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Antimicrobial Resistance of Pathogenic Bacteria Isolated from Selected Aquaculture Sites in Awka, Southeast, Nigeria

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Abstract

The study examined the antimicrobial resistance of pathogenic bacteria isolated from some selected fish farms in Awka, Nigeria. This study assessed the physicochemical and bacteriological quality of pond water across four aquaculture sites. The water temperature and pH were within the limits set by WHO, indicating. Temperature varied between 29.00 ± 2.83 °C and 30.00 ± 2.83 °C; pH Levels is 6.70 ± 0.28 to 7.60 ± 0.28. All ponds were contaminated with heterotrophic bacteria and coliforms. Total Heterotrophic Count of 7.2×10^5 to 1.8×10^6 CFU/ml, with Concrete Pond 2 having the highest contamination. Total Coliform Count: between 25 to 160 CFU/100ml; Earthen Pond 2 was the most contaminated. Faecal Coliform Count is 0 to 120 CFU/100ml. Dominant species included: *E. coli, Acinetobacter* spp., *Klebsiella* spp., *Staphylococcus* spp., *Bacillus* spp., *Aeromonas* spp., *Micrococcus* spp., *Shigella* spp. Concrete Pond 1, 3 and 4 had the highest loads of heterotrophic and coliform bacteria. Concrete Pond 2 was comparatively less contaminated. *Staphylococcus* spp. and *Bacillus* spp. exhibited greater resistance across all samples. All ponds were contaminated with potentially pathogenic bacteria, posing risks to fish health, yield, and public health. Therefore, regular monitoring is essential for safeguarding aquaculture and public wellbeing.

Keywords: Aquaculture, Antimicrobial resistance, Microbial Load, Conrete Pond, Physiochemical.

Introduction

One of the most global heath challenges is antibiotic resistance, with aquaculture systems been recognized as serious reservoirs of antibiotics resistance microorganisms. (World Health Organization [WHO], 2020; Food and Agriculture Organization [FAO], 2020). Addressing bacterial issues, antibiotics are extensively utilized to avoid tackling illnesses caused by microorganism. It was estimated that country like China, the total antibiotics production kept increasing and in 2020, it was up to 223000 tons. Some of these antibiotics were used for animal farming, (https://www.chinabgao.com/k/kangshengsu /66064.html accessed on 25 August 2024). The emergence of antibiotic-resistant microorganisms occurs after the long-term use of antibiotics and this can enter aquatic systems, like rivers and lakes thereby causing selective pressure on bacteria, leading to the enrichment of antibiotic resistance microorganisms (Omotoso *et al*., 2025)

The increase in public realization about the loss of potency of antibiotics due to overuse has led groups such as the public health specialists, consumer groups, and environmentalists to challenge antibiotic usage in livestock, poultry and fish farming. Dramatic increase in population of antibiotic-resistant bacterial strains is believed to be because of excessive use of antibiotics in fish farming and other animal food, and this is now threatening human health (Apenteng *et al*., 2017). Also, non-hygienic and stressful conditions present in aquaculture facilities increased the risk of bacterial infections among aquaculture fish (Aina and Olaleye, 2023). In fish feed, high amount of antimicrobials are used for purposes of prevention and curation in aquaculture worldwide because of this, fish and fish products are potential health risk to humans and the environment since important bacteria which are pathogenic to humans (Gufe *et al*., 2019). Also, the safety of eating fish from contaminated fishponds cannot be assured. Therefore, the need to study the pathogenic bacterial isolates from fishponds and their antibiotic resistance pattern against some clinically used antibiotics is paramount. This will enable us to know the clinical importance of bacteria and the potential risk that consumption of fish from contaminated ponds cause to human health.

Materials and Method

Study Location

The study was carried out on two farms, Ayom's fish farm and Modozie fish farm located at Aroma and Agu-Awka respectively in Awka South Local government area of Anambra state, Southeast, Nigeria.

Isolation and Identification of the Bacteria

Sample Collection

Pond water sample were aseptically taken from the four different ponds using sterile screw cap bottles. The chemical parameters of the pond were also taken, which include pH, atmospheric temperature and the water temperature. A total number of four water samples were collected from the ponds at depths 1 m within the catfish ponds, at the outlets from the catfish ponds in Awka, Anambra State. The water samples were transported to the Microbiology laboratory of Nnamdi Azikiwe University, Awka, in an ice-packed container for microbiological analysis within 8 hours of collection.

Samples from different ponds were homogenized and 1 ml each of the water samples was transferred into 9 ml of normal sterile saline and then serially diluted. 1 ml of the samples from 10^{-1} to 10^{-3} of the dilution test tubes were inoculated into Nutrient agar, MacConkey agar, Eosin methylene blue agar and Salmonella Shigella agar using pour plate method and were incubated at 37°C for 24 hours. A control was equally prepared without adding the sample. The bacterial colonies were counted using a colony counter and expressed in colony forming unit per ml (CFU/ml).

$$\text{Colony forming unit / ml} = \frac{N}{V \times D}$$

N = Average number of colonies, V = Aliqout volume, D = Dilution factor

The bacteria isolates were counted using a colony counter and sub-cultured on a freshly prepared nutrient agar and incubated for 24 hours at 37°C to obtain a pure culture (Cheesbrough, 2006).

Characterization and Identification of the Bacterial Isolates

The pure cultures were identified and characterized macroscopically, microscopically (Gram's staining) and using biochemical tests (Catalase test, Oxidase test, Coagulase test, Citrate test, Urease production, Methyl Red-Vogues Proskauer (MRVP), and Sugar Fermentation test) and comparison with those of known taxa of Bergey's manual of determinative Bacteriology (Cheesbrough, 2006).

Antibiotic Sensitivity Testing

Antibiotics sensitivity patterns of the bacterial isolates were determined using the disk diffusion method (Bauer et al., 1966). The disks were placed on Muller-Hinton agar plates that were inoculated with the broth culture of the test organisms. The plates were incubated at 37^0C for 48hr, after which zones of

inhibition were examined and interpreted based on the national committee for Clinical and Laboratory Standards institute (CLSl, 2007) criteria as sensitive, intermediate and resistant

Results

The present study was conducted to isolate resistance pathogenic bacteria from fishponds. The results of chemical analysis showed that the atmospheric temperature of the pond water analyzed ranged from 30.20±0.28 to 30.50±0.71. The atmospheric temperatures were within the permissible limits of WHO. Concrete pond 1 and concrete pond 2 with the highest mean value of 30.50±0.71 each shows no significant difference with other samples ($p>0.05$).

The water temperature of the pond water analyzed ranged from 29.00±2.83 to 30.00±2.83. Concrete pond 1 and concrete pond 2 with the highest mean value of 30.50±2.83 each shows no significant difference with other samples ($p>0.05$). (Table 1)

The results of bacterial count showed that all the sample collection sites (concrete 1, concrete 2, concrete pond 1 and concrete pond 4) were contaminated with heterophic bacterial and coliform but not with Salmonella and Shigella. Total heterophic count ranged from 1.5×10^6 to $7.2 \times 10^5$and concrete pond 1 was most contaminated by heterophic bacterial (7.2×10^5). Total coliform ranged from 25 to 160cfu/100ml and concrete pond 4 was most contaminated. Total faecal coliform ranged from 0 to 120cfu/100ml and concrete pond 4 was most contaminated as shown in (Table 2).

Morphological and biochemical characteristics of the bacterial isolates showed that the predominant bacteria isolated were *E. coli, Acinetobacter spp, Klebsiella spp, Staphylococcus spp, Bacillus spp, Aeromonas spp, Micrococcus spp* and *Shigella spp.* concrete pond 4 is most contaminated by various microorganisms. *E. coli Acinetobacter spp, Klebsiella spp, Staphylococcus spp, Bacillus spp, Aeromonas spp, Micrococcus spp* and *Shigella spp* were all found in concrete pond 4. The same groups of microorganisms were found in earthen pond 1 except *Shigella spp.* Concrete Pond 2 has only *Klebsiella spp, Staphylococcus spp, Bacillus spp, Aeromonas spp* and *Micrococcus spp* while concrete pond 1 has all the organisms found in concrete pond(Table 4). Table 5 showed inhibition of organisms isolated from concrete pond 1 against conventional antibiotics. The results showed that Perfloxacin, Gentamycin, Ciprofloxacin, Streptomycin and Septrin were active against *E. coli*. Gentamycin, Ciprofloxacin, arvid and Augmentin were active against *Klebsiella spp.* Only Ciprofloxacin was active against *Staphylococcus spp* and *Bacillus spp*. Perfloxacin, Gentamycin and Augmentin were active against *Aeromonas spp*. Ampiclox, Zinnacef, Ciprofloxacin, Streptomycin and Erythromycin were active against *Micrococcus spp*. Perfloxacin, Gentamycin, Ciprofloxacin, Streptomycin and Septrin were active against *Shigella spp*.

Table 6 showed inhibition of organisms isolated from concrete pond 2 against conventional antibiotics. The results showed that Gentamycin, Ciprofloxacin, Tarvid and Augmentin were active against *Klebsiella spp*. Only Ciprofloxacin was active against *Staphylococcus spp* and *Bacillus spp*. Perfloxacin, Gentamycin, Ciprofloxacin and Augmentin were active against *Aeromonas spp*. Ampiclox, Zinnacef, Ci Table 7 showed inhibition of organisms isolated from concrete pond 3 against conventional antibiotics. The results showed that Perfloxacin, Gentamycin, Ciprofloxacin, Streptomycin and Septrin were active against *E. coli*. Gentamycin, Ampiclox, Ciprofloxacin, Augmentin and Nalidixic Acid were active against *Acinetobacter spp*. Gentamycin, Ciprofloxacin, Tarvid, Augmentin and Nalidixic Acid were active against *Klebsiella spp.* Only Ciprofloxacin was active against *Staphylococcus spp* and *Bacillus spp*. Perfloxacin, Gentamycin and Augmentin were active against *Aeromonas spp*. Ampiclox, Zinnacef, Ciprofloxacin, Streptomycin and Erythromycin were active against *Micrococcus spp*. profloxacin, Streptomycin and Erythromycin were active against *Micrococcus spp*.

Table 8 showed inhibition of organisms isolated from concrete pond 4 against conventional antibiotics. The results showed that Perfloxacin, Gentamycin, Ciprofloxacin, Streptomycin and Septrin were active against *E. coli*. Perfloxacin, Gentamycin, Ciprofloxacin, Augmentin and Nalidixic Acid were active against *Acinetobacter spp*. Gentamycin, Ciprofloxacin, Tarvid and Augmentin were active against

Klebsiella spp. Only Ciprofloxacin was active against *Staphylococcus spp* and *Bacillus spp*. Perfloxacin, Gentamycin and Augmentin were active against *Aeromonas spp*. Ampiclox, Zinnacef, Ciprofloxacin, Streptomycin and Erythromycin were active against *Micrococcus spp*. Perfloxacin, Gentamycin, Ciprofloxacin, Streptomycin, Septrin and Ampicilin were active against *Shigella spp*.

Table 1: Chemical Parameter of the pond water

Ponds	Atmospheric Temperature	Water Temperature	Water pH
Concrete pond 1	$30.50^{a}\pm0.71$	$30.00^{a}\pm2.83$	$7.60^{a}\pm0.28$
Concrete pond 2	$30.50^{a}\pm0.71$	$30.00^{a}\pm2.83$	$7.70^{a}\pm0.28$
Concrete pond 3	$30.20^{a}\pm0.28$	$29.00^{a}\pm2.83$	$6.70^{b}\pm0.28$
Concrete pond 4	$30.20^{a}\pm0.28$	$29.00^{a}\pm2.83$	$7.00^{ab}\pm0.28$

^{abc}Means with different superscripts on the same column are significantly different at $p\leq0.05$.

Table 2: Total Heterotrophic Bacteria Count, Salmonella/Shigella Count and Coliform Count of the Pond Water.

Pond	Total Heterophic Count (CFU/ml)	Salmonella/Shigella (CFU/100ml)	Total Coliform Count (CFU/100ml)	Total Fecal Coliform Count (CFU/100ml)
Concrete pond 1	7.2×10^5	0	72	50
Concrete pond 2	1.8×10^6	0	25	0
Concrete pond 3	1.5×10^6	0	150	100
Concrete pond 4	1.7×10^6	0	160	120

CFU: colony forming unit

Table 3: Occurrence of Bacterial Isolate from Fishpond Water

Bacterial isolates	Concrete pond 1	Concrete pond 2	Concrete pond 3	Concrete pond 4
E. coli	+	-	+	+
Acinetobacter spp	-	-	+	+
Klebsiella spp	+	+	+	+
Staphylococcus spp	+	+	+	+
Bacillus spp	+	+	+	+
Aeromonas spp	+	+	+	+
Micrococcus spp	+	+	+	+
Shigella spp	+	-	-	+

Table 4: Biochemical Characteristics of Isolated Microorganisms

Probable Organisms	Gram Reaction	Motility	Catalase	Coagulase	Indole	M.R	Citrate	Oxidase	V.P	Urease	Sugar Fermentation				
											Glc	**Mal**	**Lac**	**Suc**	**Gal**
E.coli	- rods	+	+	-	+	+	-	-	-	-	AG	AG	AG	AG	AG
Acinetobacter spp	- rods	+	+	-	-	-	+	-	+	-	AG	AG	-	AG	-
Klebsiella spp	- rods	-	+	-	-	-	+	-	-	+	AG	AG	AG	AG	AG
Staphylococcus spp	+ cocci	-	+	+	-	+	+	-	+	+	A+	A+	A+	A+	A+
Bacillus spp	+ rods	+	+	-	-	-	+	-	+	-	A+	AG	-	A+	A+
Aeromonas spp	- rods	+	+	-	+	+	+	+	-	+	AG	AG	AG	AG	AG
Micrococcus spp	+ cocci	-	+	-	+	+	+	+	-	+	AG	A+	AG	AG	A+
Shigella spp	- cocci	-	+	-	-	+	-	-	-	-	A+	A+	-	-	A+

Key: + = Positive, - = Negative, GLC = Glucose, LAC = Lactose, MAL = Maltose, FRC = Fructose, SUC = Sucrose,

Table 5: Antibiotic Sensitivity Test of the Isolates from concrete pond 1

Antibiotic	***E.coli***	***Klebsiella spp***	***Staphylococcus spp***	***Bacillus spp***	***Aeromonas spp***	***Micrococcus spp***	***Shigella spp***
PEF	12mm	Nil	Nil	Nil	10mm	Nil	10mm
CN	12mm	17mm	Nil	Nil	12mm	Nil	13mm
APX	Nu	Nu	Nil	Nil	Nu	11mm	Nu
Z	Nu	Nu	Nil	Nil	Nu	10mm	Nu
AM	Nu	Nu	Nil	Nil	Nu	Nil	Nu
R	Nu	Nu	Nil	Nil	Nu	Nil	Nu
CPX	9mm	15mm	16mm	13mm	Nil	9mm	6mm
S	11mm	Nil	Nil	Nil	Nil	10mm	10mm
SXT	10mm	Nil	Nil	Nil	Nil	Nil	4mm
E	Nu	Nu	Nil	Nil	Nu	7mm	Nu
OFX	Nil	11mm	Nu	Nu	Nil	Nu	Nil
AU	Nil	12mm	Nu	Nu	15mm	Nu	Nil
CEP	Nil	Nil	Nu	Nu	Nil	Nu	Nil
NA	Nil	Nil	Nu	Nu	Nil	Nu	Nil
PN	Nil	9mm	Nu	Nu	Nil	Nu	Nil

KEY: PEF = Perfloxacin 10µg, CN = Gentamycin 10µg, APX = Ampiclox 30µg, Z = Zinnacef 20µg, AM = Amoxacilin 30µg, R = Rocephin 25µg, CPX = Ciprofloxacin 10µg, S = Streptomycin 30µg, SXT = Septrin 30µg, E = Erythromycin 10µg, OFX= Tarvid 10µg, AU = Augmentin 30µg, CEP = Ceporex 10µg, NA = Nalidixic Acid 30µg, PN = Ampicilin 30µg, Nil = no zone of inhibition, Nu = Not Used.

Table 6. Antibiotic Sensitivity Test of the Isolates from concrete pond 2

Antibiotic	***Klebsiella spp***	***Staphylococcus spp***	***Bacillus spp***	***Aeromonas spp***	***Micrococcus spp***
PEF	Nil	Nil	Nil	12mm	Nil
CN	15mm	Nil	Nil	11mm	Nil
APX	Nu	Nil	Nil	Nu	11mm
Z	Nu	Nil	Nil	Nu	10mm
AM	Nu	Nil	Nil	Nu	Nil
R	Nu	Nil	Nil	Nu	Nil
CPX	16mm	14mm	12mm	10mm	9mm
S	Nil	Nil	Nil	Nil	9mm
SXT	Nil	Nil	Nil	Nil	Nil
E	Nu	Nil	Nil	Nu	6mm
OFX	12mm	Nu	Nu	Nil	Nu
AU	11mm	Nu	Nu	17mm	Nu
CEP	Nil	Nu	Nu	Nil	Nu
NA	Nil	Nu	Nu	Nil	Nu
PN	10mm	Nu	Nu	Nil	Nu

KEY: PEF = Perfloxacin 10µg, CN = Gentamycin 10µg, APX = Ampiclox 30µg, Z = Zinnacef 20µg, AM = Amoxacilin 30µg, R = Rocephin 25µg, CPX = Ciprofloxacin 10µg, S = Streptomycin 30µg, SXT = Septrin 30µg, E = Erythromycin 10µg, OFX= Tarvid 10µg, AU = Augmentin 30µg, CEP =

Ceporex 10µg, NA = Nalidixic Acid 30µg, PN = Ampicilin 30µg, Nil = no zone of inhibition, Nu = Not Used

Table 7. Antibiotic Sensitivity Test of the Isolates from Concrete Pond 3

Antibiotic	***E.coli***	***Acinetobacter spp***	***Klebsiella spp***	***Staphylococcus spp***	***Bacillus spp***	***Aeromonas spp***	***Micrococcus spp***
PEF	14mm	Nil	Nil	Nil	Nil	11mm	Nil
CN	15mm	15mm	19mm	Nil	Nil	14mm	Nil
APX	Nu	17mm	Nu	Nil	Nil	Nu	12mm
Z	Nu	Nil	Nu	Nil	Nil	Nu	13mm
AM	Nu	Nil	Nu	Nil	Nil	Nu	Nil
R	Nu	Nil	Nu	Nil	Nil	Nu	Nil
CPX	9mm	11mm	12mm	18mm	15mm	Nil	11mm
S	11mm	Nil	Nil	Nil	Nil	Nil	15mm
SXT	10mm	Nil	Nil	Nil	Nil	Nil	Nil
E	Nu	Nu	Nu	Nil	Nil	Nu	10mm
OFX	Nil	Nil	12mm	Nu	Nu	Nil	Nu
AU	Nil	15mm	14mm	Nu	Nu	15mm	Nu
CEP	Nil	Nil	Nil	Nu	Nu	Nil	Nu
NA	Nil	10mm	Nil	Nu	Nu	Nil	Nu
PN	Nil	Nil	8mm	Nu	Nu	Nil	Nu

KEY: PEF = Perfloxacin 10µg, CN = Gentamycin 10µg, APX = Ampiclox 30µg, Z = Zinnacef 20µg, AM = Amoxacilin 30µg, R = Rocephin 25µg, CPX = Ciprofloxacin 10µg, S = Streptomycin 30µg, SXT = Septrin 30µg, E = Erythromycin 10µg, OFX= Tarvid 10µg, AU = Augmentin 30µg, CEP = Ceporex 10µg, NA = Nalidixic Acid 30µg, PN = Ampicilin 30µg, Nil = no zone of inhibition, Nu = Not Used

Table 8. Antibiotic Sensitivity Test of the Isolates from Concrete Pond 4

Antibiotic	***E.coli***	***Acinetobacter spp***	***Klebsiella spp***	***Staphylococcus spp***	***Bacillus spp***	***Aeromonas spp***	***Micrococcus spp***	***Shigella spp***
PEF	16mm	8mm	Nil	Nil	Nil	12mm	Nil	13mm
CN	15mm	18mm	17mm	Nil	Nil	12mm	Nil	16mm
APX	Nu	19mm	Nu	Nil	Nil	Nu	15mm	Nu
Z	Nu	Nil	Nu	Nil	Nil	Nu	11mm	Nu
AM	Nu	Nil	Nu	Nil	Nil	Nu	Nil	Nu
R	Nu	Nil	Nu	Nil	Nil	Nu	Nil	Nu
CPX	10mm	11mm	15mm	16mm	18mm	Nil	10mm	10mm
S	14mm	Nil	Nil	Nil	Nil	Nil	12mm	15mm
SXT	10mm	Nil	Nil	Nil	Nil	Nil	Nil	7mm
E	Nu	Nu	Nu	Nil	Nil	Nu	6mm	Nu
OFX	Nil	Nil	11mm	Nu	Nu	Nil	Nu	Nil
AU	Nil	18mm	12mm	Nu	Nu	15mm	Nu	Nil
CEP	Nil	Nil	Nil	Nu	Nu	Nil	Nu	Nil
NA	Nil	13mm	Nil	Nu	Nu	Nil	Nu	Nil
PN	Nil	Nil	9mm	Nu	Nu	Nil	Nu	6mml

KEY: PEF = Perfloxacin 10µg, CN = Gentamycin 10µg, APX = Ampiclox 30µg, Z = Zinnacef 20µg, AM = Amoxacilin 30µg, R = Rocephin 25µg, CPX = Ciprofloxacin 10µg, S = Streptomycin 30µg, SXT = Septrin 30µg, E = Erythromycin 10µg, OFX= Tarvid 10µg, AU = Augmentin 30µg, CEP = Ceporex 10µg, NA = Nalidixic Acid 30µg, PN = Ampicilin 30µg, Nil = no zone of inhibition, Nu = Not Used.

Discussion

Findings from this work showed that there are presence of antibiotic-resistant bacteria in fishponds. These agrees with the result of Maduwube (2024) which stated that the total heterotrophic bacteria count revealed a relatively high bacteria concentration in the fishpond water though the bacteria concentration varies with ponds. Adebami *et al.,* (2010) highlighted that heterotrophic bacterial counts range from 7.30 x 10^5 cfu/ml to 1.6 x 10^6 cfu/ml and that this could be due to duration of fishpond and the quality and physiochemical properties of water used in fishpond.

Omotoso *et al.,* 2025 explained that high bacterial loads and total bacterial loads and total coliforms counts in fishpond exceeds world health standard (WHO, 2020) and this shows poor quality of water serious public health risks.

Table 3 shows that occurrence of bacteria isolates from fishpond water and the bacteria isolates from fishpond and the bacteria include *E. coli, Actinetobacter sp, Klebsielle sp, Staphylococcus sp, Bacillus sp, Aeromonas sp, Micrococus sp* and *Shigella Sp*. This is in line with the work of Omotoso *et al.,* 2025. In their research, they highlighted that the presence of *E. coli*, *Salmonella* and other pathogens underscores the risks aquaculture creates to aquatic ecosystem as well as human health. They also explained that the isolation of *Aeromonas* sp. and *Salmonella* sp. have been associated with contamination with human and animal activities.

Also Lopes *et al.,* 2022 and Chen *et al.,* 2022 stated that the prevalence of these pathogens in fishponds indicates poor hygiene and waste disposal practices.

This work also determines the antibiotic resistance among these bacteria isolates. These bacteria are slightly susceptible to pefloxacin, ciprofloxacin and gentamicin and resistance to other antibiotics as shown in tables 5 to 8. This agrees with the work of Faruk *et al*., 2021 who agreed that resistance is due to selective pressure from extensive antibiotic exposure and gene transfer in aquatic environment.

Amugui *et al.,* 2022 explained that the misuse of fluroquinolones (ciprofloxacin) is also one of the contributions of resistant strains. The contamination of fishpond environment by mounting resistance, *E. coli* serves as a reservoir to resistance genes, and this can spread to human pathogens thereby becoming public health challenge (Shen *et al*., 2020)

Conclusion

These findings shows the urgent need for proper measures to control antibiotic use in aquaculture, upgrade wastewater management and encourage long-lasting aquaculture practices to reduce the spread of antibiotics resistance.

Recommendation

Fishponds should be properly and strategically constructed in a way to reduce exposure to pollutants and some weeds that institutes harmful microorganisms in the pond.

Government should enforce guidelines for aquaculture through NAFDAC to minimize the overuse of antibiotics in fishponds. Fish farmers should also endeavor to use good quality water and fish feed. They should also adopt the method of draining their ponds regularly and checkmate public access to fish farms to enable in the prevention of contamination.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional or non-professional conflict that would have appeared to influence the work reported in this paper.

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