

# Antimicrobial-resistant *Escherichia coli* Isolated from Wild Birds in Newfoundland, Canada Differ Greatly Based on Host Origin and Anthropogenic Interactions

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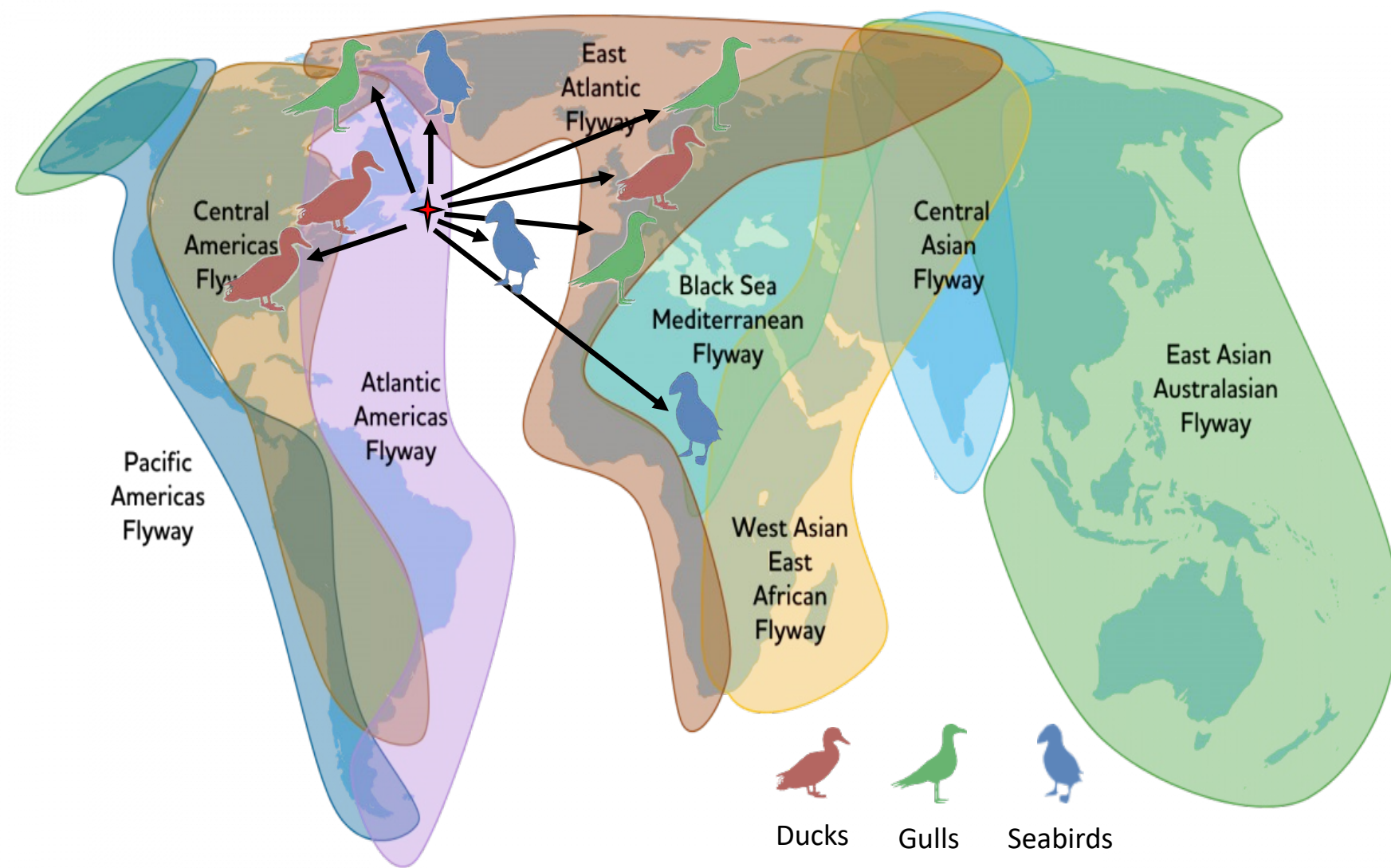
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## Background

- Antimicrobial resistance (AMR) is an ever-present and growing problem in the treatment of infectious diseases, with infections caused by drug-resistant microbes projected to kill 10 million people per year by 2050.<sup>1</sup>
- There has been little previous research on AMR in wild birds, starting roughly 15 years ago, with most of these being small in scale dealing with only a few locations or one or a few species. Of these, only a handful of studies have looked into AMR bacteria carried by ducks and seabirds.
- An increasing number of studies have identified wild birds, particularly gulls, as serving as important vectors by which AMR bacteria are spread, including on global scales<sup>2,3,4</sup>, highlighting their important role in the One Health framework.
- There has been no previous research on AMR bacteria found in wild birds in Eastern Canada, and minimal along Eastern North America. As Newfoundland, Canada is frequently visited by a wide range of duck, gull, and seabird species and is connected with several global migratory routes, it is the perfect place to perform this work.

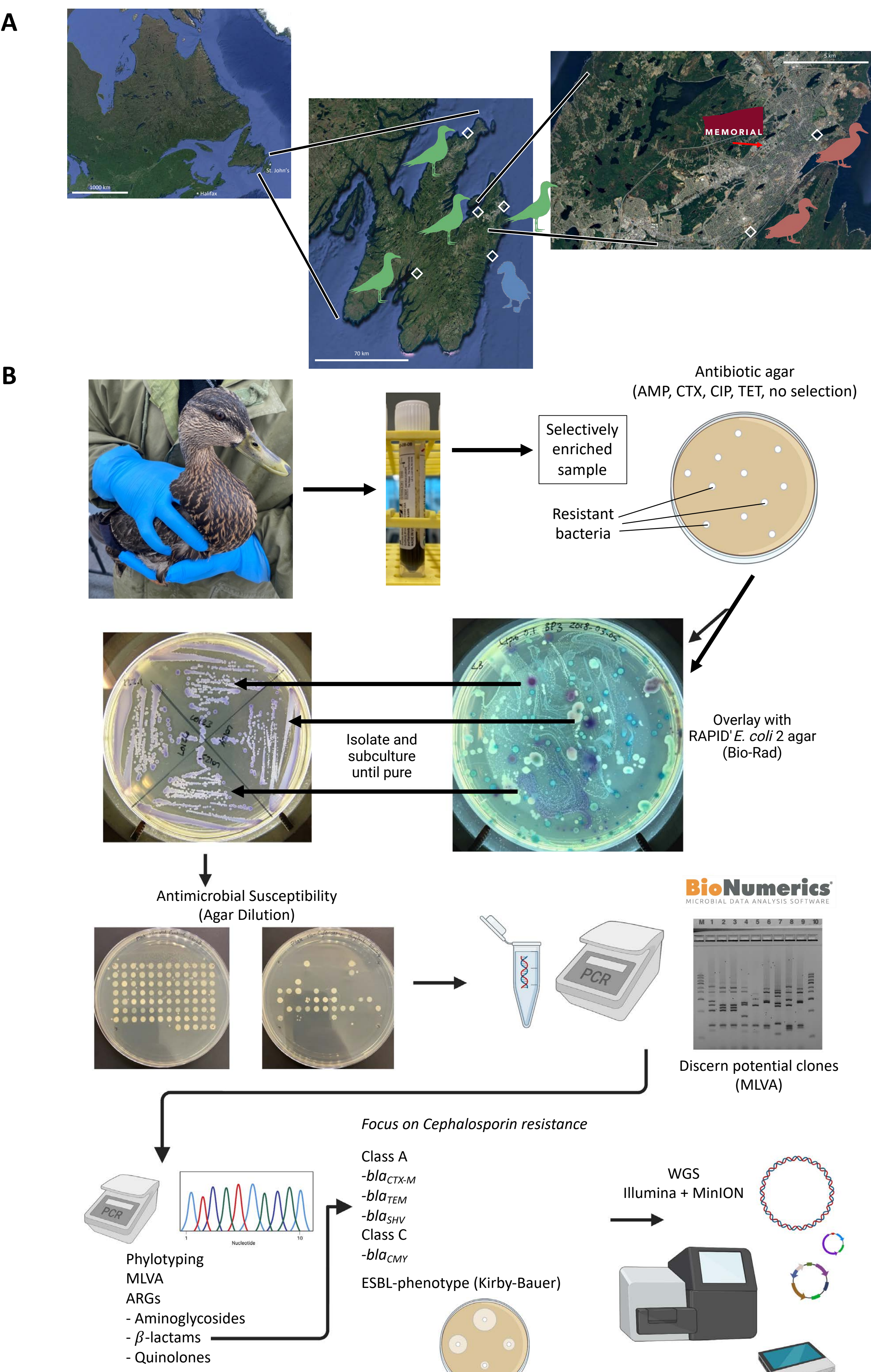
**FIGURE 1 |** Wild birds found in Newfoundland travel from a variety of places around the Atlantic Ocean. Ducks, gulls, and seabirds found here visit from elsewhere in North America, the Arctic, Europe, and Africa at various times of the year.



## Objectives

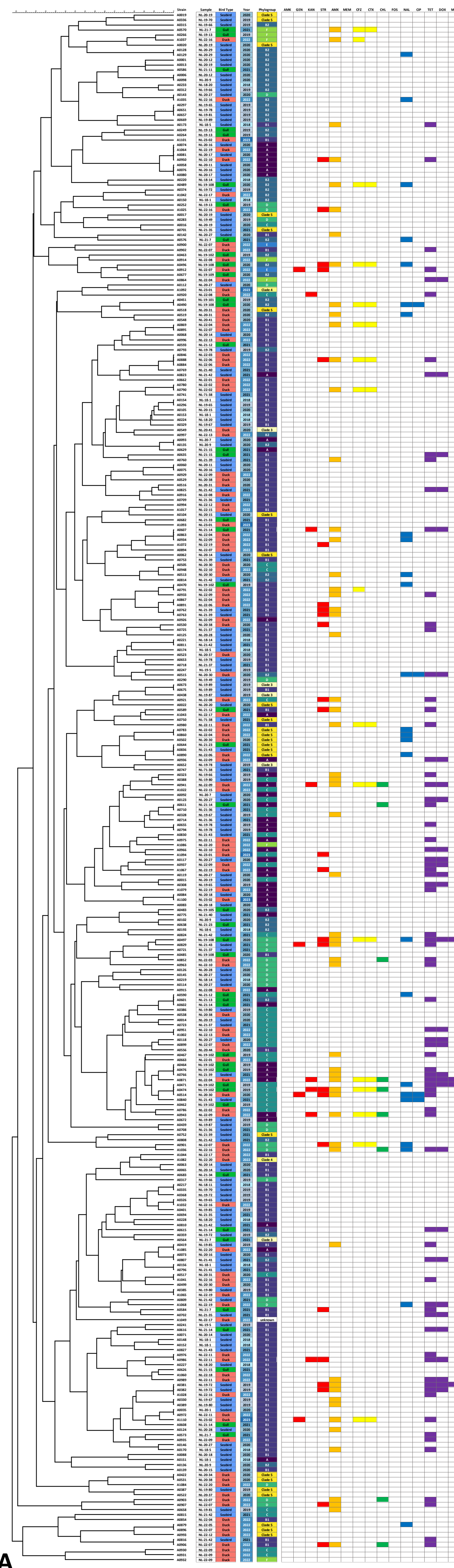
- To investigate the presence, abundance, and diversity of AMR *E. coli* from wild birds across geographic and temporal scales.
- To characterize the molecular determinants of resistance through targeted resistance gene screening for select classes of antimicrobials, and later through whole-genome sequencing.
- To identify potential sources of these microbes and the persistence of clonal groups in different species and relate this to the lifestyles of their host.
- To assess risk of infection by drug resistant microbes and the role of wild birds as sources and vectors across broad geographic and temporal scales.

## Methods

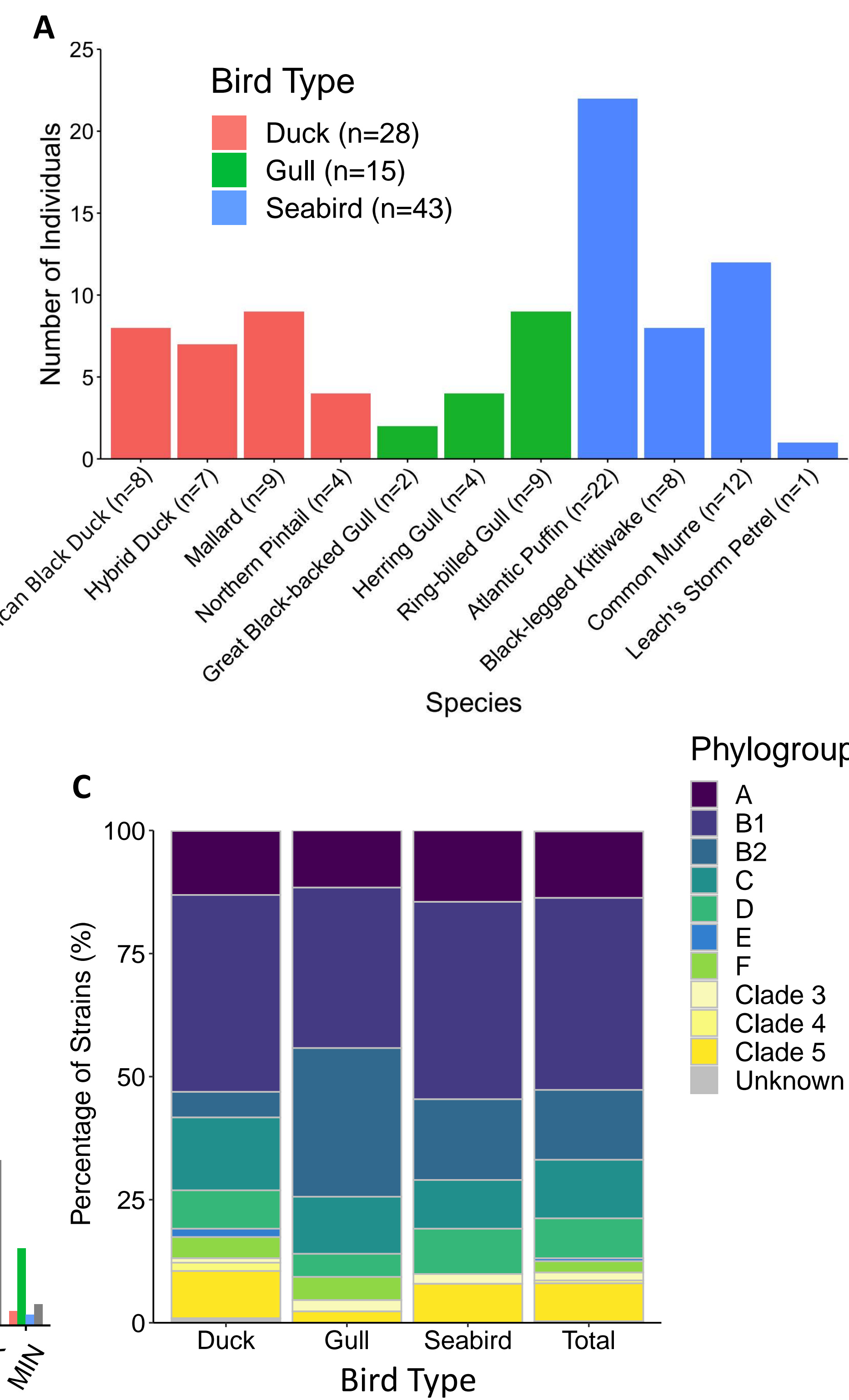


**FIGURE 2 |** (A) Bird types and their respective sampling locations; cloacal swabs were obtained from 242 individuals between 2018 and 2023. (B) Overview of the sample processing methodology and molecular characterization workflow.

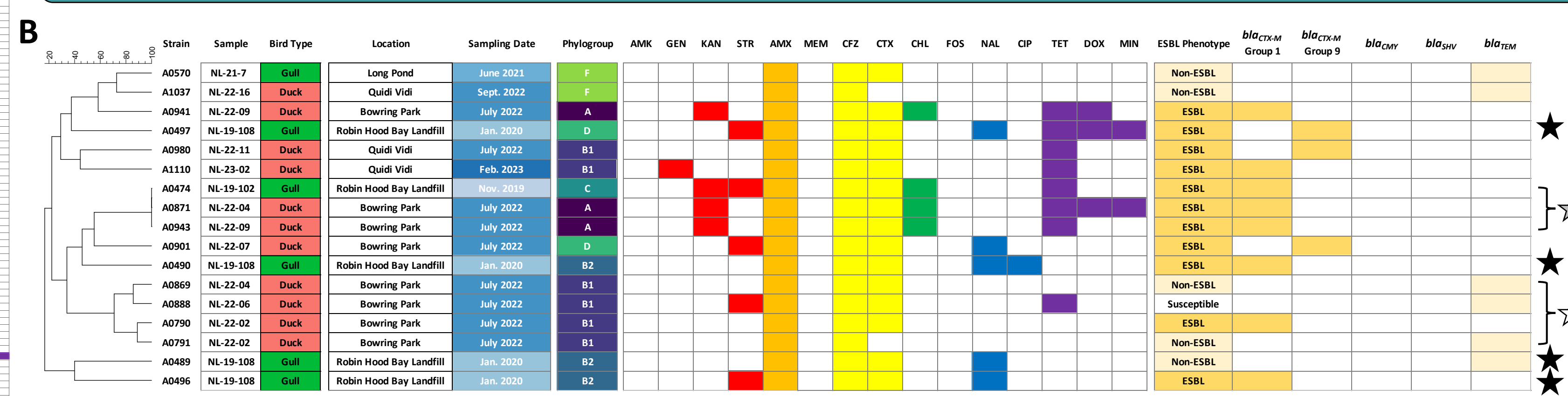
## Results



**FIGURE 3 |** (A) Number and species of ducks, gulls, and seabirds from which *E. coli* were obtained. *E. coli* were isolated from each species with differing success. (B) Rates of antimicrobial resistance among all 310 strains; antimicrobials are ordered by drug class and generation. Resistance was generally low, being highest to tetracycline and amoxicillin across all bird types. (C) Phylogroup distribution of strains from each bird type. Although there is some variation, phylogroup distribution is similar for each group.



## Dendrograms and Cephalosporin-resistance Focus



- Some interesting patterns can be observed among cephalosporin-resistant strains:
- ★ Multiple unique strains were found in the same individual.
  - ☆ Very similar strains, but different phenotypic resistance.
  - ☆ Very similar strains in different ducks from the same time and place, but differed in phenotypic resistance and ARGs.

**FIGURE 4 |** Dendrograms constructed from VNTR analysis (MLVA<sup>5</sup>) of (A) all 310 strains and (B) the 17 cephalosporin-resistant strains using BioNumerics (UPGMA with Dice algorithm). Bird type and phylogroup colours match those of above (Figure 3). Antimicrobial susceptibility profiles are indicated where coloured blocks indicate presence of phenotypic resistance, and they are coloured by drug class. ESBL-phenotypes and cephalosporin-resistance genes detected are as indicated in the last panel of (B).

## Discussion

- We processed 138 swabs and learned that significant and visible fecal material is needed, and successfully cultured *E. coli* from 86 birds (62%). 840 strains were isolated, and after analysis of potential clonal strains, we were left with 310 unique strains.
- We found an incredible diversity among our *E. coli* collection, with some sharing as little as ~5% similarity by MLVA.
- Ducks carried more unique strains, while gulls had less, and seabirds carried usually just a few.
- We isolated multiple unique strains from the same individual, therefore the bird microbiome is not clonally dominant. For example a female MALL from Bowring Park in August 2022 carried 11 unique *E. coli* strains. She was also co-infected with H9NX avian influenza.
- We observed no major division by bird type/species or by year, indicating that the *E. coli* communities and sources of infection are diverse and varied.
- We identified AMR *E. coli* in several Atlantic puffin chicks that had not yet left the burrow, indicating infection via interaction with their parents, highlighting the complexity of understanding infection dynamics.

- From the resistance phenotypes and the mobile resistance genes identified, horizontal gene transfer is likely contributing to the patterns observed.
- Only 5.5% of strains were resistant to a cephalosporin (17 of 310), however despite this, they were still very genotypically diverse.

## Future Work and Open Questions

- Molecular characterization of these strains is still ongoing, with WGS of a subset of isolates in the coming month. Network and relatedness and SNP analyses will be performed to further understand the complex *E. coli* collection and to specifically address objectives 3 and 4.
- There are many open questions we hope to address, such as:
  - What are the source(s) of infection/colonization and where is this occurring.
  - To what extent is there spread of AMR bacteria between individuals and across species or types of birds.
  - What role do birds found in Newfoundland have as vectors of AMR bacteria across broad geographic scales, potentially with global implications.
  - If wild birds can serve as sentinels of what's in the environment – if we know source of infection, can we intervene?

## Acknowledgements and References



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