



Assessment of Bacterial Contamination of Fish from Aquaculture Sources and Its Public Health Implications in Abakaliki, Ebonyi State, Nigeria

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ABSTRACT: The objective of this paper was assessment of bacterial contamination of fish from aquaculture sources and its public health implications in Abakaliki, Ebonyi state, Nigeria using appropriate standard procedures. Colony enumeration of bacteria isolates from fish harvested in Abakaliki revealed a high Bacteria count of 6.5×10^7 cfu/g, and lowest of 1.9×10^3 cfu/g, while 4.09×10^4 is the average mean. The isolates were identified and characterized using standard microbiological procedures. *Staphylococcus aureus* was the most prevalent bacterium (26%), *Vibrio cholera* (22%), *Shigella* (13%) while the least *Salmonella* has (9%). The Gram positive bacteria (*Staphylococcus aureus*) were highly susceptible to ciprofloxacin (100 %) and ofloxacin (100 %) but highly resistant to ampicillin (100 %). Gram negative pathogens (*Escherichia coli*, *Vibrio cholera* and *Shigella* species) were highly susceptible to perofloxacin (100 %) and Ofloxacin (100 %). It also showed high resistance to amoxicillin (100 %), chloramphenicol (100 %), gentamicin (100 %), nitrofurantoin (100 %). The high prevalence of antibiotic-resistant bacteria in this study is a serious concern as the resistance patterns of *E. coli*, *S. aureus*, *Salmonella* species, *Vibrio cholerae* and *Shigella* can have implications on human health, thereby suggesting that fish processors and vendors should improve handling hygiene and consumers should also handle fish properly in order to minimize possible health hazards.

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Over the last 35 years, Nigeria's aquaculture production has increased by 12% per year, from 6,000 tonnes in 1980 to approximately 307,000 tonnes in 2016 (Worldfish, 2018). Nigeria is the largest fish

producer in sub-Saharan Africa, accounting for 52% of all fish farmed in the region (Bordas *et al.*, 2021). Fish are omnivorous and are reared in farms such as ponds, cages and pens. They are bony fish who seen

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the body surface, fins and barbells are covered with skin composed of non-keratinized stratified squamous epithelial cells. The skin is an organ that interacts with the environment and plays an important homeostatic role, including protection, perception, communication, excretion, locomotion, regulation, respiration, thermoregulation and immunity (Bordas *et al.*, 2021). Fish has served as a fundamental food source for humanity for centuries and continues to play a vital role in dietary practices. It is recognized as an economical source of high-quality protein accessible globally (Cluces and Ward, 2008). The consumption of fish is favored due to its high biological value, characterized by excellent protein retention, low cholesterol levels, and the presence of essential amino acids (FAO, 2020). Additionally, fish represents a significant source of income for both fishermen and aquaculture practitioners, providing employment opportunities in developing nations (Felix *et al.*, 2018). Over the last few decades, the fisheries and aquaculture sectors have experienced substantial growth, with total production, trade, and consumption reaching a historic peak of 179 million tons in 2018 (FAO, 2013). Freshwater rivers and lakes host complex microbial flora that includes both real aquatic organisms and materials derived from land, animals and plants. The initial microbial community on the surface of the fish body is directly related to the surrounding water, while the bacterial community in the digestive tract corresponds to the state of the fish body. The first point of contact of many bacteria in the water is the skin. The combination of bacteria on the skin of the fish leads to dermatitis, regardless of fish size, causing colonization with many infectious bacteria, leading to high osmotic life, increased energy expenditure, poor movement due to poor mucus production, inadequate swimming, increased water consumption due to increased color change and poor quality of oxygen consumption (Noga, 2019) and (Ibrahim and Mesalhy, 2020). These skin lesions can negatively impact the performance and productivity of affected fish. In response, farmers and veterinarians often resort to various antimicrobial agents to address bacterial infections. However, there have been instances of treatment failures, frequently attributed to the development of resistance among the pathogens involved (Olatoye and Basiru, 2019). Understanding these dynamics is crucial for effective management. Globally, the use of antibiotics as a tool to promote the development of aquaculture and the potential for pathogens to spread in soil and water appears to be the cause of many drug reactions in animals and humans (Cabello, 2020). This use is expected to lead to the development of many resistant drugs. This is because antibiotics are used at sub-

therapeutic doses, leading to resistance. Therefore, most fish farmers meet this growth demand by adding antibiotics to stimulate growth for both treatment and conservation purposes (FAO, 2005). Sub-therapeutic use of antibiotics in animals for treatment, prophylaxis or growth purposes can result in the transfer of resistance genes from animals to humans, thus creating a reservoir of resistant bacteria (Angulo *et al.*, 2004). Subsequent infection of fish with antibiotic-resistant bacteria can pose a threat to public health as they can transmit to the important diseases to the same individuals (O'Brien, 2002). Due to the emergence of resistant bacteria, antibiotics used to treat infectious diseases in humans are becoming increasingly expensive, costly and ineffective (FAO, 2013). To this end, bacterial pathogens are a great threat, it is on the above ground that the need arises to carry out the study. However, the study will be of immense benefit to policy maker in aquaculture, biologist and fish farmers sectors to have a comprehensive report and data, the study will as well help researchers to have a clear evidence and knowledge gap on bacteria associated with fish. Hence, the objective of this paper is to assess the bacterial contamination of fish from aquaculture sources and its public health implications in Abakaliki, Ebonyi State, Nigeria

MATERIALS AND METHOD

Study area and description: The study was carried out in Abakaliki Local Government Area of Ebonyi State, Nigeria. Ebonyi State is located in the southern part of Nigeria, approximately between longitudes 7°30' and 7°E and, latitude 5°40' and 6°45' N. It has a population of 149,683, and a land mass of about 5,935 square kilometers (NPC census, 2006). Ebonyi State is bounded to the east by Enugu State to the west by Cross River State and to the north by Benue State, and south by Abia State respectively as shown in fig. 1.

Sample Collection: a total of ten samples was collected, 5 fishes and 5 pond water. The fishes were collected through the help of fish handheld net of about 5-5 feet was used during the fish capturing. The purpose of using handheld fishing net is to avoid injury that might occur during the fish collection. The fishes collected from different farms in the same location were transported to the laboratory in a bowl containing water to avoid the fish dying before commencement of practical. A sample was collected from the intestine of the fish using swab stick after dissecting with blade. After the sample collection, it was inoculated into a nutrient broth in an incubator overnight at 37°C. *Method of Pond Water Collection:* About 200ml volume of water was collected in a

sterile bottles and transported to the laboratory at approximately 8⁰C and measured in 5ml in a reagent bottle, this was processed within 4 hours at Applied Microbiology Complex of Ebonyi State University Abakaliki

Serial Dilution for Pond Water, Sample C: Using 5ml syringe, 1ml of pond water was taken and mixed homogeneously with distilled water in the first test tube (1st dilution factor) and 1ml of water was taken the 1st dilution factor to the 2nd dilution factor which was mixed again and 1ml was taken from the 2nd dilution factor to the 3rd dilution factor. We did 3 fold serial dilution for pond water. After the dilution, 1ml was taken from the mixture and was pour into Petri dish, Five (5) Petri dish since the water sample is 5 samples. Then Nutrient agar was poured into the

plates and allowed to gel for some minutes and was incubated over night at 37⁰C. The measurement of 1g of soil was measured from each of the 5 soil sample provided and was poured into a test tube containing distilled water, it was mixed homogeneously using each of the test tube, 1ml of the water was transferred to the next test tube containing distilled water until 5 fold serial dilution was completed. After the dilution, 0.5ml was measured and poured into the 5 petri dish for the five samples, and then nutrient agar was poured into each of the petri dish and was allowed to gel and then incubated overnight at 37⁰C. The number of samples collected include: (i) Pond Water (200ml) (ii) Fish (5) The sand sample was collected from each of the four corner of the pond. Each collected samples were identified and labeled accordingly Bergey and Holt, (1994).

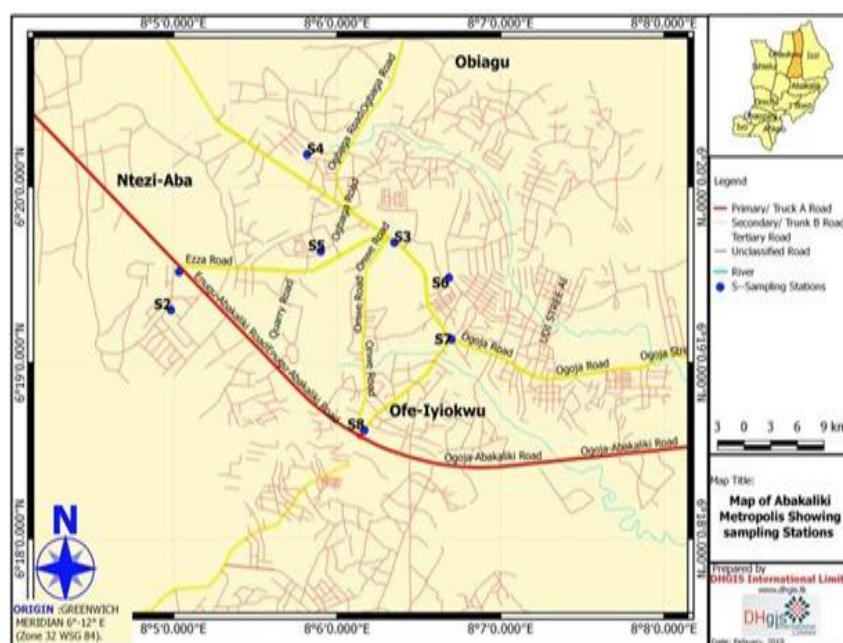


Fig 1: Map of Abakaliki L.G (source Wikipedia)

Statistical Analysis: The raw data on the bacteria isolate obtain in the course of the study was presented as mean in tables while relevant data was interpreted using simple descriptive statistics such as minimum, maximum, and one way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

According to Ibemenuga and Okeke (2019), research report has shown many infectious diseases that have been associated with the consumption of contaminated fish like; cholera, dysentery, diarrhea, typhoid and other life-threatening chronic diseases. Consequently, infectious disease outbreak observed in different communities of the world as a result of

eating infected fish mostly by biological agent which have further threaten the global health system (FAO, 2020).

Colony enumeration is very useful in the understanding of the contamination level by different microorganisms from a sample drawn from a particular habitat. In this study, the enumeration of bacteria isolates from fish harvested in Abakaliki revealed high Bacteria count of 6.5×10^{-4} CFU/g, and lowest of 1.9×10^{-4} CFU/g, while 4.09×10^{-4} is the average mean as shown in Table 1. The results indicated a high microbial load exceeding the safety standards recommended by the World Health Organization for both water and fish samples. This

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elevated level may be linked to human activities such as washing, bathing, indiscriminate waste disposal, and open defecation in proximity to the riverbanks, as well as flooding in the Abakaliki metropolis that drains into the river (Igwe *et al.*, 2024). The variation in bacterial loads among the 40 selected freshwater and domesticated fish samples aligns with the understanding that the presence of bacteria in natural aquatic ecosystems is influenced by the degree of contamination and the balance established between bacterial proliferation and their elimination in that environment (Lejeune *et al.*, 2001). The total bacteria count of catfish water samples was higher than that of WHO standard (1.0×10^3 CFU/100 ml). This may be due to the fact that people living around the Abakaliki metropolis use the Ebonyi River as a dumping ground, open defecation, and lack of proper waste management. This study agrees with Atribom and Kolndadacha (2014), Fidelis, (2019), Novotny (2019), and Olugbojo and Ayoola (2020). This means that most people in the area consider the river as a dumping ground for all types of waste. The bacterial load levels in this study are in line with the study by Amuneke *et al.* (2020) who studied the bacteriological profiles of selected fish species and water samples from Otuocha River in Anambra State, where the bacterial counts were from water samples. When counted in Petri plates 10^{-2} and 10^{-1} , the total bacterial count (TBB) was higher than the World Health Organization (WHO) standard (1.0×10^3 CFU/ml) (1.42×10^5 and 1.72×10^4 CFU/ml/100ml). The differences in the commonly reported bacterial load number

may be due to differences in sanitary conditions in the study areas, and the bacterial count ratios may be explained by differences in sampling locations, different development level occurring each area, and the methods used. In this study, the microscopic analysis revealed *Escherichia coli* was pink colored, with smooth surface, *Staphylococcus aureus* appeared in golden yellow color, *Shigella specie* was pink in color, salmonella black in color, and *Vibrio cholera* golden yellow in color table 2. The morphological characteristics of microorganisms is very crucial to the further understanding of target microbe(s) as it reveals the physical characteristics of such organisms at the preliminary steps. The bio-chemical test showed that *Escherichia coli*, *Samonella* specie, *Shigella* specie, were negative to catalase, while *Vibrio cholera* was positive to catalase test. These observations were in line with reports of Ogbukagu, (2020), respectively who observed similar microorganisms.

Table 1: The Bacteria colony count of the isolate from fish harvested from Abakaliki

S/N	NO. OF COUNT	Cfu/ml
1	22	2.2×10^{-4}
2	31	3.1×10^{-4}
3	35	3.5×10^{-4}
4	48	4.8×10^{-4}
5	65	6.5×10^{-4}
6	63	6.3×10^{-4}
7	52	5.2×10^{-4}
8	22	2.2×10^{-4}
9	52	5.2×10^{-4}
10	19	1.9×10^{-4}
Average mean		4.09×10^{-4}

Table 2: Morphological and Biochemical Characteristics of Bacteria species Isolated from fish Samples in Abakaliki Metropolis.

Morphological characteristics			Microscopic characteristics		Biochemical test							Isolated organisms
SN	Shape	Colour	Gram RXN	Motility Test	OX Test	IND Test	CAT Test	M-red Test	CIT Test	CO	VP Test	
1	Rod	Pink	-	+	-	+	+	+	-	-	-	<i>Escherichia Coli</i>
2	Rod	Yellow	-	+	+	+	+	+	-	-	-	<i>V. cholerae</i>
3	Rod	Pale white & black edges	-	+	-	-	+	+	-	-	-	<i>Salmonella species</i>
4	Rod	Large pink	-	+	-	-	+	+	-	-	-	<i>Shigella spp</i>
5	cocci	Golden yellow	+	-	-	-	-	-	+	+		<i>S. aureus</i>

Key: RXN= Reaction, OX = Oxidase, IND= Indole, CAT=Catalase, CO= Coagulase M=Methyl, CIT= Citrate and VP=VogesProskauer, += positive, - = negative

The frequency analysis indicated that *Escherichia coli* exhibited a 30% occurrence rate, *Staphylococcus aureus* at 26%, *Vibrio cholerae* at 22%, *Shigella* at 13% and *Salmonella* at 9% table 3. This finding aligns with the work of (Ogbukagu, 2020) who recorded different proportions of bacterial isolates.

The high levels of microorganisms found in catfish samples from Abakaliki L.G. collected from different rivers and ponds reflect the waste disposal and management practices of the local population. This observation supports the findings of (Dalgaard *et al.*, 2006) who emphasized that aquatic environmental

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pollution, especially fecal pollution, is primarily a result of human activities. The presence of *E. coli* in food or water indicates potential gastrointestinal disease and poses a risk of transmission of antibiotic resistance in aquatic bacteria to humans (Igwe *et al.*, 2024). *E. coli*, *Klebsiella*, *V. cholerae*, *Salmonella*, and *S. aureus* has been shown to survive and replicate in the gastrointestinal tract and tissues of fish, posing a long-term risk of human disease (Udeze *et al.*, 2012). Supporting this study, (Allamin *et al.*, 2015) reported that the most frequently isolated species in their study was *Aeromonassobria* with an overall prevalence of 98%, followed by *Klebsiella spp.* with 67%, *Salmonella spp.* with 66%, and *Staphylococcus* species at 25%, which represented the lowest prevalence in pond water sources in Kaduna State, Nigeria. The detection of *E. coli*, *Klebsiella*, *V. cholerae*, *Salmonella*, *S. aureus*, *Proteus* and *Brucellosis* in fish may be attributed to the indiscriminate disposal of human and animal waste, as well as other environmental pollutants into ponds and rivers, or through the runoff of land surfaces into water bodies during the rainy season (Cabral, 2010). *Staphylococcus aureus* as the only gram negative was 100 % susceptible to ciprofloxacin, ofloxacin, levofloxacin, 50 % susceptible to ceftriaxone, amoxicilin, 16.7 % susceptible to cephaloxin, 83.3 % and susceptible to gentamycin. But was 100 % resistant to ciprofloxacin, ofloxacin, levofloxacin, 50 % resistant to ceftriaxone, amoxicilin, 83.3 % resistant to ceftriaxone, 16.7 % resistant to gentamycin, and 66.7 % resistant to coxacin table 4. This aligns with the report of Albuquerque *et al.* (2007) and Ekhsuehi, *et*

al. (2018), who worked on bacteriological quality and antibiotic of isolates from potable water Sources in Ekosodin community, Benin City, Nigeria and tested susceptibility and resistance of *Staphylococcus* species on 20 different antibiotics where microbe showed resistance to some antibiotics and was susceptible to other antibiotics. However, the high *Staphylococcus aureus* resistant in this study is in agreement with the work Rahman,(2021), who Investing in sustainable resistant *Staphylococcus aureus* strains isolated from a fish market and from fish handlers.

Table 3 The Percentage Frequency occurrence of the bacterial species implicated in a fresh fish harvested in Abakaliki.

S/N	Bacterial Isolates	% Frequency Occurance
1	<i>Staphylococcus aureus</i>	6(26)
2	<i>E.coli</i>	7(30)
3	<i>Vibrio cholera</i>	5(22)
4	<i>Shigellai</i>	3(13)
5	<i>Salmonella</i>	2(9)

Key: *E. coli* = *Escherichia coli*, % = percentage

Table 4 Antibiotic Susceptibility Profile of Gram positive Bacteria isolate implicated in fresh fish harvested in Abakaliki

Antibiotic Concentrations (ug)	<i>Staphylococcus aureus</i>	
	S (%)	R (%)
CPX(10)	6(100)	0(0.0)
CX(30)	1(16.7)	5(83.3)
Ct(200)	3(50)	3(50)
GN(10)	5(83.3)	1(16.7)
OF(10)	6(100)	0(0.0)
CL(20)	1(16.7)	5(83.3)
E(10)	3(50)	3(50)
LEV(20)	6(100)	0(0.0)
AM(30)	3(50)	3(50)
CL(30)	2(33.3)	4(66.7)

KEY: s = susceptibility, r = resistance, % = percentage

Table 5 Antibiotic Susceptibility Profile of Gram negative Bacteria isolate implicated in fresh fish harvested in Abakaliki

Antibiotics Concentration (ug)	<i>Vibro cholera</i>		<i>E.coli</i>		<i>Salmonella Species</i>		<i>Shigella species</i>	
	S(%)	R(%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
CPX (10)	3(60)	2(40)	5(71.4)	2(28.6)	1(50)	1(50)	3(100)	0(0.0)
MP (10)	0(0.0)	5(100)	2(28.6)	5(71.4)	1(50)	1(50)	0(0.0)	3(100)
ST (30)	0(0.0)	5(100)	1(14.3)	6(85.7)	0(0.0)	2(100)	0(0.0)	3(100)
AX (30)	0(0.0)	5(100)	0(0.0)	7(100)	0(0.0)	2(100)	0(0.0)	3(100)
CT(30)	4(80)	1(20)	1(14.3)	6(85.7)	0(0.0)	2(100)	0(0.0)	3(100)
PEF(30)	5(100)	0(0.0)	4(57.1)	3(42.9)	2(100)	0(0.0)	2(66.7)	1(33.3)
C (30)	0(0.0)	5(100)	0(0.0)	7(100)	0(0.0)	2(100)	0(0.0)	3(100)
OF (30)	5(100)	0(0.0)	6(85.7)	1(14.3)	1(50)	1(50)	3(100)	0(0.0)
GN (30)	2(40)	3(60)	2(28.6)	5(71.4)	0(0.0)	2(100)	0(0.0)	3(100)

Key: *E.coli* =*Escherichiacoli*, s = susceptibility, r = resistance, % = percentage.

Table 5 showed, *Vibro cholera* isolated from fish harvested in Abakaliki was 100 % resistant to meropenem, streptomycin, amoxicilin and chloramphenicol, 40 % resistant to ciprofloxacin, 20 % resistant to ceftriaxone, 60 % resistant to gentamycin, 0 % resistant to pefloxacin, and ofloxacin. But 100 % susceptible to pefloxacin and

ofloxacin, 0 % susceptible to meropenem, streptomycin, amoxicilin and chloramphenicol, 60 % susceptible to ciprofloxacin, 80 % susceptible to ceftriaxone, and 40 % susceptible to gentamycin. *Escherichia coli* was 100 % resistant to amoxicilin and chloramphenicol, 28.6 % resistant to ciprofloxacin, 71.4 % resistant to meropenem,

gentamycin, 85.7 % resistant to streptomycin, ceftriaxone, 14.3 % resistant to ofloxacin, 42.9 % resistant to pefloxacin, and 28.6 % resistant to ciprofloxacin. But was 0 % susceptible to amoxicillin, chloramphenicol, 14.3 % susceptible to streptomycin, and ceftriaxone, 71.4 % susceptible to ciprofloxacin, 28.6 % susceptible meropenem and gentamycin. *Salmonella* specie was 100 % resistant to streptomycin, amoxicillin, ceftriaxone, chloramphenicol and gentamycin, 50 % resistant to ciprofloxacin, meropenem and ofloxacin, 0 % resistant to pefloxacin. But was 0 % susceptible to streptomycin, amoxicillin, ceftriaxone, chloramphenicol and gentamycin, 50 % susceptible to ciprofloxacin, meropenem and ofloxacin and 100 % susceptible to chloramphenicol. *Shigella* specie was 0% resistant to ciprofloxacin and ofloxacin, 100 % resistant to meropenem, streptomycin, amoxicillin, ceftriaxone, chloramphenicol and gentamycin, 33.3 % resistant to pefloxacin but was 100 % susceptible to ciprofloxacin and ofloxacin, 66.7 % susceptible to pefloxacin, and 0 % susceptible to meropenem, streptomycin, amoxicillin, ceftriaxone, chloramphenicol, and gentamycin. The observation here is similar to the report of Okeh *et al.* (2022) who tested susceptibility and resistance of *Escherichia coli*, *Shigella* specie, and *Salmonella* specie on 10 different antibiotics where these microbes showed resistance to some antibiotics and susceptible to other antibiotics in Ebonyi state. Similar observations have been reported in previous studies, including those by Kinge *et al.* (2010) and Grema *et al.* (2015). This is further corroborated by Overdeest *et al.* (2011), who noted a significant increase in antibiotic resistance among enteric bacteria over the past decade. The current results indicate a concerning rise in antibiotic resistance among bacterial isolates from fish and aquatic environments, consistent with findings by Albuquerque *et al.* (2007), which highlighted a growing trend of antibiotic resistance in bacterial isolates from fish. While the use of antibiotics in human medicine has played a role in the emergence of resistant bacteria, the application of antibiotics in animal husbandry has exacerbated this issue, complicating treatment options for human diseases (Novotny *et al.*, 2004). Notably, the documented transfer of resistant bacteria from aquatic animals to humans via the food production chain poses a significant public health risk (Grema *et al.*, 2015). Certain pathogenic bacteria, including *Escherichia coli*, *Staphylococcus aureus*, and *Shigella*, have been linked to skin ulcers, with these isolates demonstrating multidrug resistance likely due to the misuse of antimicrobial agents in aquaculture. To mitigate this issue, it is essential to enhance the sanitary conditions of fish rearing practices by

adhering to established standards, such as utilizing high-quality water, employing feeds with superior microbial quality, regularly draining pond water, and restricting public access to these ponds. Furthermore, the longstanding practice of using antibiotics as growth promoters in aquaculture should be actively discouraged.

Conclusion: This study has therefore proven the need for the adoption of proper hygienic measures and also hygienic education for fish handlers/traders and consumers. The resistance patterns as observed in this study imply that some components of resistance are likely related to environmental origins and may disseminate without the selective pressure of antibiotic use. There is a need to research more on the antimicrobial susceptibility and surveillance in aquatic environments where fish from pond and other fish habitat are obtained for human consumption.

Declaration of Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: Data are available upon request from the corresponding author.

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