin	Lactose	Arabinose	Inositol	Sucrose	Amygdalin	Glucose	Rhamnose	Mannose
Lb. MRS A 30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S. MRS A 44	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S. MRS C 30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lb. MRS D 30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S. MRS D 44	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lb. MRS E 30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lb. MRS F 30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lb. MRS G 30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S. MRS H 30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S. MRS TS 30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S. VF b 30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S. VF J 30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S. M17 C 44	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
S. M17 D 44	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

S: Cocci LAB strains, Lb: Bacilli LAB strains; (+): Positive reaction; (-): Negative reaction (No fermentation).

Although the results varied among the lactic acid bacteria (LAB) strains, all were capable of fermenting a range of simple saugars, disacharides and heterosides, including lactose, glucose, arabinose, sucrose and amygdalin. The strains' ability to ferment these sugars is linked to their production of various enzymes (Suvorov *et al.*, 2019; Razni *et al.*, 2024). However, only Lb. MRS E 30 could ferment xylose, while none of the strains fermented sorbose, starch, or cellulose. This is due to the presence or absence of specific enzymes and transport systems, such as sorbose reductase, amylase, cellulase, xylose isomerase, or xylose kinase (Gunkova *et al.*, 2021).

Regarding esculin hydrolysis, the results indicated that all the isolated LAB strains were able to degrade esculin, except for three strains: Lb. MRS D 30, Lb. MRS G 30 and S. MRS H 30.

Acidifying power

Fig 2 shows the evolution of titratable acidity over 6 hours of incubation.

Fig 2 shows that the production of lactic acid varied from medium to high across different strains. Notably, the following cocci LAB strains-S. M17 D 44, S. M17 C 44, S. MRS D 44, S. MRS VFb 30, S. MRS VFj 30 and S. MRSA44- demonstrated significant acidifying ability, producing between 2.5 and 3.6 g/L of lactic acid after 6 hours of incubation, which was higher compared to the bacilli LAB strains. These results corroborate those obtained by Kandler and Weiss (1986); Belhamra (2017) who reported that lactobacilli exhibit a slow acidification profile. This may explain the lower acidifying capacity of the lactobacilli isolated from *kadid*.

The acidifying activity of each strain depends on its ability to break down and assimilate nutrients, as well as the presence or absence of transport systems for these nutrients (Albenzio *et al.*, 2001). Additionally, rapid growth and acidification capacity are essential criteria for selecting an LAB starter, as they help prevent spoilage and the proliferation of pathogens (Boubakri *et al.*, 2022).

For probiotics to deliver health benefits, they must endure various physical and chemical challenges, such as exposure to acid and bile in the small intestine. Thus, evaluating the bile and acid tolerance of lactic acid bacteria intended for probiotic use is essential (Timothy and Oyedokun, 2021). Additionally, LAB strains are capable of fermenting lactose into lactic acid (Ketrouci *et al.*, 2021).

Amylolytic, proteolytic and lipolytic activity testing

Table 4 presents the results for the amylolytic, proteolytic and lipolytic activities of LAB strains isolated from *Kadid*.

The physiological and technological characteristics of the isolated LAB strains showed that, out of 14 strains, 5 were protease and gelatinase producers, with proteolysis zone diameters ranging from 10 to 12 mm. Additionally, all strains exhibited lipolytic activity, while none demonstrated amylolytic activity. According to Gunkova *et al.* (2021), lactic acid bacteria are unable to ferment polysaccharides like

starch due to the absence of the required hydrolytic enzymes, a finding supported by Giraud *et al.* (1994), who reported that amylase synthesis is a rare characteristic of LAB strains, which aligns with our results.

The proteolytic activity is a key desirable trait for comple-mentary cultures, as it can contribute to aroma production, flavor enhancement, cell growth and increased inhibitory activity in the fermented final product (Zaaraoui *et al.*, 2021). Shirai *et al.* (2001) and François *et al.* (2007) showed that lactic acid bacteria are unable to synthesize several amino acids; nonetheless, they are well adapted to a protein-rich environment due to a complex bacterial proteolytic system. It is also important to note that species with high acidifying activity do not

necessarily exhibit the highest proteolytic activity (Zaaraoui *et al.*, 2021).

The lipolytic activities of microorganisms are important during the maturation stages of certain food products, as these activities generally contribute to the development of various flavors (Ortiz De Apodaka *et al.*, 1993; Krockel, 2013).

Antibiotic susceptibility testing of isolated LAB strains

Table 5 displays the antibiotic resistance profiles of the LAB strains.

The antibiotic resistance results varied among the strains, but we were able to identify a common profile across the LAB strains. The antibiotic susceptibility profile

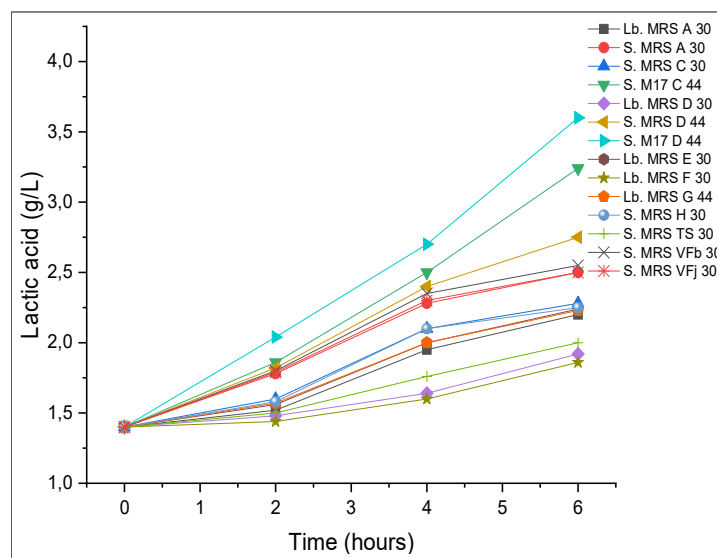


Fig 2: Titratable acidity kinetics of skim milk as a function of time ($\text{g.L}^{-1}.\text{h}^{-1}$).

Table 4: Technological suitability of LAB strains isolated from *kadid*.

Technol. A LAB	Proteolytic activity (mm)	Gelatin hydrolysis	Lipolytic activity	Amylolytic activity
Lb. MRS A 30	10 (+)	+	+	-
S. MRS A 44	12 (+)	+	+	-
S. MRS C 30	- (PZ < 5 mm)	-	+	-
Lb. MRS D 30	- (PZ < 5 mm)	-	+	-
S. MRS D 44	10 (+)	+	+	-
Lb. MRS E 30	- (PZ < 5 mm)	-	+	-
Lb. MRS F 30	- (PZ < 5 mm)	-	+	-
Lb. MRS G 30	- (PZ < 5 mm)	-	+	-
S. MRS H 30	- (PZ < 5 mm)	-	+	-
S. MRS TS 30	- (PZ < 5 mm)	-	+	-
S. VF b 30	- (PZ < 5 mm)	-	+	-
S. VF J 30	- (PZ < 5 mm)	-	+	-
S. M17 C 44	12 (+)	+	+	-
S. M17 D 44	12 (+)	+	+	-

Technol. A: Technological ability, LAB: Lactic acid bacteria, PZ: Diameter of the proteolysis zone (in mm); S: Cocci LAB strains, Lb: Bacilli LAB strains, (-): Negative reaction, (+): Positive reaction.

Table 5: Multidrug-resistant profiles of LAB strains isolated from *kadid*.

LAB strains	Antibiotic resistance profiles
Lb. MRS A 30	AUG-CM-E-PEF-TE-COT-AMP-OX-AMX-C-VA-CIP-GEN-S <i>Susceptible</i> to P-OF
S. MRS A 44	AUG-CM-PEF-COT-OX <i>Susceptible</i> to E-TE-AMP-P-AMX-OF-C-VA-CIP-GEN-S
S. MRS C 30	CM-PEF-TE-COT-AMP-OX-AMX <i>Susceptible</i> to AUG-E-P-OF-C-VA-CIP-GEN-S
Lb. MRS D 30	PEF-COT-AMP-OX-AMX-OF-VA-GEN <i>Susceptible</i> to AUG-CM-E-TE-P-C-CIP-S
S. MRS D 44	PEF-COT-OX <i>Susceptible</i> to AUG-CM-E-TE-AMP-P-AMX-OF-C-VA-CIP-GEN-S
Lb. MRS E 30	E-PEF-COT-AMP-OX-AMX-VA <i>Susceptible</i> to AUG-CM-TE-P-OF-C-CIP-GEN-S
Lb. MRS F 30	AUG-CM-E-TE-COT-AMP-OX-AMX-C-GEN <i>Susceptible</i> to PEF-OF-P-VA-CIP-S
Lb. MRS G 30	AUG-PEF-COT-AMP-OX-AMX-OF-VA <i>Susceptible</i> to CM-E-TE-P-C-CIP-GEN-S
S. MRS H 30	AUG-PEF-TE-COT-AMP-OX-AMX-OF-VA <i>Susceptible</i> to CM-E-P-C-CIP-GEN-S
S. MRS TS 30	AUG-PEF-TE-COT-AMP-OX-AMX-OF-VA-GEN-S <i>Susceptible</i> to CM-E-TE-P-C-CIP
S. VF b 30 and S. VF J 30	AUG-PEF-COT-OX-VA <i>Susceptible</i> to CM-E-TE-AMP-P-AMX-OF-C-CIP-GEN-S
S. M17 C 44	CM-PEF-COT-OX-AMX-VA-GEN <i>Susceptible</i> to AUG-E-OF-TE-AMP-P-C-CIP-S
S. M17 D 44	AUG-CM-E-PEF-COT-OX-S <i>Susceptible</i> to TE-AMP-P-AMX-OF-C-VA-CIP-GEN

LAB: Lactic acid bacteria.

of the bacilli LAB strains showed resistance to *AMP-AMX-OX-COT-VA* and sensitivity to *P-CIP-S*. In contrast, the cocci LAB strains exhibited resistance to *PEF-COT-OX* and sensitivity to *P-E-C-CIP-OF*.

According to Mahajan *et al.* (2022), antibiotic susceptibility should be considered an essential criterion for the safety assessment of probiotics. This aligns with the findings of other researchers, such as Botes *et al.* (2008), Abid (2015), Makete *et al.* (2017), Zommiti *et al.* (2017) and Sharma *et al.* (2021), who share the same view that lactic acid bacteria are sensitive to penicillin. Additionally, they reported the natural resistance of a significant range of lactic acid bacteria to antibiotics, specifically pefloxacin and ciprofloxacin, which are considered intrinsic resistance traits in bacilli LAB strains, such as *Lactobacillus* spp.

Based on the results of antibiotic resistance patterns, growth characteristics, acidification ability, NaCl tolerance, moderate protein hydrolysis and the absence of virulence factors (such as hemolysis), the isolated LAB strains may be considered potential candidates for use in the food and biotechnology fields.

CONCLUSION

Fourteen LAB strains, including five bacilli and nine cocci, were isolated from *kadid*. Six cocci strains, particularly S. M17 D 44, demonstrated strong acidifying activity and promising technological characteristics. Antibiotic resistance testing showed resistance to 3-14 out of 16 antibiotics, while all isolates were γ -hemolytic, indicating potential as probiotic starter cultures. These strains offer significant potential for biopreservation and other technological applications.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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