

ISOLATION, IDENTIFICATION AND ANTIBIOGRAM STUDY OF BACTERIA ASSOCIATED WITH QUAIL FECES IN SYLHET SADAR, BANGLADESH

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Abstract

The current research was conducted for isolation, identification of isolates, and antibiogram study of quail feces isolated from different quail farms and hatchery in Sylhet, Bangladesh. The data used for this study were randomly collected from three different farms and hatchery in the Sylhet district. A total of 90 samples (30 samples from each farm) were settled for bacterial isolation. In this study, A series of culture and biochemical tests were carried out for the isolation and identification of isolated isolates. The antibiotic sensitivity tests of *E. coli*, *Salmonella* spp., and *Pseudomonas* spp. were determined using the Kirby Bauer disc diffusion method. Out of 90 samples, *E. coli* was found 74 (82.22%), and the highest number of 26 (86.67%) was observed in Shibganj Bazar Sylhet. The second most pathogen of *Salmonella* spp. was recorded in Hashem quail and poultry breeders with the prevalence of 23 (76.67%), whereas the lowest number of *Pseudomonas* spp. was obtained in sarkar quail and poultry hatchery 16.67%, respectively. The antibiotic susceptibility test exposed that *E. coli* were 100% sensitive to Colistin sulfate and Neomycin, whereas cloxacillin showed 100% resistance. *Salmonella* spp. and *Pseudomonas* spp. were shown 100% resistant to Amoxicillin, Ampicillin, and cloxacillin. We isolated the three most pathogenic isolates from different farm and hatchery, and these bacteria create a severe public health risk.

1 Introduction

Bangladesh is an agriculture-based country. About 80% of people of the country are dependent on agriculture either directly or indirectly. Livestock is one of the most important parts of agriculture. The livestock sector is the integral sector of the agro-based economy (Modak et al.,

2012). But the quail is a very newbird that is rearing on a commerciallevel. Quail farming in Bangladesh is growing gradually because of its high rate, pre-sexual maturity, pleasantmeat, high egg production rate, and less floor space for maintenance. In Bangladesh, poultry, eggs, and meataccount for around 38% of total animal protein(Khatun et al., 2020). Quail maybe two sources of income in addition to chicken andducks for its immense potentiality for meat and egg production. At present, quail production has commerciallystarted in small ranges in Dhaka, BAU poultry farm, Mymensingh, Manikgonj, TMSS NGO in Sylhet, Barisal, andanotherdistrict in Bangladesh (Banglapedia). However, the rise of quail manufacturing is being seriously wastedbysomemanagementfactors,includingvariousfatalcontagious,noncontagious,andbloodsucki ngsicknesses (Barnes 1987). Colibacillosis, Salmonellosis, ulcerative enteritis, chicken cholera, quail bronchitis, Newcastle disease, andquail pox are the most prevalent infectious diseases associated with quail production. A disease outbreak canresult in high flock mortality as high as 50% to 90%, negatively affecting a grower's economic bottom line (DeJongDozieret al., 2010). Inpoultry, *E. coli* infections can cause egg peritonitis, omphalitis, coligranuloma, enlarged head syndrome, cellulitis,and colisepticaemia, as well as bird death. This disease is an extensive, severe form of infection characterized bythe existence of*E.coli* inblood andtheconsequence oforgans like theliver, spleen,andheart (Barnes et al., 2003).Therespiratorytract and air sacs are the most prevalent sites of infection, and it is usually due to a mycoplasma orvirusinfection. Affected birds show signs of perihepatitis, pericarditis, and air sacculus. Reduced food consumption,listlessness, ruffled three feathers, laborious quick breathing, and a peculiar"snicking" are all symptoms ofcolisepticemia (Mdegela et al., 2000).

Salmonellosis is another crucial complication that causes severe financial loss due to fatality and decreased eggproduction (Khan et al., 2005). Salmonellae may be involved in a variety ofpathogenic processes inhumans and animals,including poultry. Affected birds appear to be depressed and can show signs of scour. Post-mortem lesionsinclude acute dehydration, septicemia, acute enteritis and classical caecal cores of white caseous materials (Johnston, A. 2007).The mortality rate can be reasonably high, reaching 15% in rare cases. *Pseudomonas* spp. showed resistanceagainst most of the antibiotics, and it is clinically significant. Some species of *Pseudomonas* are opportunisticpathogenic for humans and animals and poultry like *P. aeruginosa*; they also play an essential role in spoilage offoods, dairy products, meat, poultry, and eggs. The clinical cases have accomplished the characterizationofmicroorganismslike *E. coli*, *Salmonella* spp., and *Pseudomonas* spp. in quail birds. For the proper treatment and control of any disease,theisolation,identification,andcharacterizationofnormalbacterialfloraareessentialbecause theywill behelpfulforthe selectionofantibioticsand vaccines.

Immunogenicity of New castledisease vaccine, fowl cholera vaccine, and invivo growthand kinetics ofvirulentNewcastlediseasevirusinsomequailpopulationofBangladeshwereperformed (Ara et al., 2009).Nostudyhasbeenorganized yet on the prevalence of bacterial disease of quail concerning age, sex, verity location, and season.

Totreat bacterial disease, farmers are indiscriminately using a different types of antibiotics

resulting in antibiotic resistance. For this reason, the priority is to isolate and molecular characterization of the causal agent of the diseases and take adequate control measures against that disease. So the current research is planned to characterize the bacterial pathogen in the quail birds of Bangladesh by using selective antibiotics to control the effectiveness of isolates. The research objective was to isolate and identify the bacterial isolates from Quail fecal sample and their antibiogram profile at Sylhet Sadar.

2 Materials and methods

2.1 Study area selection

The current investigation was carried out in Sylhet to extract and identify bacteria from quail feces, as well as to conduct an antibiogram study on bacteria from different quail farms and hatcheries. The research was conducted during the period of 2014-2015 in the Department of Microbiology and Immunology, Faculty of Veterinary and Animal Science, Sylhet Agricultural University (SAU).

2.2 Sample collection

A total of 90 feces samples of quail were randomly collected from three different farms and hatchery, including Sarkar Quail and Poultry Hatchery, Kashem Quail and Breeder Hatchery, and Shibganj Bazar in Sylhet, Bangladesh. 30 samples were collected from each farm and hatchery.

2.3 Isolation and identification of bacteria

After collection, samples were transferred into the laboratory and primarily cultured on nutrient agar and nutrient broth media. Next, different culture media such as MacConkey agar, EMB agar, SS agar, BA, BGA, and MSA (Hi media, India) were used for subculture and pure culture for pure colony isolation of suspected isolates. At that point, all cultured plates were incubated at 37°C for 24 hours with the aseptic condition. In this study, pure bacterial cultures were acquired based on Bergey's manual of determinative bacteriology (1994). Bacterial pure cultures were stored at -80°C with 20% glycerol for further use. A group biochemical test such as indole, MR, VP, oxidase, catalase, motility test, simons citrate, and five sugar fermentation tests was used by following standard protocols (Cheesbrough M. 2003).

2.4 Antibiogram study of isolated bacteria

Eight commercially available antibiotics were used for antibiotic susceptibility tests in this study. According to the guidelines and recommendations of CLSI, the antibiotic sensitivity of isolates using Mueller-Hinton agar (Hi-Media, India) plate by disk diffusion method (CLSI).

2013). The isolates resistant to three or more antibiotics were classified as multidrug-resistant (MDR) strains. Ciprofloxacin (5 µg), Amoxicillin (10 µg), Ampicillin (10 µg), Erythromycin (15 µg), Colistin sulphate (25 µg), Neomycin (30 µg), Chloramphenicol (30 µg), Cloxaciline (5 µg) (Hi media, India) were used for antibiogram study. The zone of inhibition is measured by the guideline of CLSI (CLSI, 2009).

3 Results

3.1 Prevalence of isolated bacteria from different quail farms and hatchery

In this research, we collected a total of 90 samples from three different quail and poultry hatcheries. 30 samples were randomly collected from each farm. Out of 30 Sarkar quail and poultry hatchery samples, *E. coli* were positive for 20 (76.67%), *Salmonella* spp. 20 (66.67%), *Pseudomonas* spp. 5 (16.67%). In Kashem Quail and

Breeder Hatchery, 25 (83.33%) *E. coli*, 23 (76.67%) *Salmonella* spp., and 6 (20%) *Pseudomonas* spp. were identified. In Shibgang Bazar, 26 (86.67%) *E. coli*, 22 (73.33%) *Salmonella* spp., and 7 (23.33%) *Pseudomonas* spp. were isolated in this study. The highest percentage (86.67%) of *Escherichia coli* was found in Shibganj Bazar, the highest percentage (76.67%) of *Salmonella* spp. was found in Kashem Quail and Breeder Hatchery, and the highest percentage (23.33%) of *Pseudomonas* spp. was found in Shibganj Bazar. The prevalence results are shown in Table 1.

Table no.1: Prevalence of isolated bacteria from different quail farms and hatchery

SL.NO	Farm/Hatchery	No. of case	<i>E. coli</i>		<i>Salmonella</i> spp.		<i>Pseudomonas</i> spp.	
			No. of positive case	%	No. of positive case	%	No. of positive case	%
1	Sarkar Quail and Poultry Hatchery	30	23	76.67	20	66.67	5	16.67
2	Kashem Quail and Breeder Hatchery	30	25	83.33	23	76.67	6	20

3	Shibganj Bazar	30	26	86.67	22	73.33	7	23.33
Total		90	74	82.22				

In this study, different media were used for cultural and biochemical tests. *E. coli* gives large pink color colonies on MacConkey agar and Shows metallic sheen colonies on EMB agar. In the biochemical test, *E. coli* gives positive results for the Indole test, MR test, VP test. *Salmonella* spp. shows in black center colonies on SS agar and give positive biochemical test for MR, Citrate, and catalase test. *Pseudomonas* spp. express pink yellow colonies in MacConkey agar and sticky circular colonies on EMB agar. Biochemical test positive for catalase and motility test. All bacterial cultural and biochemical test results are shown in Figures 1, 2 and 3.



Figure 1: *E. coli* on MacConkey agar with pink colony (left) and EMB agar with metallic sheen colony (right)



Figure 2: *Salmonella* spp. on EMB agar with colorless colony (left) and on SS agar with black center colony (right)

**Figure**

3: *Pseudomonas* spp. on EMB agar with blue color stick colony (left) and hemolytic colony on blood agar (right)

3.2 Antibigram profile of the isolated bacteria

Commercially available eight antibiotics were used for antibiotic susceptibility pattern of isolated *E. coli*, *Salmonella* spp., and *Pseudomonas* spp. We selected each of five isolates for this antibiogram study. Three sensitivity profiles, such as resistant (R), intermediate (I), and sensitive (S), were observed in all isolates tested against antibiotics. *E. coli* were 100% sensitive to Colistin sulfate and Neomycin followed by ciprofloxacin, chloramphenicol, and amoxicillin 60%, respectively.

In case of *Salmonella* spp. Colistin sulphate and ciprofloxacin were 100% sensitive. *Pseudomonas* spp. were 100% sensitive to ciprofloxacin and Colistin sulphate, whereas Amoxicillin, Ampicillin, and Cloxaciline were 100% resistant. The summary of antibiogram profiles is presented in Table 2 and figure 5, 6 and 7. The Clinical and Laboratory Standard Institute evaluated antibiotic sensitivity tests according to the disc diffusion method (CLSI. 2013).

Table 2: Antibigram profiles of *E. coli*, *Salmonella* spp. and *Pseudomonas* spp.

Antimicrobial agents	Symbol	Disc concentration (µg/ml)	<i>E. coli</i> (n=5)			<i>Salmonella</i> spp. (n=5)			<i>Pseudomonas</i> spp. (n=5)		
			R	I	S	R	I	S	R	I	S
Ciprofloxacin	CLIP	5 µg	1	1	3	0	0	5	0	0	5
Amoxicillin	AML	10 µg	5	0	0	5	0	0	5	0	0
Ampicillin	AMP	10 µg	4	0	1	5	0	0	5	0	0
Erythromycin	E	15 µg	3	2	0	3	0	2	4	1	0
Colistin sulphate	CT	25 µg	0	0	5	0	0	5	0	0	5
Neomycin	N	30 µg	0	0	5	0	2	3	0	2	3
Chloramphenicol	C	30 µg	1	2	3	0	3	2	4	1	0

Cloxaciline	OB	5µg	5	0	0	5	0	0	5	0	0
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R=Resistant;I=Intermediate;S=Sensitive.

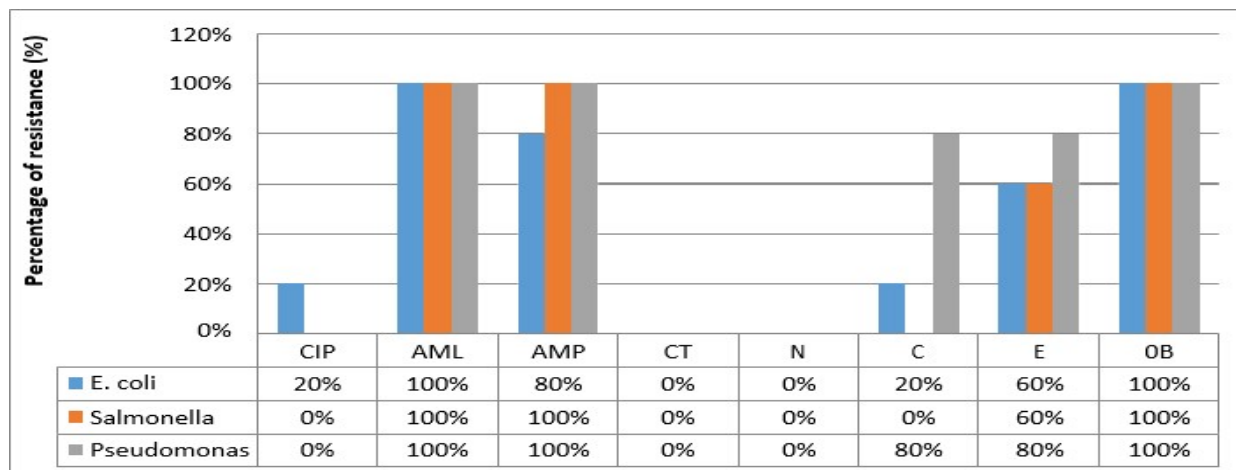


Figure5: Graphical presentation of resistance to antibiotics in *E.coli*; *Salmonella* spp. and *Pseudomonas* spp.

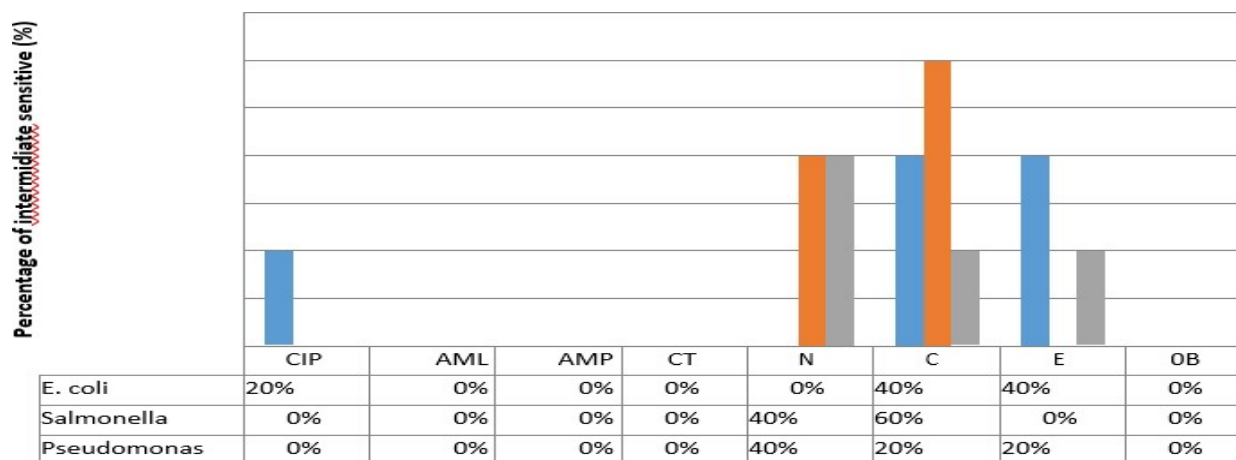


Figure6: Graphical presentation of intermediate sensitivity to antibiotics in *E.coli*; *Salmonella* spp. and *Pseudomonas* spp.

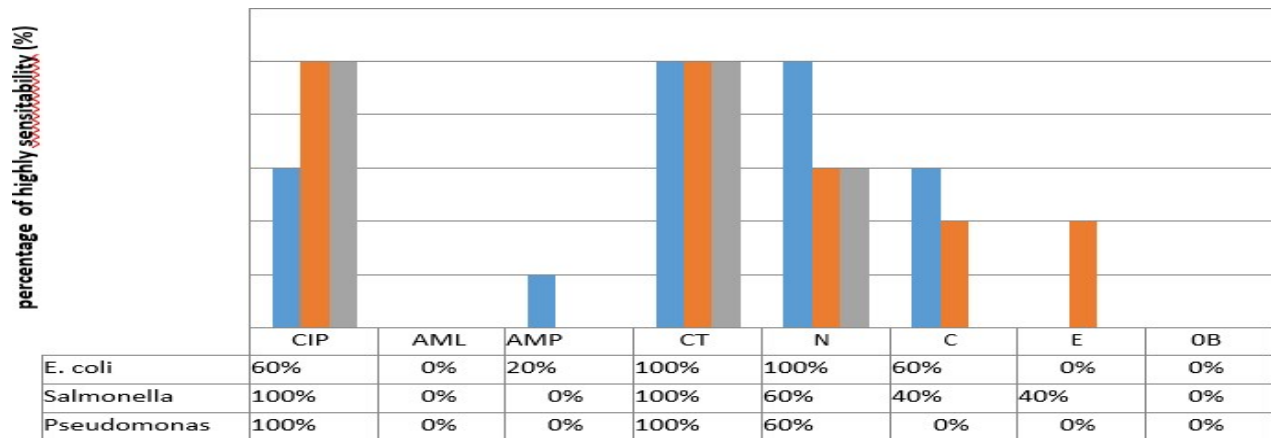


Figure7: Graphical presentation of sensitivity to antibiotics in *E. coli*, *Salmonella* spp., and *Pseudomonas* spp.

4 Discussion

Bangladesh's economy is based on agriculture. More than 70% of families in Bangladesh's rural areas raise poultry. Poultry production, particularly in animal husbandry, serves a crucial role in producing protective food in human nutrition. Quail is one of the species of poultry. The current investigation was carried out to isolate and identify bacteria found in the feces of quail birds, and an antibiotic sensitivity test was also performed. The research work was carried out at Sarkar Quail and Poultry Hatchery, Kashem Quail and Breeder Hatchery, and Shibganj Bazar, Sylhet and in the laboratory of Microbiology and Immunology Department, Faculty of Veterinary and Animal Science, Sylhet Agricultural University, Sylhet. Total 90 feces samples were collected from Sarkar Quail and Poultry Hatchery, Kashem Quail and Breeder Hatchery, and Shibganj Bazar (30 samples from each farm). The numbers of positive feces samples of Quail from Sarkar Quail and Poultry Hatchery, Kashem Quail and Breeder Hatchery and Shibganj Bazar for *E. coli* were 23, 25, and 26 respectively, and their percentage were 76.67%, 83.33%, and 86.67%, respectively and the results were compared with earlier results (El-Dosoky et al., 1999; Burns et al., 2003).

The numbers of positive feces samples of Quail from Sarkar Quail and Poultry Hatchery, Kashem Quail and Breeder Hatchery and Shibganj Bazar for *Salmonella* spp. were 20, 23, and 22 respectively and their percentage were 66.67%, 76.67% and 73.33% respectively and the results were compared with earlier results (El-Dosoky et al., 1999; Burns et al., 2003). The numbers of positive feces samples of Quail from Sarkar Quail and Poultry Hatchery, Kashem Quail and Breeder Hatchery and Shibganj Bazar for *Pseudomonas* spp. were 5, 6 and 7 respectively, and their percentage were 16.67%, 20.00%, and 23.33% respectively and the results were compared with earlier results (El-Dosoky et al., 1999; Burns et al., 2003). The colonies characteristic of *E. coli* on different culture media such as NA, MAC, EMB agar were identical to other findings (Husseina et al., 2008; Nazir et al., 2005). The cultural and biochemical test results of *E. coli* were reported as (Cheesbrough M. 2003). *Salmonella* spp. colony characteristics on NA, SS agar, were equivalent to other authors

(Khan et al., 2005; Mondal et al., 2008). According to (Jonget al., 2012) *E. coli* bacteria demonstrated full-sugar fermentation by producing acid and gas. *Pseudomonas* spp. is usually found in the urinary bladder, respiratory tract, wounds infection, and blood, but it can also be found elsewhere in the body. In this experiment, the bacteria was found mainly in tracheal swabs supported by (Ogunleye, A. O. 2012). *Pseudomonas* spp. Found positive in catalase test, no acid and gas producing in sugar fermentation test and negative in indole, methyl red, and VP test; the conditions described by (Ogunleye, A. O. 2012). On blood agar, a hemolytic reaction was observed by (Ogunleye, A. O. 2012). Antibigram study explained that *E. coli* were found 100% sensitive to Colistin sulphate and Neomycin; followed by Ciprofloxacin and chloramphenicol 60%, Ampicillin 20% whereas 100% resistant to Amoxicillin and Cloxacillin and 80%, 60%, and 20% case of resistance found against Ampicillin; Erythromycin respectively. The results were related to other researchers (Lee et al., 2005, Hossneera et al., 2007, Akond et al., 2009; Ozaki et al., 2011).

In case of *salmonella* spp. found 100% sensitive to Ciprofloxacin, Colistin sulphate, which partly agrees with (Khan et al., 2005), 60% sensitive to Neomycin; 40% sensitive to Chloramphenicol, and Erythromycin and 0% sensitive to Amoxicillin, Ampicillin, Cloxacillin. The organism was intermediate sensitive to Chloramphenicol and Neomycin 60% and 40%, respectively. *Salmonella* spp. is resistant to Amoxicillin, Ampicillin, Cloxacillin (100%). Else that 60% cases of resistance were found against Erythromycin. *Pseudomonas* spp. was found 100% sensitive to Ciprofloxacin and Colistin sulfate followed by 60% sensitive to Neomycin whereas 100% resistant to Amoxicillin, Ampicillin, Cloxacillin followed by 80% resistance was found against Chloramphenicol and Erythromycin. Quail birds and their egg's nutritional value is very high and are rich in protein and poultry meat.

The emergence of multidrug-resistant bacteria is concerning. This resistance may acquire access in the environment and humans and animals, potentially resulting in difficulties in treating bacterial infections with medicines. There is minimal research conducted in quail feces in Bangladesh, and it's an outstanding significance if we conduct good research about this and find out new research output. Our research has many limitations and a lack of proper funding; we did not characterize pathogenic isolates by PCR techniques. Further research needs for the proper characterization of isolates.

5 Conclusion

Quail feces samples from three different quail farms and hatchery are the main reservoirs of isolated isolates such as *E. coli*, *Salmonella* spp., and *Pseudomonas* spp. In this research, we identified multidrug resistance *E. coli*. Customer consumes quail bird meat, and it creates a public health problem due to the antibiotic resistance character of *E. coli*, *Salmonella* spp., and *Pseudomonas* spp. Because of antibiotic resistance, the treatment of quail bird disease is complex. Proper control measures should be taken urgently against antimicrobial resistance of bacteria soon.

List of abbreviations

MA= MacConkey Agar. NA= Nutrient Agar, EMB= Eosin Methylene Blue, BGA= Brilliant Green Agar, BA= Blood Agar, SS= Salmonella - Shigella, MR= Methyl Red, VP= Voges-Proskauer, MDR=Multidrug Resistance, SAU= Sylhet Agricultural University.

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Nothing is disclosed.

Conflict of interests

The author has no conflict of interest.

Author's contribution

Conceptualization, methodology and supervision: Dr. A.T.M Mahbub-E-Elahi. Resources: Md. Abdullah Al-Amin, Md. Safwan Hussain. Data collection: Md. Hasibul Hasan and Harunur Rashid. Manuscript drafting: All authors contribute to prepare manuscript and finally manuscript written by Md. Aoulad Hosen. Review: manuscript reviewed by Dr. A.T.M Mahbub-E-Elahi. Plagiarism and grammar check: Md. Aoulad Hosen. Final correction: Md. Aoulad Hosen, Md. Hasibul Hasan finally corrected the manuscript. Before final submission and publication, all authors read the manuscript and approve.

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