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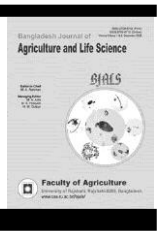

Antibiogram Profile of *Escherichia coli* Isolated from Pangas (*Pangasius sutchi)* Reared in Feed-Based Aquaculture Systems

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ABSTRACT

The study was conducted to isolate and identify the *E. coli* bacteria from pangas catfish (*Pangasius sutchi*) raised in feed-based aquaculture systems and to determine the level of antibiotic susceptibility rates against commonly used antibiotics in aquaculture. The fish samples were collected from different feed-based aquaculture ponds in the Rajshahi region, Bangladesh. The laboratory tests were performed in the laboratory of the Department of Fisheries at the University of Rajshahi from January to March 2024. The bacteria were isolated by selective enrichment and cultured on different agar media following the standard methods. The bacterial isolates were confirmed based on their morphological and biochemical characteristics using different biochemical tests. The antibiotic susceptibility pattern was studied by disc diffusion method with a panel of nine antibiotics viz., penicillin (10µg), vancomycin (30µg), ciprofloxacin (5µg), gentamicin (10µg), ampicillin (25µg), rifampicin (5µg), kanamycin (5µg), erythromycin (15µg), and amoxicillin (10µg). The identified *E. coli* isolates revealed resistance rates of 88.89%, 66.67%, 55.56%, 77.78%, 11.11%, 50.0% and 33.33% to penicillin, vancomycin, ampicillin, rifampicin, kanamycin, erythromycin, and amoxicillin, respectively. On the other hand, *E. coli* isolates revealed intermediate resistance rates of 11.11%, 22.22%, 44.44%, 22.22%, 27.78%, 50.0% and 66.67% to penicillin, vancomycin, ampicillin, rifampicin, kanamycin, erythromycin, and amoxicillin, respectively. The isolates also showed sensitivity rates of 11.11%, 100%, 100.0%, and 61.11% to vancomycin, ciprofloxacin, gentamicin, and kanamycin, respectively. The results of the study indicated that *E. coli* bacteria present in *P. sutchi* reared in feed-based aquaculture in Rajshahi were found to be resistant to commonly used antibiotics. If proper management is not followed, fish farmers and consumers could face additional challenges due to the resistant bacteria.

Introduction

Aquaculture continues to be the fastest-growing animal food-producing sector, accounting for about 46% of the total food fish supply to meet the protein needs of the increasing global population (FAO, 2016).

There has been a general increase across all continents in aquaculture's share of total fish production due to various intensifications (FAO, 2016). Aquaculture plays a vital role in socio-economic and livelihood development in Bangladesh. Presently, the widespread adoption of feed-based commercial fish farming with a variety of species (carp, catfish, tilapia, etc.) is evident. Pangas (*Pangasius sutchi*) is one of the important species thathas expanded tremendously throughout the country, associated with the commercialization of aquaculture. This fish is considered one of the key species in feed-based aquaculture farming because of its fast growth, year-round production, and high productivity

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(Abedin *et al*., 2020). However, to achieve more production, farmers have adopted indiscriminate and unplanned use of feed that affects the environment, ultimately increasing stress on the fish and enhancing their susceptibility to various pathogens, especially bacteria, which lead to diseases in fish. Several pathogenic bacteria are associated with fish and the fish culture environment, among which *Escherichia coli* is significant because some strains can be transferred to humans and cause foodborne diseases. To minimize the detrimental effects of pathogenic bacteria, various types of antimicrobial agents, apart from antibiotics, have been used by farmers for treatment for a long time. Antibiotics are the most significant type of antibacterial agent for combating bacterial infections (Mog *et al*., 2020). In modern culture systems, antibiotics are employed prophylactically and therapeutically to protect fish from bacterial diseases and promote growth (Avsever*et al*., 2010). However, most farmers in our country do not have adequate knowledge about the stability and efficacy of antibiotics (Mog *et al*., 2020). They are often influenced by extensive promotional campaigns from pharmaceutical companies, which encourage the indiscriminate use of antibiotics without understanding their necessity and proper dosage. Such uncontrolled antibiotic practices within the aquaculture system may contribute to the rise of antimicrobial resistance (AMR). Unfortunately, the number of antibiotic-resistant mutants of *E. coli* in the aquatic environment is increasing day by day.

The occurrence of antimicrobial resistance indicates the possibility of transferring antimicrobial resistance (AMR) genes from the aquatic environment to humans who are in close contact with the fish (Jacobs and Chenia, 2007). Thus, regular investigations into the susceptibility patterns of antibiotics used in aquaculture ponds are essential to develop awareness about safe fish production and minimize public health risks. However, several studies have been conducted on the susceptibility patterns of antibiotics used in aquaculture ponds by different authors (Kerigano*et al.*, 2023;Ayoub *et al.*, 2021; Hamom *et al.*, 2020; Begum *et al.*, 2019), but the susceptibility pattern of antibiotics used in catfish culture in the Rajshahi region is scarce. Therefore, the present investigation was conducted to isolate and identify *E. coli* bacteria from pangas (*P. sutchi*) raised in a feed-based aquaculture system and to determine the levels of antibiotic susceptibility rates of the isolated bacteria against commonly used antibiotics.

Materials and methods

Study design

The entire study was divided into four steps. The first step involved the collection of samples. The second step involved the preparation of bacterial samples and culture media for the isolation of suspected bacteria. The third step included the identification of the bacterial isolates through testing characteristics. Lastly, the fourth step included the determination of the antimicrobial sensitivity pattern of the suspected bacteria.

Collection and preparation of the sample

The fish samples (*P. sutchi*) were collected from various feed-based culture ponds located in the Rajshahi region, Bangladesh. During collection, fish samples were handled to avoid touch, and an icebox was used to maintain a cold chain. The collected samples were analyzed in the Quality Control Laboratory in the Department of Fisheries at the University of Rajshahi for three months (January to March 2024). The intestine, gut, and muscle of the fish were used for microbiological testing. Ten (10) grams of samples were homogenized using a stomacher machine with 90 milliliters of freshly prepared 0.1% peptone salt solution.

Selective enrichment and isolation of bacteria

One (1) mL of the prepared samples was inoculated into test tubes containing 9 mL of MacConkey Broth with Neutral Red. After inoculation, the test tubes were incubated at 37°C for 24 hours for bacterial growth. Then, one loop of enriched bacteria from MacConkey broth was streaked onto selective and non-selective agar media, including nutrient agar, MacConkey agar (MCA), Eosin Methylene Blue (EMB) agar, Blood Agar (BA), and Tryptone Soya Agar (TSA), respectively. After streaking, all the media were incubated at 37°C for 24 hours.

Identification of bacteria

Colony morphology, including shape, size, surface texture, edge and elevation, color and opacity, pigmentation of the suspected colonies on different agar media developed after 24 hours of incubation, was carefully observed. Various biochemical tests, namely Catalase, Coagulase, Methyl Red (MR), Voges-Proskauer (VP), Indole, Triple Sugar Iron (TSI) agar slant reaction, and Citrate Utilization test, were performed to identify the bacteria.

Antibiotic sensitivity test

The antimicrobial sensitivity testing of isolated bacteria was conducted using the Kirby-Bauer disc diffusion method. A total of nine antibiotic sensitivity discs, including Penicillin (10 µg), Vancomycin (30µg), Ciprofloxacin(5 µg), Erythromycin (15 µg),

Gentamicin (10 µg), Ampicillin (25 µg), Amoxicillin (10 µg), Rifampicin (5 µg), and Kanamycin (5 µg), were utilized for the examination to determine the antibiotic susceptibility patterns of the isolated bacteria. The bacterial isolates were spread onto Mueller-Hinton Agar (MHA) plates using a swab, which resulted in heavy growth. Then, the antibiotic discs were placed aseptically on the surface of the agar plates. The plates were incubated at 37ºC for 24 hours. The zone of inhibition was measured using slide calipers, and the isolates were classified as resistant, intermediate, or sensitive to a particular antibiotic based on the standard interpretation updated according to the Clinical and Laboratory Standards Institute guidelines.

Data analysis

Documented data were sorted, tabulated, and analyzed with the help of computer software MS-Excel (Microsoft Office Professional Plus 2019).

Results

Isolation and identification

All the samples were bacteriologically tested and resulted positive for *E. coli* bacteria, which were identified through morphological characteristics, including colony color, shape, and transparency in different media as well as a series of biochemical tests such as catalase, coagulase, MR-VP, indole, TSI, and citrate utilization (Tables 1 & 2 and Plate 1 & 2).

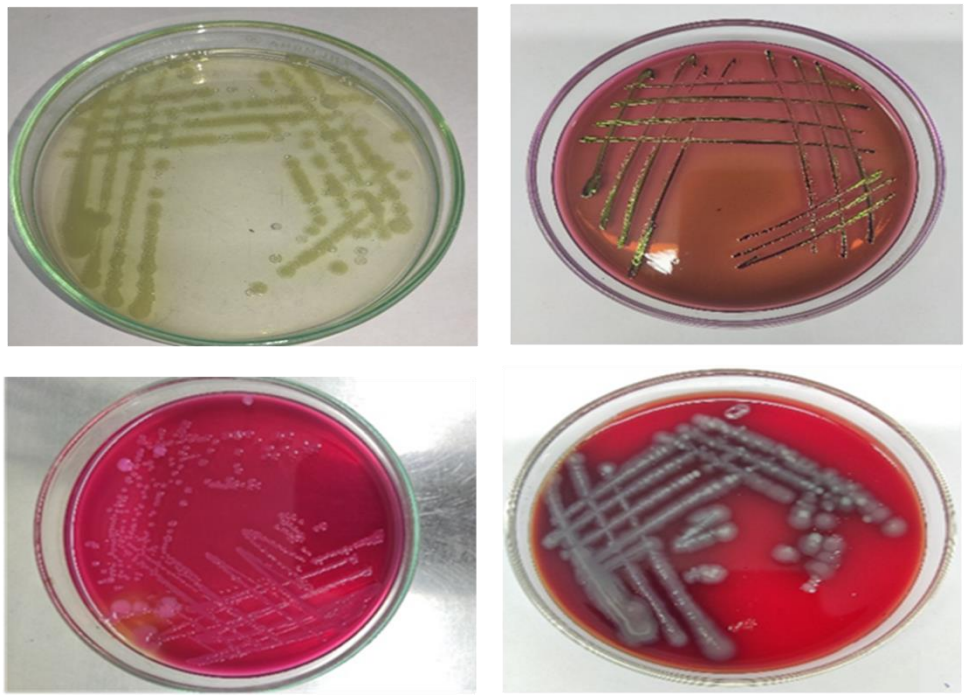

Plate 1: Morphological characteristics of *E. coli* isolates in different agar media

Table 1: Summary of cultural characteristics of *E. coli* on the different agar media

Name of the media	Characteristics
Nutrient Agar (NA)	Round, smooth, and whitish-gray colonies
Mac Conkey Agar (MCA)	Smooth, pink colonies
Blood agar (BA)	Circular, smooth, and whitish-gray in colonies
Eosin Methylene Blue (EMB) Agar	Smooth, circular metallic green sheen colonies

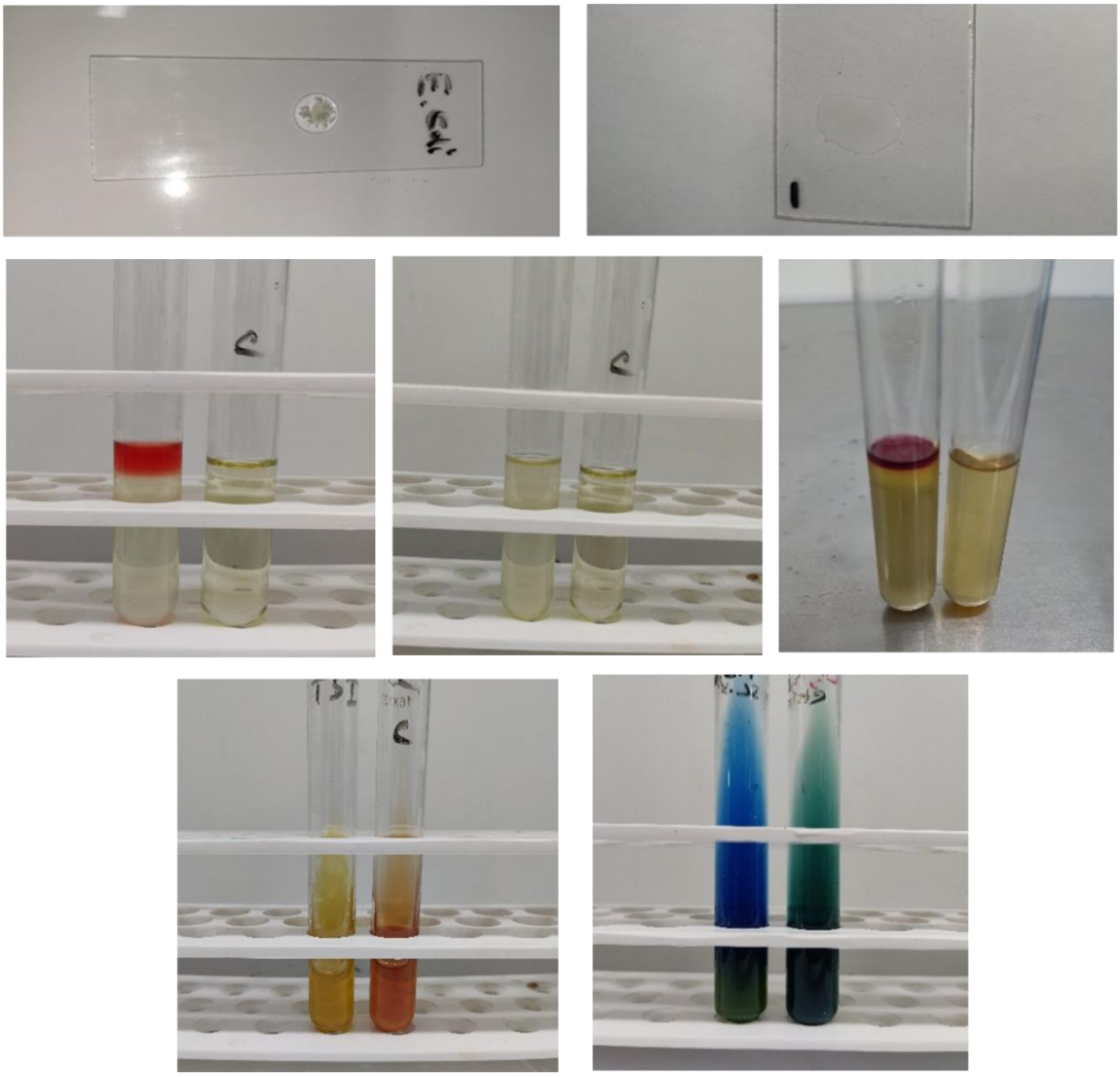

Plate 2: Biochemical tests results of *E. coli*

Table 2: The summary of biochemical tests result of the isolated *E. coli*

Test name	Result
Catalase test	+ve
Coagulase test	-ve
Methyl Red (MR) test	+ve
Voges-Proskauer (VP) Test	-ve
Indole Test	+ve
Triple Sugar Iron (TSI) Test	+ve
Citrate Utilization	+ve

Antibiotic sensitivity test

The results of the antibiotic sensitivity pattern of the isolated *E. coli* are shown in Table 3, Fig.1 and Plate 3. The isolated *E. coli* showed 88.89%, 66.67%, 0.0%, 55.56%, 77.78%, 11.11%, 50.0% and 33.33% resistant to penicillin, vancomycin, ciprofloxacin, gentamicin, ampicillin, rifampicin, kanamycin, erythromycin, and amoxicillin, respectively. On the contrary, 0.0%, 11.11%, 100%, 100%, 0.0%, 0.0%, 61.11%, 0.0% and 0.0% sensitive to penicillin, vancomycin, ciprofloxacin, gentamicin, ampicillin, rifampicin, kanamycin, erythromycin and amoxicillin, respectively. Intermediate sensitivity was also observed in the isolate to the antibiotics, and those were 11.11%, 22.22%, 0.0%, 0.0%, 44.44%, 22.22%, 27.78%, 50.0% and 66.67% to penicillin, vancomycin, ciprofloxacin, gentamicin, ampicillin, rifampicin, kanamycin, erythromycin, and amoxicillin, respectively (Table 3 and Fig. 1).

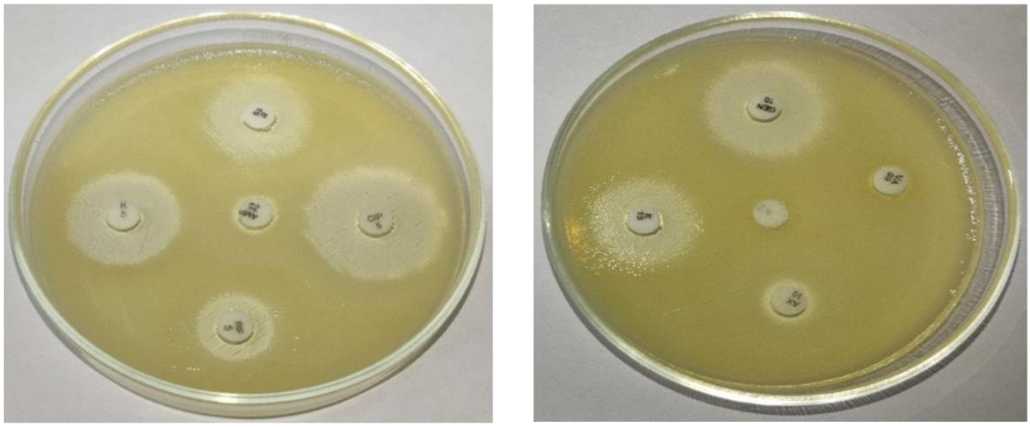

Plate 3: Antibiotic sensitivity and resistant pattern of *E. coli* on Mueller Hinton Agar (MHA) media

Table 3: Results of antibiotic sensitivity pattern of *E. coli* isolated from *P. sutchi*

Name of Antibiotic	**Dose (µg)**	**Antibiotic sensitivity pattern (%)**		
		Resistant	**Intermediate sensitive**	**Sensitive**
Penicillin	10.	88.89	11.11	0.0
Vancomycin	30	66.67	22.22	11.11
Ciprofloxacin	5	0.0	0.0	100.0
Gentamicin	10	0.0	0.0	100.0
Ampicillin	25	55.56	44.44	0.0
Rifampicin	5	77.78	22.22	0.0
Kanamycin	5	11.11	27.28	61.11
Erythromycin	15	50.0	50.0	0.0
Amoxicillin	10	33.33	66.67	0.0

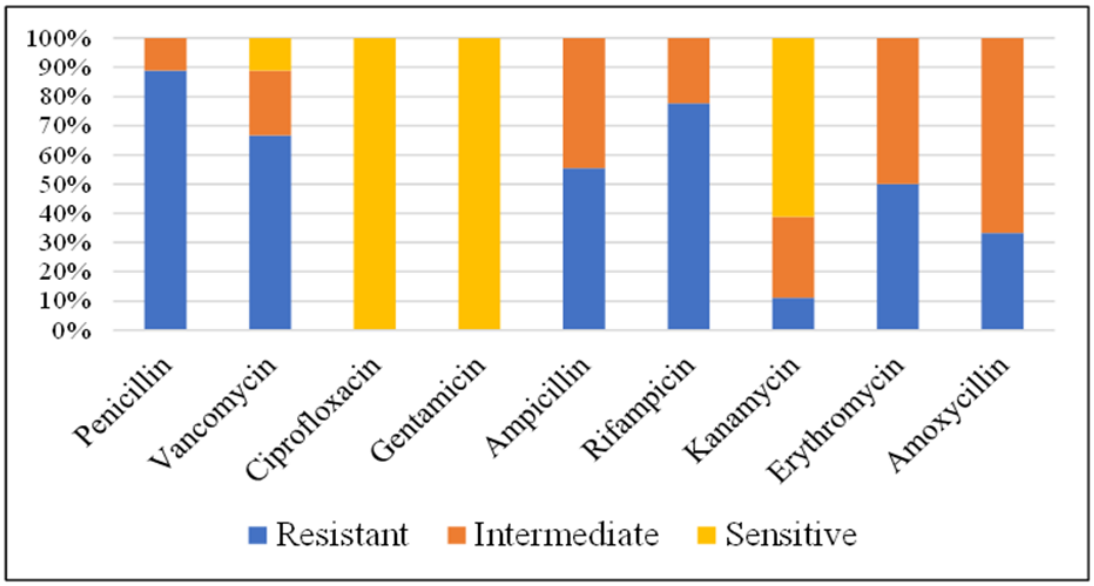

Fig. 1. Antibiotic sensitivity pattern of *E. coli* isolated from *P. sutchi*

Discussion

Pangas (*P. sutchi*) is considered an important catfish species in Bangladesh. Pangas farming often involves high stocking densities and feeding rates, which can lead to increased organic matter in the water. This organic matter can contribute to the growth and survival of *E. coli*. The presence of *E. coli* in catfish, especially high levels or certain strains, can pose a risk to both fish and human health. It's a common indicator of fecal contamination and the potential presence of other harmful bacteria. It can be introduced through various sources such as contaminated water, contaminated fish feeds, feces from other animals, and even wild birds. Runoff from pastures and farmland, or flooding, can carry *E. coli* into catfish ponds. Various studies have been conducted to understand the prevalence, antibiotic resistance, and potential health risks associated with *E. coli* in catfish (Ngoc *et al*., 2022; Khan *et al*., 2023; Amin *et al*., 2024) and other fish species (Begum *et al*., 2019; Kusunur*et al*., 2022; Rana *et al*., 2025). However, in the present study, the presence of *E. coli* in all the samples of muscles, liver, and intestine of apparently healthy *P. sutchi* from the feed-based aquaculture ponds was confirmed by the various morphological and biochemical studies. Its presence in aquaculture settings can indicate potential issues with water quality and hygiene of the selected farms. The presence of *E. coli* in intestines, gills, liver, spleen, and brain of different freshwater fish species (Al-Harbi, 2003; Guzman *et al*., 2004) and culture ponds is a common occurrence, but it's crucial to monitor its levels and implement appropriate management strategies to mitigate potential risks to fish health and food safety.

While the presence of *E. coli* doesn't solely indicate the fish is unsafe to eat, it's a food safety concern due to potential antibiotic resistance. *E. coli* is a common bacterium found in pangas fish and their culture environment, and studies have shown that a significant portion of *E. coli* isolates from the ponds exhibit antibiotic resistance. In present study, the resistance analysis of *E. coli* to various antibiotics showed that the isolates were found to be varying degree of resistant to penicillin, rifampicin, vancomycin, ampicillin, erythromycin, and amoxicillin. The present findings are identical with the report of Karki *et al*. (2013) who found all isolates from fish revealed resistance to penicillin andsensitivity to gentamicin. The *E. coli* isolates in this study were sensitive to ciprofloxacin, gentamicin, and kanamycin. This is in line with the findings of Abraham *et al*. (2011) and Al-Salauddin*et al*. (2015) whose findings were similar to bacterial human pathogens highly sensitive to ciprofloxacin and gentamycin. The isolates of *E. coli* were observed to be multidrug-resistant, which may pose a severe threat to public health. The antibacterial resistance observed in the isolated *E. coli* might be due to the

indiscriminate use of these antibacterial agents in fish where they were cultured. In Bangladesh, the issue of multidrug-resistant strains of *E. coli* is frequently raised due to the abuse of antibiotics (Islam *et al*., 2016).

Conclusion

To minimize the detrimental effects of bacterial infection, various types of antimicrobial agents have been used for treatment purposes in our country for a long time. The present study concluded that *E. coli* bacteria present in pangas catfish (*P. sutchi*) raised in a feed-based aquaculture system in the Rajshahi region were found to be resistant to commonly used antibiotics except ciprofloxacin, gentamicin, and kanamycin. Therefore, due to the widespread emergence of resistant bacteria, it is crucial that susceptibility testing is performed routinely to ensure the effective use of antibiotics and to support the development of sound treatment guidelines.

Authors' Contribution

Conceptualization, MKK and MMR; Methodology, MKK and MMR; Investigation, MKK and IKS; Write-up, MKK and IKS; Supervision, MMR. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interests.

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