Detection of bactericidal activity of camel's milk compared with raw and processed cow's milk against pathogenic bacteria

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Objective
The aim of this study was to determine the antimicrobial activity of camel's milk compared with three types of raw and processed cow's milk as a natural safe way to overcome pathogenic bacteria instead of using chemotherapy, which leads to the phenomenon of microbial resistance.
Materials and methods
A total of 16 milk samples were collected; four samples from four healthy camels (4 years old), four fresh cow milk samples, four pasteurized milk samples, and four packed buttermilk samples. The camel's and cow's milk were boiled before the investigation. Bacterial isolation from these samples was carried out on specific media. The antibacterial activity for each milk type was assessed against seven Gram-positive and Gram-negative bacterial strains previously examined for its multidrug resistance activity. The minimal inhibitory concentration and minimal bactericidal concentration of each milk type was also determined. Transmission electron microscope was used for the highly affected bacterial strain for each of the milk types.
Results and conclusion
The four milk sample types were free from bacterial contamination. They all possessed antimicrobial activity but not for all seven examined bacterial strains. The strains affected were *Escherichia coli* and *Klebsiella pneumoniae* by camel's milk and *E. coli* and *Streptococcus faecalis* by the other three cow's milk types. Pasteurized milk and buttermilk showed the higher effects. Minimal inhibitory concentration and minimal bactericidal concentration for the milk types ranged between 10 and 80%. Transmission electron microscope studies on the bacteria affected revealed damage in bacterial cell wall and disturbance in cell protein content. It can be concluded that milk can be used *in vitro* as a natural safe way to overcome some pathogenic bacteria instead of using antibiotics.

Keywords:
antimicrobial activity, buttermilk, camel's milk, minimal inhibitory concentration, pasteurized, transmission electron microscope

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Introduction

The milk of mammals is protected to different extents against microbial contaminations by natural inhibitory systems, including the lactoperoxidase/thiocyanate/hydrogen peroxide system, lactoferrins, lysozyme, immunoglobulins, and free fatty acids [1–3]. The concentration and the activity of each of these antimicrobial systems/substances depend on the animal species and on the stage of lactation [4].

In particular, the levels of lysozyme and lactoferrins in camel milk are reported to be two and three times higher than those in cow's milk [2,5]. Camel milk can be kept for longer periods compared with cow's milk when refrigerated, and even with the desert heat it does not spoil shortly [6].

Camel milk also has valuable nutritional properties as it contains a high proportion of antibacterial substances and a higher concentration of vitamin C in comparison with cow milk [7,8]. In average, camel milk contains more proteins and whey protein compared with cow milk [9].

The ability of camel milk to inhibit the growth of pathogenic bacteria and its relation to whey lysozyme has been demonstrated in previous study [10].

Pasteurization of milk has been practiced as the most effective method of reducing the risk for contamination and spreading of disease. Although pasteurized milk is expected to have a shelf life of 14–20 days, the shelf life of pasteurized milk stored at ambient temperature is dependent upon the efficiency of pasteurization process [11].

This study was conducted to examine the purity of boiled raw camel's and cow's milk, pasteurized cow's milk, and packed buttermilk from bacterial contaminants and to determine the antimicrobial activity of these types of milk, which is considered as a natural safe way to overcome pathogenic bacteria instead of using chemotherapy, which leads to the phenomenon of microbial resistance.

Materials and methods

Milk sample preparation

Camel's milk

Camel's milk samples were collected early in the morning from camel farm in Kerdasa, Giza, Egypt. Milk was collected from four healthy camels (4 years old) by means of hand milking in sterile screw bottles and kept in cool boxes until transported to the laboratory.

Cow's milk

Raw, pasteurized, and packed buttermilk samples were collected from a dairy shop, Giza, Egypt. Milk was collected (four samples each) in sterile screw bottles and also kept in cool boxes until transported to the laboratory.

Both camel's milk and raw cow's milk were boiled at 100°C for 10 min before usage.

Isolation and identification of bacteria from the examined milk types

Isolation and identification of Gram-negative bacterial pathogens were carried out following aseptic sampling techniques [12,13]. Briefly, a loopful (0.01 ml) of milk sample was streaked on 7% blood agar (base blood agar; Oxoid, Germany) and incubated aerobically at 37°C. The plates were checked for bacterial growth after 24, 48, and 72 h to rule out slow-growing microorganisms, and subcultured on blood agar at 37°C for 24 h to obtain pure culture. A single colony from a pure culture was then subjected to Grams' stain to observe morphological characteristics and transferred to brain heart infusion and MacConkey agar to be grown for further analysis. Identification of bacteria to the species level was carried out using a conventional method of biochemical reaction.

Bacterial strains

The following bacterial strains were used (ATCC, USA): *Pseudomonas aeruginosa* (ATCC: 27853), *Proteus vulgaris* (ATCC: 13315), *Salmonella typhi* (ATCC: 14028), *Escherichia coli* (ATCC: 25922), *Klebsiella pneumoniae* (ATCC: 13883), *Staphylococcus aureus* (ATCC: 25923), and *Streptococcus faecalis* (ATCC: 29212). They were subcultured on Nutrient agar (Lab M, UK) and incubated aerobically at 37°C. The organisms were maintained in the laboratory on nutrient agar slopes at 4°C [14].

Antimicrobial susceptibility test

Antimicrobial susceptibility was assessed using the disk diffusion method, and the results were interpreted using the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards, NCCLS) break point criteria [15]. Antimicrobial drugs included the penicillin group (amoxicillin 30 μg), the glycopeptide group (vancomycin 30 μg), aminoglycosides (amikacin 30 μg), cephalosporin (cephradine 30 μg), and carbapenem (imipenem 10 μg). Multidrug resistant strains were detected and defined as strains that were nonsusceptible to at least one agent in three or more antimicrobial with group [16].

Screening for the milk's type antibacterial activity

Antibacterial activity was tested using the agar well diffusion method on nutrient agar media using milk dilutions (10–100%). The wells were made using a sterile borer and were filled with 0.45 μl of each concentration. The antibacterial assay plates were incubated at 37°C for 24 h. The diameter of the zones of inhibition around each well was taken as a measure of the antibacterial activity.

Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded [17].

Minimal inhibitory concentration and minimal bactericidal concentration

The minimal inhibitory concentration (MIC) was determined as the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in a broth dilution susceptibility test. The minimal bactericidal concentration (MBC) was determined, after determining the results for the MIC, as the lowest concentration that achieved a 99.9% decrease in viable bacteria. The MBC can be determined from broth dilution MIC tests by subculturing on agar medium without disinfectant and incubating at 35°C for 16–20 h according to the macrodilution method described by the NCCLS [18]. The experiments were conducted in triplicate.

Transmission electron microscope examination

Conventional transmission electron microscope (TEM) is frequently selected to visualize the ultrastructural damage on both cell wall and cytoplasmic membrane of entire microbes [19].

At ultrastructural level, a simple negative staining for TEM of bacterial cells can report evidence on the mechanism of membrane disruption by antimicrobial proteins and peptides [20]. The highly affected bacterial strain was scanned to determine the milk type that had greater effect on the bacterial structure. Ultrathin sections of ~75–90 μm thickness were prepared and stained with uranyl acetate and lead citrate. This was examined in TEM lab FA-CURP, Faculty of Agriculture, Cairo University, using a TEM (JEM-1400 TEM; Jeol, Japan). Images were captured using a CCD camera with 1632 × 1632 pixels format as side mount configuration manufactured; this camera uses a 1394 fire wire board for acquisition.

Results

All examined milk sample types were free of microbial contamination when cultured to isolate bacterial contaminants. These samples were then ready to investigate its antibacterial effect against the seven tested bacterial strains.

Antimicrobial susceptibility test was performed for the seven tested bacterial strains (Table 1).

Table 1 shows that only *St. faecalis* was the multidrug resistant strain. All tested strains were sensitive to the carbapenem group (imipenem). It was also noted that 85.7% of the strains were sensitive to the aminoglycoside and cephalosporin groups, whereas 71.4% of the strains were sensitive to the penicillin group. However, only 57.1% of them were sensitive to the glycopeptide group.

The agar well diffusion method was carried out to investigate the effect of the different milk types against the tested bacterial strains (Tables 2–5). The camel's milk was only effective on *E. coli* and *K. pneumoniae*, whereas raw cow's milk, pasteurized milk, and packed buttermilk were effective only on *E. coli* and *St. faecalis*.

The 40 and 70% concentrations of camel's milk were the highly effective concentrations against *E. coli* and *K. pneumoniae*, respectively (Table 2).

The 40 and 90% concentrations of cow's milk were the most effective concentrations against *E. coli* and *St. faecalis*, respectively (Table 3).

Table 1 Antibiogram of the tested strains to different antibiotics and detection of multidrug resistant ones

Bacterial strains	Antibiotics [mean diameter of inhibition zone (mm)]				
	Penicillin group	Carbapenem group	Cephalosporin group	Aminoglycoside group	Glycopeptide group
	Amoxil	Imipenem	Cephradine	Amikacin	Vancomycin
Pseudomonas aeruginosa	30	44	33	28	28
Proteus vulgaris	28	32	25	28	16
Salmonella typhi	12	38	28	22	00
Escherichia coli	23	32	30	18	00
Klebsiella pneumoniae	22	18	35	25	18
Staphylococcus aureus	32	40	15	28	18
Streptococcus faecalis	00	25	13	00	10

Table 2 Determination of the effect of camel's milk on the tested bacterial strains

Bacterial strains	Milk dilutions [inhibition zone diameter (mm)]									
	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
Pseudomonas aeruginosa	00	00	00	00	00	00	00	00	00	00
Proteus vulgaris	00	00	00	00	00	00	00	00	00	00
Salmonella typhi	00	00	00	00	00	00	00	00	00	00
Escherichia coli	20	18	22	25	15	16	18	18	21	20
Klebsiella pneumonia	00	00	00	00	00	00	22	20	20	16
Staphylococcus aureus	00	00	00	00	00	00	00	00	00	00
Streptococcus faecalis	00	00	00	00	00	00	00	00	00	00

Table 3 Determination of the effect of raw cow's milk on the tested bacterial strains

Bacterial strains	Milk dilutions [inhibition zone diameter (mm)]									
	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
Pseudomonas aeruginosa	00	00	00	00	00	00	00	00	00	00
Proteus vulgaris	00	00	00	00	00	00	00	00	00	00
Salmonella typhi	00	00	00	00	00	00	00	00	00	00
Escherichia coli	16	18	18	22	20	18	16	16	20	20
Klebsiella pneumonia	00	00	00	00	00	00	00	00	00	00
Staphylococcus aureus	00	00	00	00	00	00	00	00	00	00
Streptococcus faecalis	00	00	00	00	00	00	00	00	16	15

All concentrations of pasteurized milk affected *St. faecalis* and *E. coli* (Table 4). The 30% concentration was the highly effective concentration on both organisms.

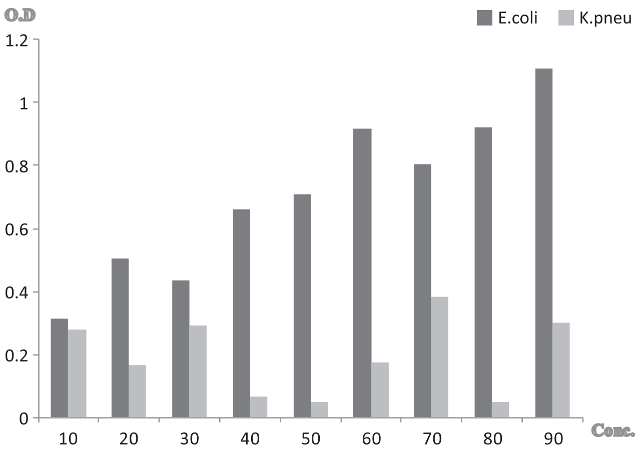
All concentrations of buttermilk affected *St. faecalis* and *E. coli*, especially at concentrations 20 and 90%, respectively (Table 5).

From Table 2, it is clear that camel's milk affected *E. coli* and *K. pneumonia*. The growth rate of both organisms were also measured using a spectrophotometer (optical density), and then the MIC and MBC were determined for each of them (Fig. 1).

K. pneumoniae was highly affected by camel's milk compared with *E. coli* (Fig. 1). The 10% concentration was the MIC of camel's milk to *E. coli*, whereas it was 80% for *K. pneumoniae*. To determine the MBC for both of the MIC, they were subcultured on nutrient broth media and it was found that the MBC was 10% for camel's milk to *E. coli* and 80% for *K. pneumoniae*.

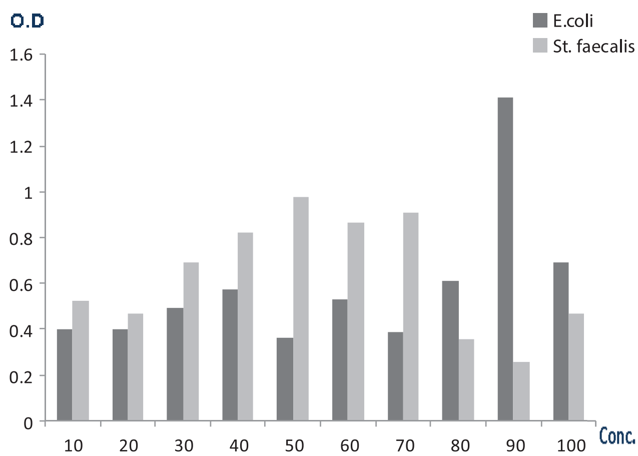
Raw cow's milk, pasteurized milk, and packed buttermilk affected only *E. coli* and *St. faecalis*, and so they were chosen to investigate the effect of these three milk types on them measured using a spectrophotometer (optical density), and the MIC and MBC for both of them were also determined (Tables 3–5 and Figs 2–4).

Figure 1



Determination of the effect of camel's milk on *Escherichia coli* and *Klebsiella pneumoniae* at different concentrations to detect the minimal inhibitory concentration. OD, optical density.

Figure 2



Determination of the effect of raw cow's milk on *Escherichia coli* and *Streptococcus faecalis* at different concentrations to detect the minimal inhibitory concentration. OD, optical density.

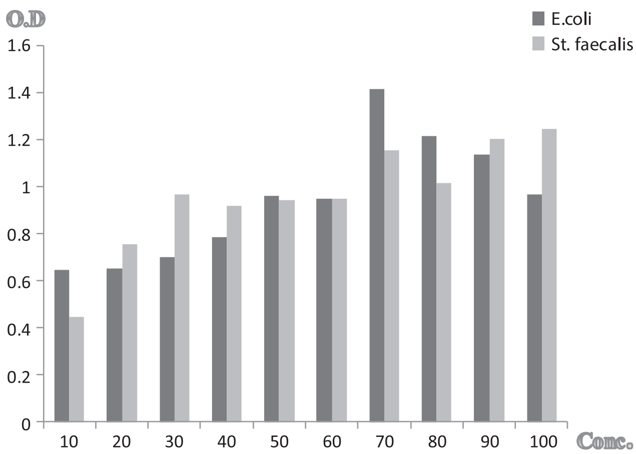
Table 4 Determination of the effect of pasteurized milk on the tested bacterial strains

Bacterial strains	Milk dilutions [inhibition zone diameter (mm)]									
	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
Pseudomonas aeruginosa	00	00	00	00	00	00	00	00	00	00
Proteus vulgaris	00	00	00	00	00	00	00	00	00	00
Salmonella typhi	00	00	00	00	00	00	00	00	00	00
Escherichia coli	25	21	32	30	26	24	24	22	22	30
Klebsiella pneumonia	00	00	00	00	00	00	00	00	00	00
Staphylococcus aureus	00	00	00	00	00	00	00	00	00	00
Streptococcus faecalis	21	18	24	11	14	15	14	16	13	13

Table 5 Determination of the effect of packed buttermilk on the tested bacterial strains

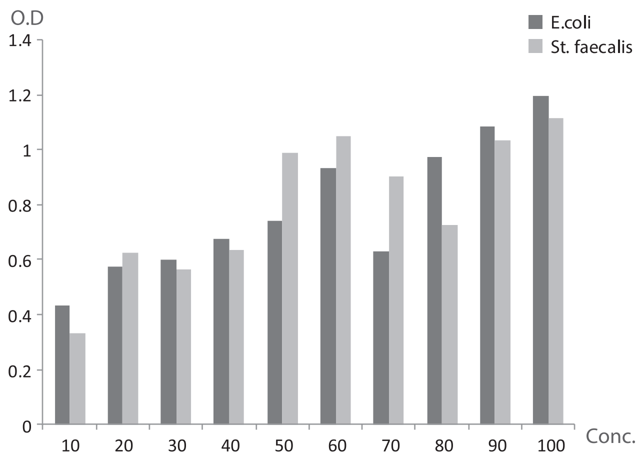
Bacterial strains	Milk dilutions [inhibition zone diameter (mm)]									
	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
Pseudomonas aeruginosa	00	00	00	00	00	00	00	00	00	00
Proteus vulgaris	00	00	00	00	00	00	00	00	00	00
Salmonella typhi	00	00	00	00	00	00	00	00	00	00
Escherichia coli	23	20	23	23	20	24	22	22	**25**	24
Klebsiella pneumonia	00	00	00	00	00	00	00	00	00	00
Staphylococcus aureus	00	00	00	00	00	00	00	00	00	00
Streptococcus faecalis	19	22	15	20	16	18	20	18	12	12

Figure 3



Determination of the effect of pasteurized milk on *Escherichia coli* and *Streptococcus faecalis* at different concentrations to detect the minimal inhibitory concentration. OD, optical density.

Figure 4



Determination of the effect of packed buttermilk on *Escherichia coli* and *Streptococcus faecalis* at different concentrations to detect the minimal inhibitory concentration. OD, optical density.

E. coli was the highly affected strain compared with *St. faecalis* except at high concentrations of 70–100% (Fig. 2). MIC of pasteurized milk on both *E. coli* and *St. faecalis* was found to be 10 and 80%, respectively, and they were detected as the MBC also for both organisms.

The effect of pasteurized milk on both *E. coli* and *St. faecalis* was nearly the same (Fig. 3). The MIC and MBC for both of them were found to be 10%.

The effect of packed buttermilk on both *E. coli* and *St. faecalis* was nearly the same (Fig. 4). MIC and MBC were found to be 10% for both organisms.

E. coli was chosen as one of the strains affected by the three cow milk types. It was scanned using TEM to see the effect of each milk type on the bacterial cell structure. *K. pneumoniae* was also chosen as one of the affected strains to be scanned before and after treatment with camel's milk (Fig. 5).

There was high effect of different milk types on internal and cell wall structure of both scanned organisms (Fig. 5).

Discussion

There is an urgent need to find new antimicrobials to treat bacterial pathogens. It is generally well established that the food constituents can be used to reduce the risk of developing or aggravating human disease conditions. In this regard, functional foods had emerged as adjuvant or alternative to chemotherapy, especially in the prevention and management of human diseases and for maintaining optimum health state [21]. Interest in camel milk usage for human nutrition is increasing due to its distinct composition and unique biofunctional properties [22].

In this study the boiled camel and cow milk and packed pasteurized and buttermilk samples were free from bacterial contamination. This is in agreement with the findings of Garedew *et al.* [23], who found that all pasteurized and packed milk samples taken from various supermarkets and restaurants at different shelf life were culture negative for Gram-negative staining bacteria. This may be explained by the effectiveness of pasteurization at the processing plant that minimized the chance of postpasteurization contamination.

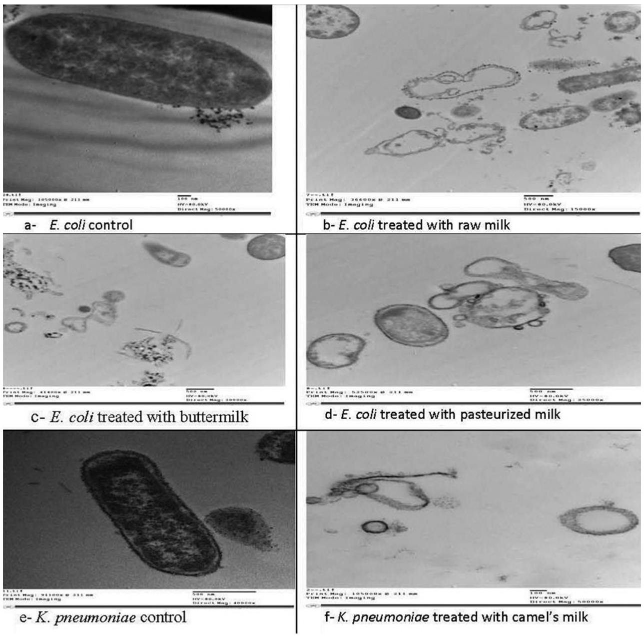
The results of this study indicated that camel's milk had antibacterial activity against *E. coli* and *K. pneumoniae*. Different concentrations were used, and it was found that 40 and 70% were the highly effective concentrations on both organisms, respectively. *E. coli* was the common organism affected by the four types of milk tested. It was found that pasteurized cow's milk was the highly effective milk type against this organism.

The concentration and the activity of lactoferrins and lysozyme (antibacterial agents) differ in cow's and camel's milk [4].

Lactoferrin works as an antimicrobial compound through chelating the iron ion, making this essential ion unavailable to the invading pathogens. There have been many reports on the antibacterial effects of lactoferrin of different origins [24–26].

The mean concentrations of lactoferrin in normal and mastitic cow milk have been reported previously and

Figure 5



Transmission electron microscope for *Escherichia coli* before and after treatment with different cow's milk types and for *Klebsiella pneumoniae* before and after treatment with camel's milk: (a) *Escherichia coli* control, (b) *E. coli* treated with raw milk, (c) *E. coli* treated with pasteurized milk, (d) *E. coli* treated with packed buttermilk, (e) *K. pneumonia* control, and (f) *K. pneumonia* treated with camel's milk.

found to be 2.23 and 2.70, respectively (concentrations are expressed in the logarithmic form) [27,28].

A previous study was conducted by Al-Majali *et al.* [29] on the lactoferrin concentrations isolated from camel's milk with and without mastitis and showed that all tested bacterial isolates were resistant to the camel lactoferrin except 20 *S. aureus* isolates, two *Streptococcus agalactiae*, and 12 streptococci other than *S. agalactiae* (growth was not inhibited at 50 mg/ml lactoferrin concentration). Lactoferrin failed to inhibit any of the *Arcanobacterium pyogenes* and *E. coli* isolates. The most sensitive isolate to lactoferrin was one of the *S. aureus* isolate with an MIC value of 0.006 mg/ml.

Elagamy [30] concluded that heating did not cause any alteration in the antibacterial activity of camel milk. This was approved by the present study, which indicated antibacterial effect of boiled cow's and camel's milk.

In contrast to the result of this study, Cardoso *et al.* [31] reported that cow milk had little antimicrobial effects compared with camel milk. Moreover, the inhibitory effect of camel's and cow's milk against several bacterial species has been reported by many researchers [1,5,32]. Lysozyme was suggested to be the main component responsible for the inhibitory activity [10]. Lysozyme is one of the most ubiquitous antibacterial molecules that exerts broad spectrum antimicrobial action. It has muramidase activity against Gram-positive bacteria [33].

Lactoferrin also present in milk represents one of the first defense systems against microbial agents. Lactoferrin affects the growth and proliferation of a variety of infectious agents, including both Gram-positive and Gram-negative bacteria, viruses, protozoa, or fungi. Its ability to bind free iron, which is one of the elements essential for the growth of bacteria, is responsible for the bacteriostatic effect of lactoferrin [34]. These studies could discuss the effect of the four milk types tested in this study against *E. coli* and *K. pneumoniae* scanned by means of TEM, which indicated disruption of internal and cell wall structure of both treated strains.

Conclusion

Milk can be used *in vitro* as antimicrobial natural product. Pasteurized and packed buttermilk had greater effect on bacteria compared with raw cow and camel milk. Milk can affect bacterial cell by destructing the cell wall and clearing the internal cell components. Further study is needed to extract the chemically effective substance present in these milk types and determine its concentration to explain why some milk concentration had effect and others did not.

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Conflicts of interest

There are no conflicts of interest.

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