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Bacteriological Quality of Environmental Samples and Patterns of Antibiotics Resistance of *Klebsiella* and *Enterobacter* Species from an Abattoir in Ibadan, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author OIF designed the study and the protocol. Authors OIF and FAA managed the literature searches, data collection and wrote the first draft of the manuscript. Both authors read and approved the final manuscript

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ABSTRACT

Antibiotics have been used appropriately and inappropriately in animal husbandry. In abattoir, faeces generated as wastes may contain coliforms and/or antibiotics which seep through the soil into ground water or runoff into surface water, thereby contaminating the water. This study was designed to determine the bacteriological quality of environmental samples from an abattoir area in Ibadan, Nigeria and the antibiotic-susceptibility pattern of *Klebsiella* and *Enterobacter* species isolated. Well water, pond water, wastewater, cow dung and soil samples were collected, the total heterotrophic bacteria count and total coliform count of the samples were carried out while *Klebsiella* and *Enterobacter* species were isolated using MacConkey agar and Eosin Methylene Blue Agar. The antibiotic susceptibility test of the isolates was carried out using the disc diffusion technique.
The total heterotrophic bacteria count (2.1×10^7 cfu/ml) of the wastewater sample was the highest followed by that of well 7 with a count of 7.4×10^4 cfu/ml. While the highest total coliform count of

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2.7×10^7 cfu/ml was from the wastewater, the counts from well 9, well 7, well 1 and the cow dung were 1.2×10^5cfu/ml, 1.0×10^5cfu/ml, 8.8×10^4cfu/ml and 1.0×10^4cfu/ml respectively. A total of 94 *Klebsiella* and *Enterobacter* species were isolated and were mostly resistant to ampicillin (76.6%) and cefotaxime (55.2%), while the least resistance was to ciprofloxacin (4.3%). The high total heterotrophic bacteria count and high total coliform count observed from this study coupled with the presence of multiple antibiotic resistant *Klebsiella* and *Enterobacter* species poses a grave danger to the health of the public if necessary precautions are not taken.

Keywords: Abattoir; bacteriological quality; antibiotic resistant; multiple resistant environment.

1. INTRODUCTION

The occurrence and spread of antibiotic resistant bacteria has continued to remain one of the public health problems all over the world, and one of the recognised reservoirs for antibiotic resistant bacteria harbouring antibiotic resistant genes is the aquatic ecosystems [1-2]. The use of antibiotics either appropriately or inappropriately in animal husbandry is common. One of the wastes being generated in abattoir and its environment is the animal faeces which could contain coliforms and/or antibiotics; these can seep through the soil into ground water or runoff into surface water thereby contaminating the water bodies. Water pollution takes place when extraneous materials enter water bodies and change the natural ecosystem of the water, thus interfering with the water use by the society [3]. Water pollution can either be of point source or non-point source. Point sources of pollution occur when pollutants are emitted directly into the water body e.g., from industrial sewage or municipal wastewater pipes, while non-point sources is when pollutants are delivered indirectly through environmental changes such as pollution from urban runoff [4]. The pollution of surface water which is the natural habitat of aquatic animals could have a consequential impact on human either directly or indirectly because nearly 1% of the world's fresh water which is about 0.007% of all water on the earth surface is readily accessible for direct human use [4-5].

In abattoir and its environment, a large volume of water is used during animal processing and cleaning. The disposal of abattoir effluent into drains and streams is a common practice and has both health and environmental hazards to the people downstream [6]. For instance, it has been identified that one of the important major risk factors to public health in the southwest Nigeria is improper management of abattoir activities [7]. Wastewater generated from abattoirs has been reported to contain varieties of pathogenic microorganisms which are directly introduced into neighbouring water bodies such as streams, ponds and rivers. These water bodies are used by local communities for domestic activities such as cooking, washing and bathing [8]. Moreover, wastes generated during the operations in the abattoir have been reported to be potential sources of public health hazards especially with respect to the enteric pathogens and contamination of water bodies [9]. Potential health risks from waterborne pathogens can exist in water contaminated by abattoir effluents [10]. This study was therefore designed to determine the bacteriological quality of environmental samples and antibiotic-susceptibility pattern of *Klebsiella* and *Enterobacter* species isolated from different samples collected from an abattoir area in Ibadan, South-western Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area and Study Sites

This study was carried out in Akinyele abattoir which is located in Akinyele Local Government Area of Oyo State in Nigeria. This is the second largest abattoir in the city of Ibadan. Samples were collected from nine wells, pond water, abattoir wastewater; cow dung and soil from dump sites. All the sampling points were within the abattoir environment. Wells 1, 2 and 9 are sources of drinking water for human and livestock as well as for the processing of slaughtered animals. Wells 3, 4, 5, 6, 7 and 8 are sources of drinking water for livestock and for processing of animals after slaughtering. Wells 4, 7 and 8 had no well casing but wells 4 and 8 had planks fitted around their rims, while wells 1, 2, 3, 5, 6 and 9 had well casings. Well 1 is located close to a pit latrine, while the casing of well 4 was not properly fitted allowing seepage from the environment to drain into it. Wastewater from the slaughter slab drains into an unlined drainage without receiving any form of treatment. The dump sites in the abattoir area were located haphazardly.

2.2 Sample Collection

Samples were collected between the months of May and July 2015, using standard methods. Soil, cattle's faecal samples, wastewater and water samples were collected in sterile containers. The water samples were placed in ice pack and transported to the Microbiology Laboratory, University of Ibadan and processed within 5 hours of collection.

2.3 Enumeration of Bacteria Counts of the Isolates

The media use for enumeration of the total heterotrophic bacteria count was nutrient agar while MacConkey agar was used for the isolation of the coliforms. The samples were analysed using the standard serial dilution method and culture media inoculated using the standard pour plate technique. Distinct colonies of coliforms were sub-cultured on Eosin Methylene Blue (EMB) Agar. Presumptive identification was based on the appearance of large mucoid colonies that are pink to purple for *Klebsiella* and *Enterobacter* species. Identification of the isolates were done using various morphological, biochemical and sugar fermentation tests as previously described [11] and interpreted using the Bergey's Manual of Determinative Bacteriology [12].

2.4 Antibiotics Susceptibility Test

Antibiotic susceptibility test was carried out on the isolates using disc diffusion technique as previously described by Clinical Laboratory Standard Institute [13]. The following antibiotics discs obtained from Oxoid, (UK) were used for the susceptibility test: tetracycline (30 µg), oxytetracycline (30 µg), nalidixic acid (30 µg), nitrofuratoin (300 µg), amikacin (30 µg), ceftazidime (30 g), cefotaxime (30 µg) chloramphenicol (30 µg), ampicillin (10 µg), and ciprofloxacin (5 µg). Sterile wire loop was used to pick 2-3 pure colonies from a 24 hours old culture on nutrient agar plates, suspended in sterile normal saline and the suspension properly mixed using a vortex mixer and the turbidity adjusted to 0.5 McFarland's standard. The organisms were evenly spread on the entire surface of freshly prepared Muller Hinton agar plates with sterile swab sticks. Using sterile forceps, the antibiotic discs were placed aseptically on the inoculated Muller Hinton agar plates. The plates were incubated for 18-24 hours at 37°C and the diameter of the zones of inhibition around each of the discs was measured to the nearest millimeter. The zones of inhibition were interpreted according to CLSI standard [13].

3. RESULTS

The total heterotrophic bacteria counts (THBC) and the total coliform counts (TCC) obtained from the samples are as shown in Table 1. The counts showed that the highest (2.1×10^7 cfu/ml) was from the wastewater sample followed by that of well 7 (7.4×10^4 cfu/ml). However, the least (3.6×10^2 cfu/ml) THBC was from well 5. While the highest TCC (2.7×10^7 cfu/ml) was from the wastewater, the least TCC (3.4×10^2 cfu/ml) was from well 2.

Table 1. Total heterotrophic bacteria count and total coliform count of the environmental samples

Sample	THBC	TCC
Well 1	1.3×10^3	8.8×10^4
Well 2	2.2×10^3	3.4×10^2
Well 3	5.0×10^3	6.0×10^3
Well 4	1.4×10^4	2.8×10^3
Well 5	3.6×10^2	ND
Well 6	ND	ND
Well 7	7.2×10^4	1.0×10^5
Well 8	1.2×10^4	ND
Well 9	3.0×10^3	1.2×10^5
Wastewater	2.1×10^7	2.2×10^7
Pond water	8.4×10^3	1.0×10^3
Cow dung	1.2×10^4	1.0×10^4
Soil	1.7×10^4	4.9×10^3

Key: ND: Not determined

A total of 94 isolates were obtained from the different samples comprising 16 (17%) *Enterobacter* species, 31 (33%) *K. pneumonia*, 17 (18.1%) *K. oxytoca* and 30 (32%) other *Klebsiella* species. The frequency of occurrence of the *Enterobacter* and *Klebsiella* spp. showed that the highest occurrence was from well 1 and the soil samples. While from the sample of well 1, seven (7.4%) *k. pneumonia*, four (4.3%) *K. oxytoca* and one (1.1%) *Enterobacter* sp. were isolated; four (4.3%) *K. pneumonia*, one (1.1%) *K. oxytoca*, four (4.3%) other *Klebsiella* spp. and three (3.2%) *Enterobacter* sp. were obtained from the soil sample. A total of eight isolates were also obtained from the samples of well 3 including three (3.2%) *Enterobacter* spp., one (1.1%) *K. pneumonia* and four (4.3%) other *Klebsiella* spp. whereas, from well 5, two (2.1%), two (2.1%) and four (4.3%) *Enterobacter* spp., *K. pneumonia* and other *Klebsiella* spp. respectively were obtained. The least occurrence was from well 2 in which only one (1.1%) *K. pneumonia* was isolated. The highest occurrence rate (3.2%) for *Enterobacter* spp. was from well 3 and the soil while for *K. pneumonia* (4.3%), it was from well 8 and the soil sample, but for *K. oxytoca* (4.3%), it was from wells 1 and 9. However, the highest occurrence rate for the other *Klebsiella* spp. (4.3%) was from wells 3, 4, 5, 8 and the soil (Table 2).

The pattern of resistance of the isolates showed that 72 (76.6%) were resistant to ampicillin comprising 14 (87.5%) *Enterobacter* sp., 19 (61.3%) *K. pneumonia*, 10 (58.8%) *K. oxytoca* and 29 (96.7%) other *Klebsiella* species. Furthermore, resistance of the isolates to cefotaxime was 52 (55.2%) and these included 11 (68.8%) *Enterobacter* species, 17 (54.8%) *K. pneumoniae*, 10 (58.8%) *K. oxytoca* and 14 (46.7%) other *Klebsiella* species. In addition, the least resistance observed in this study (4.3%) was to ciprofloxacin as only four (4.3%) of the other *Klebsiella* species showed resistance (Table 3).

The different patterns of multiple antibiotic resistance observed in this study is shown in Table 4. There were six different antibiotype observed in *K. pneumonia*. Two of the isolates obtained from well 8 showed resistant to a combination of three antibiotics including AMP, CXM and F, while one isolate each obtained from the wastewater and pond water samples were resistant to a combination of four antibiotics (AMP, NA, CXM and F). Furthermore, for *K. oxytoca*, there were five different antibiotypes from which it was observed that one isolate each from the wastewater and pond water showed resistant to a combination of four antibiotics (AMP, NA, CXM, and F). while one isolate each form well 1 and wastewater were resistant to a combination of five antibiotics. In addition, one of the isolates from well 7 showed resistance to a combination of seven antibiotics (AMP, NA, C, OT, TE, CIP and AK). However, for the *Enterobacter* species, only two antibiotypes were obtained and these were resistance to a combination of three antibiotics. While two isolates from well 3 showed resistance to a combination of AMP, CXM and F, one isolate from well 7 showed resistance to AMP, CXM and CAZ (Table 4).

Table 2. Frequency of occurrence of *Klebsiella* and *Enterobacter* species isolated from Akinyele abattoir samples n (%)

Sources	*Enterobacter* spp.	*K. pneumonia*	*K. oxytoca*	*Klebsiella* spp.	Total
Well1	1 (1.1)	7(7.4)	4(4.3)	0(0)	12(12.8)
Well2	0(0)	1(1.1)	0(0)	0(0)	1(1.1)
Well3	3(3.2)	1(1.1)	0(0)	4(4.3)	8(8.5)
Well4	0(0)	3(3.2)	0(0)	4(4.3)	7(7.4)
Well5	2(2.1)	2(2.1)	0(0)	4(4.3)	8(8.5)
Well6	1(1.1)	1(1.1)	0(0)	3(3.2)	5(5.3)
Well7	1(1.1)	3(3.2)	0(0)	3(3.2)	7(7.4)
Well8	1(1.1)	4(4.3)	3(3.2)	4(4.3)	12(12.8)
Well9	2(2.1)	1(1.1)	4(4.3)	0(0)	7(7.4)
Pond water	1(1.1)	3(3.2)	1(1.1)	2(2.1)	7(7.4)
Waste- water	1(1.1)	1(1.1)	2(2.1)	1(1.1)	5(5.3)
Cow dung	0(0)	0(0)	2(2.1)	1(1.1)	3 (3.2)
Soil	3(3.2)	4(4.3)	1(1.1)	4(4.3)	12(12.8)
Total	(17.0)	(33.0)	(18.1)	(32.0)	94(100)

Table 3. Antibiotic-resistance of *Klebsiella* and *Enterobacter* species isolated

Antibiotics	*Enterobacter* spp. n=16	*K. pneumonia* n=31	*K. oxytoca* n=17	*Klebsiella* spp. n=30	Total n=94
Ampicillin (10µg)	14 (87.5%)	19(61.3%)	10(58.8%)	29(96.7%)	72(76.6%)
Nalidixic acid (30µg)	0(0)	3 (9.7%)	2(11.8%)	3(10.0%)	8(8.5%)
Cefotaxime (30µg)	11 (68.8%)	17(54.8%)	10(58.8%)	14(46.7%)	52(55.2%)
Chloramphenicol (30µg)	0(0)	3(9.7%)	0(0%)	4(13.3%)	7(7.4%)
Nitrofuratoin (300µg)	5 (31.3%)	7(25.6%)	1(5.9%)	4(13.3%)	17(18.1%)
Oxytetracycline (30µg)	0(0)	2(6.5%)	5(29.4%)	5(16.7%)	12(12.8%)
Tetracycline (30µg)	1 (6.3%)	3(9.7%)	4(23.5%)	3(10.0%)	11(11.7%)
Ciprofloxacin (5µg)	(0)	0(0%)	0(0%)	4(13.3%)	4(4.3%)
Ceftazidime (30µg)	1 (6.3%)	4(12.9%)	2(11.8%)	3(10.0%)	10(10.6%)
Amikacin (30µg)	0(0)	0(0%)	1(5.9%)	5(16.7%)	6(6.4%)

Table 4. Multiple antibiotic resistance patterns of the isolates obtained from the environmental samples

Phenotypes	Sample source													
	Klebsiella pneumoniae													
	W1	W2	W3	W4	W5	W6	W7	W8	W9	S	CD	WW	PW	MAR index
AMP, CXM, F	0	0	0	0	0	0	0	2	0	0	0	0	0	0.3
CXM, C, F	0	0	0	0	0	0	0	0	0	2	0	0	0	0.3
CXM, F, CAZ	0	0	0	0	1	0	0	0	0	0	0	0		0.3
AMP, NA, CXM, F	0	0	0	0	0	0	0	0	0	0	0	1	1	0.4
AMP, CXM, OT, TE	0	1	0	0	0	0	0	0	0	0	0	0	0	0.4
AMP, NA, C, F	1	0	0	0	0	0	0	0	0	0	0	0	0	0.4
	Klebsiella oxytoca													
AMP, NA, CXM	0	0	0	0	0	0	0	1	0	0	0	0	0	0.3
AMP, OT, TE	0	0	0	0	0	0	0	0	0	0	0	0	1	0.3
AMP, NA, CXM, F	0	0	0	0	0	0	0	0	0	0	0	1	1	0.4
AMP, CXM, OT, CAZ, AK	1	0	0	0	0	0	0	0	0	0	0	0	0	0.5
AMP, CXM, OT, TE, CAZ	0	0	0	0	0	0	0	0	0	0	0	1	0	0.5

Phenotypes	Sample source													
	***Klebsiella* species**													
AMP, OT, TE	0	0	0	0	0	0	0	0	0	0	1	1	0	0.3
AMP, F, CIP	0	0	0	0	1	0	0	0	0	0	0	0	0	0.3
AMP, NA, OT	0	0	1	0	0	0	0	0	0	0	0	0	0	0.3
AMP, C, OT	0	0	0	0	0	0	0	0	0	0	0	0	1	0.3
AMP, F, CAZ	0	0	0	0	0	0	0	0	0	1	0	0	0	0.3
AMP, CAZ, AK	1	0	0	0	0	0	0	0	0	0	0	0	0	0.3
CXM, F, CAZ	0	0	0	0	1	0	0	0	0	0	0	0	0	0.3
AMP, CXM, CAZ	0	0	0	0	0	0	2	0	0	0	0	0	0	0.3
AMP, C, F	0	0	0	0	0	0	0	0	0	1	0	0	0	0.3
AMP, CXM, CIP, CAZ	0	0	0	0	0	0	1	0	0	0	0	0	0	0.4
AMP, NA, C, OT, TE, CIP, AK	0	0	0	0	0	0	1	0	0	0	0	0	0	0.7
	***Enterobacter* species**													
AMP, CXM, F	0	0	2	0	0	0	0	0	0	0	0	0	0	0.3
AMP, CXM, CAZ	0	0	0	0	0	0	1	0	0	0	0	0	0	0.3

Key: 0 (none of the isolates showed resistant to the combination of the antibiotics); 2(two isolates showed resistance); W (well); CD (Cow dung); WW (Wastewater); PW (Pond water); S (Soil)

4. DISCUSSION

The THBC of 2.1×10^7 cfu/ml that was obtained from the wastewater sample is above the permitted count allowed for effluents for discharge according to the standard of the Federal Environmental Protection Agency [14]. However, this observation is comparably similar to the THBC of $2.7x10^7$cfu/ml previously reported from another study on abattoir wastewater in Lagos, a city in south western Nigeria [15]. This high THBC may be as a result of the unhygienic practices in the abattoir. The THBC (7.4×10^4 cfu/ml) obtained from well 7 is high and is above the permissible limit allowed for drinking water [14], this high count could be due to the absence of well casing and planks around the rim of the well which might have allowed over flooding of the well during rainfall.

The highest value of the TCC ($1.2\ x10^5$cfu/ml) which was obtained from the well 9 that is used for drinking purpose for both human and animals as well as for washing of dressed animals may be as a result of many factors. The well is in close proximity to the abattoir dumpsite where open defecation is practiced, some of the well casings were not properly fitted, the dump sites were haphazardly located and the untreated wastewater from the slaughtering and dressing slabs in the abattoir is washed into open drainage. Furthermore, the high TCC ($1.0\ x10^5$cfu/ml) obtained from well 7, one of the wells that the livestock drink from, may be because the well had no casing and could have been affected by flooding. The TCC count obtained from this well is in agreement with the previously reported count from a similar study carried out on well water samples from an abattoir area [16]. Specifically, the value of the TCC ($8.8\ x10^4$cfu/ml) obtained from well 1 could have resulted from the infiltration from a closely situated pit latrine.

The highest TCC ($2.2\ x\ 10^7$cfu/ml) from this study that was from the wastewater sample exceeded the recommended permissible limit of $4.2\ x\ 10^2$cfu/ml and $5.2\ x\ 10^2$cfu/ml for effluents to be discharged into water bodies and land application [14]. Furthermore, the count was also higher than the previously reported TCC of 3.2×10^4cfu/ml from the wastewater of another abattoir in the northern part of the country [17]. The high coliform count observed in this study could be as a result of the poor sanitary practice of the abattoir workers.

The frequency of occurrence (17.0%) of *Enterobacter* spp. in this study which was lower compared to that of *Klebsiella* spp. agrees with the report of a study conducted on abattoir wastewater from another city (Ado Ekiti) in southwestern Nigeria where *Enterobacter* spp. had the least frequency of occurrence among other coliforms isolated [16]. The isolation of *K. pneumonia* from all the well water samples, including those used as source of drinking water for humans and animals is of public health concern since *K. pneumonia* is an opportunistic pathogen, especially among immuno compromised individuals.

The observation from this present study that showed a high resistance of 87.5% (*Enterobacter* spp.) to ampicillin without any resistance to nalidixic acid is a finding that agrees and is comparably similar to the report of another study on ready to eat foods samples in South Africa from which 79.0% resistance of *Enterobacte*r spp. to ampicillin and 0% resistant to nalidixic acid was obtained [18]. The high level of resistance observed to ampicillin in these studies is an indication that the antibiotic might have been abused in animal husbandry and the antibiotic may not be effective for treatment of *Enterobacter* infection if it occurs. However, the resistance of *Enterobacter* spp. to tetracycline (6.3%) in this study was low, and also much lower compared to the 53.5% resistance of *Enterobacter cloacae* to the same antibiotic reported from a study on water samples collected from spring and stream in India [19]. This contrast might be due to the studied samples and the possibility of exposure of the organism to the antibiotics in the later study. However, none of the *Enterobacter* spp. showed resistance to amikacin which is also similar to the report of the study on natural sources of water from rural areas of east Sikkim in Indian [19]. This is an indication that amikacin may still be effective and a drug of choice for the treatment of *Enterobacter* infection. Moreover, the findings from this study that showed a resistance of *Enterobacter* spp. to ciprofloxacin and tetracycline being 0% and 6.3% respectively is not in agreement with the reported 44.4% and 77.8% to ciprofloxacin and tetracy cline respectively from a previous study carried out in Ogun State, Nigeria [20]. However, the observed resistance of *Enterobacter* spp. to ampicillin (100%) and ceftazidime (0%) in this present study agrees with the report of Balogun et al. [20].

Furthermore, the high resistance of *Klebsiella* spp. in this study to ampicillin as well as low

resistance to nitrofuratoin and ciprofloxacin is in agreement with a previous study carried out on status of contamination and antibiotic resistance of bacteria from well water from which high resistance of *Klebsiella* spp. to ampicillin was reported while resistance to both nitrofuratoin and ciprofloxacin was low [21]. However, while in this study, resistance of the *Klebsiella* spp. to tetracycline was very low, resistance of the bacteria to the same antibiotic in the latter study was high. Moreover, the resistance of the *Klebsiella* isolates to nalidixic acid, chloramphenicol and ciprofloxacin were comparably similar in both studies.

In addition, resistance of *Klebsiella* spp. to cefotaxime (52.6%) and ceftazidime (11.5%) as observed in this study is not in agreement with the reported resistance to the two antibiotics at the same concentrations by *Klebsiella* spp. isolated from a previous study on samples collected from River Danube [22] where resistance to cefotaxime and ceftazidime were 0.94% for the antibiotics. While the present study was on environmental samples in an abattoir that could have been impacted on by the activities at the abattoir, the latter studies was on river water samples. This could have been responsible for the observed difference. Furthermore, the observed resistance of the isolates in this present study is also not in agreement with the resistance observed to nitrofurantoin, nalidixic acid and tetracycline compared to the 100% resistance previously reported to these antibiotics from a study carried out on faeces from abattoir waste, processing water and abattoir products in the northern part of the country [23]. The difference could be as a result of the disparity in the studied samples. While the present study is comprised of different environmental samples, the latter study was purely on samples from the abattoir waste and effluent. Specifically, the antibiotic resistance observed in the bacterial isolates from this study may be due to the fact that they were exposed to the antibiotics tested. Also, the antibiotics susceptibility testing that showed some isolates across the sampling areas having MAR index greater than 0.2 could be an indication of the extent to which the organisms have been exposed to antibiotics commonly used within the study area.

5. CONCLUSION

In conclusion, the high total heterotrophic bacteria count and total coliform count as well as the presence of multiple antibiotic resistant *Klebsiella* and *Enterobacter* species obtained signifies improper abattoir waste management and possible misuse of antibiotics together with open defecation practiced in this environment could have given rise to the high occurrence of the faecal pollutants and the antibiotic resistance organisms. Thus, wastewater generated from the abattoirs should be properly treated before being discharge into the environment and the use of antibiotic should be regulated and monitored to forestall infection outbreak among the human and animal's population in the area.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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