Evidence for continental-scale dispersal of antimicrobial resistant bacteria by landfill-foraging gulls

Christina A. Ahlstrom [a,1], Mariëlle L. van Toor [b,1], Hanna Woksepp [c], Jeffrey C. Chandler [d], John A. Reed [a], Andrew B. Reeves [a], Jonas Waldenström [b], Alan B. Franklin [d], David C. Douglas [e], Jonas Bonnedahl [f,g], Andrew M. Ramey [a,*]

[a] Alaska Science Center, U.S. Geological Survey, 4210 University Drive, Anchorage, AK 99508, USA
[b] Centre for Ecology and Evolution in Microbial Model Systems, Linnaeus University, Stuvaregatan 2, Kalmar 392 31, Sweden
[c] Department of Development and Public Health, Kalmar County Hospital, Kalmar 391 85, Sweden
[d] USDA/APHIS/WS, National Wildlife Research Center, 4101 Laporte Ave, Fort Collins, CO 80521, USA
[e] Alaska Science Center, U.S. Geological Survey, 250 Egan Drive, Juneau, AK 99801, USA
[f] Department of Biomedical and Clinical Sciences, Linköping University, Linköping 581 83, Sweden
[g] Department of Infectious Diseases, Region Kalmar County, Kalmar 391 85, Sweden

ARTICLE INFO

ABSTRACT

Anthropogenic inputs into the environment may serve as sources of antimicrobial resistant bacteria and alter the ecology and population dynamics of synanthropic wild animals by providing supplemental forage. In this study, we used a combination of phenotypic and genomic approaches to characterize antimicrobial resistant indicator bacteria, animal telemetry to describe host movement patterns, and a novel modeling approach to combine information from these diverse data streams to investigate the acquisition and long-distance dispersal of antimicrobial resistant bacteria by landfill-foraging gulls. Our results provide evidence that gulls acquire antimicrobial resistant bacteria from anthropogenic sources, which they may subsequently disperse across and between continents via migratory movements. Furthermore, we introduce a flexible modeling framework to estimate the relative dispersal risk of antimicrobial resistant bacteria in western North America and adjacent areas within East Asia, which may be adapted to provide information on the risk of dissemination of other organisms and pathogens maintained by wildlife through space and time.

© 2020

1. Introduction

Antimicrobial resistance (AMR; abbreviation also used for 'antimicrobial resistant') is widespread in human and veterinary clinics, food production systems, and the environment, which may undermine the treatment of bacterial infections (Cassini et al., 2019). Through numerous pathways, AMR bacteria and genes conferring resistance are transferred among these sectors (Allen et al., 2010; Woolhouse et al., 2015), as well as among healthy community members, such that resistance may spread between the environment and humans in both directions (Chamosa et al., 2017; Forsberg et al., 2012; Gomi et al., 2017). However, quantification of exchange among sectors in each direction and specific pathways of dissemination are difficult to infer. This is largely a function of the complex and multidimensional spread of bacteria, plasmids, and resistance genes (Sheppard et al., 2016), and the generally low levels of surveillance for AMR bacteria in environmental settings.

Environmental AMR genes may be ancient (D'Costa et al., 2011) and have been found at some of the most remote locations on the planet (Hernández et al., 2010); however, AMR genes within mobile genetic elements are more prolific in the antibiotic era and at locations with anthropogenic inputs such as sewage, refuse, and livestock waste (Ma et al., 2017). This is presumably a function of increased selection for AMR in environments contaminated with antibiotic residues, biocides, and heavy metals (Gullberg et al., 2014), as well as the direct input of resistance genes into the environment from anthropogenic sources. Additionally, anthropogenic inputs into the environment may alter the ecology and population dynamics of synanthropic wild animals by providing food subsidies (Ackerman et al., 2018; Giroux et al., 2016). Some species of gulls (family Laridae) are known for their opportunistic behavior and ability to adapt to anthropogenically influenced environments (e.g. Bond, 2016; Duhem et al., 2008), and often utilize refuse and habitats receiving wastewater effluent (Weiser

* Corresponding author.
E-mail addresses: cahlstrom@usgs.gov (C.A. Ahlstrom); marielle.vantoor@lnu.se (M.L. van Toor); Hanna.Woksepp@regionkalmar.se (H. Woksepp); jeffrey.c.chandler@usda.gov (J.C. Chandler); jareed@usgs.gov (J.A. Reed); areeves@usgs.gov (A.B. Reeves); jonas.waldenstrom@lnu.se (J. Waldenström); alan.b.franklin@usda.gov (A.B. Franklin); ddouglas@usgs.gov (D.C. Douglas); jonas.bonnedahl@regionkalmar.se (J. Bonnedahl); aramey@usgs.gov (A.M. Ramey)
[1] These authors contributed equally to the development of this product.

and Powell, 2010). Consequently, these birds have been purported to acquire AMR bacteria from human inputs (Varela et al., 2015) and are therefore targeted as indicators of AMR in the environment (Ahlstrom et al., 2018; Dolejska et al., 2015). Furthermore, gulls and other wild birds may serve as important environmental reservoirs or bridge hosts of clinically relevant AMR bacteria (Franklin et al., 2020), and as vectors for long-distance dispersal of resistance determinants (Guenther et al., 2012; Lin et al., 2020). These are plausible scenarios given that gulls have previously been found to harbor high levels of clinically relevant AMR bacteria (Dolejska et al., 2015; Vittecoq et al., 2017), identified as sources of elevated coliform bacteria levels at public beaches (Nevers et al., 2018), and implicated in the local dispersal of bacteria across the landscape (Ahlstrom et al., 2019a; Alm et al., 2018). Additionally, the shedding period for enteric bacteria exhibiting AMR harbored by experimentally inoculated gulls and other water birds may be of sufficient duration to facilitate dissemination through migration (Franklin et al., 2020; Sandegren et al., 2018).

In this study, we investigated the acquisition and risk of long-distance dispersal of AMR bacteria by landfill-foraging gulls using a combination of phenotypic and genomic approaches, and satellite telemetry to track gull movements. We focused on gulls inhabiting Alaska, USA, because the state has minimal agricultural production (USDA, 2019) and is sparsely populated by humans such that communities are often separated by hundreds of kilometers. Thus, exposure of gulls to anthropogenic AMR determinants and environmental conditions that select for elevated levels of resistance are presumably limited to discrete populated areas within the state with relatively well-defined inputs (e.g., refuse and wastewater effluent). Furthermore, most gulls inhabiting Alaska migrate following the breeding season, suggesting that they may serve as a useful model for understanding dispersal of AMR bacteria among spatially distant regions.

2. Materials and methods

2.1. Study sites and sample collection

Fecal material from glaucous-winged gulls (*Larus glaucescens*), herring gulls (*Larus argentatus*), glaucous gulls (*Larus hyperboreus*), and potential hybrids of these species, was collected from seven communities in Alaska (Fig. S1) twice per year (May–June and August) in either 2016 or 2017. Samples were collected at community landfills when possible, or at other gull congregation areas (e.g. beaches) if landfills were not present or accessible. A total of 50 to 67 samples were collected at each location during each sampling period (Table S1) by inserting a sterile swab into recently deposited (i.e. wet) gull fecal material (following flushing of the flock) and placing it into a vial with chilled Luria broth (BD, Sparks, USA). GPS coordinates were recorded at each site and all samples were kept cool on ice for approximately 4–72 h until frozen at −80 °C. Human population size estimates of each community for the year 2016 were retrieved from the State of Alaska Department of Labor and Workforce Development Research and Analysis (State of Alaska Department of Labor and Workforce Development, n.d.). We incorporated the estimated population of the City of Kenai into the population size estimate for Soldotna, given these two communities form a continuous urban corridor. We assumed the influx of tourists and visiting researchers to sampling locations to be roughly proportional to (and unlikely to eclipse the importance of) the larger resident populations of these seven communities.

2.2. Bacterial culture and phenotypic AMR testing

Two different culture methodologies were employed to isolate *Escherichia coli*. A selective screening approach for enrichment of cephalosporin-resistant *E. coli*, which are of specific concern to public health, was utilized as the more sensitive method for isolating ex-

tended-spectrum beta lactamase- (ESBL) producing isolates. A second, non-selective screening approach was employed for enrichment of all *E. coli* and was utilized to assess overall resistance among viable *E. coli*. For both screening approaches, gull fecal swab samples were inoculated in 2 ml brain heart infusion (BHI) broth (Becton Dickinson, USA), supplemented with vancomycin (16 mg/l; Sigma-Aldrich, Merck, Sweden) for selection of gram-negative bacteria, and incubated for 18–24 h at 36 °C. Following incubation, 10 μl of BHI broth was streaked onto CHROMagar C3GR plates (CHROMagar, France), a selective growth medium that supports growth of bacteria with reduced susceptibility to extended-spectrum cephalosporins, and 10 μl was streaked onto Uriselect agar plates (Bio-Rad Laboratories, France), a non-selective growth medium. *E. coli* CCUG 17620 and *Klebsiella pneumoniae* CCUG 45421 strains were included as negative and positive controls, respectively. Plates were incubated in aerobic conditions for 18–24 h at 36 °C. Putative *E. coli* isolates were confirmed as *E. coli* by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Bruker Daltonics, Bremen, Germany) using a threshold criterion of >2 and database versions BDAL 5989 and BDAL 6903 (Seng et al., 2009). One *E. coli* isolate per plate was selected in most instances; however, when different colony morphologies were observed, more than one colony was retained (Fig. S2).

Antimicrobial susceptibility testing was performed on confirmed *E. coli* isolates using the following antibiotic discs: Nalidixic acid (30 μg), nitrofurantoin (100 μg), piperazillin-tazobactam (36 μg), tetracycline (30 μg), trimethoprim (5 μg), trimethoprim-sulfamethoxazole (25 μg), meropenem (10 μg), ciprofloxacin (5 μg), ampicillin (10 μg), cefadroxil (30 μg), chloramphenicol (30 μg), gentamicin (10 μg) and mecillinam (10 μg) (Thermo Fisher Scientific Oxoid Ltd, Hants, UK), according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations (EUCAST, 2013). Inhibition zone diameters were interpreted according to EUCAST breakpoints (EUCAST, 2016) for all antimicrobials except for tetracycline, for which we used the Normalized Resistance Interpretation method (Kronvall and Smith, 2016), as this antimicrobial has no defined clinical breakpoint. Additional characterization of ESBL-producing *E. coli* isolates from CHROMagar C3GR plates was performed using disc diffusion with the following five antibiotic discs: Ceftazidime (10 μg), cefotaxime (5 μg), cefepime (30 μg), cefoxitin (30 μg) and amoxicillin/clavulanic acid (30/1 μg). All phenotypic antimicrobial susceptibility testing results can be found at https://doi.org/10.5066/P9D72Q3W (Ahlstrom et al., 2020).

2.3. Genomic analysis

DNA was extracted from all *E. coli* isolates resistant to one or more antibiotics using MagnaPure compact nucleic acid isolation kits (Roche, Stockholm, Sweden). Multiplexed DNA libraries were prepared using NexteraXT or Nextera DNA Flex library preparation kits (Illumina, San Diego, USA) according to manufacturer's instructions. Whole genome sequencing was performed using MiSeq or HiSeq 4000 (Illumina) platforms to generate 250 or 150 base pair paired-end reads, respectively.

Reference mapping to the *E. coli* K-12 reference genome (NC_000913.3) was performed to quality check sequencing data. Reads were mapped using BWA (Li and Durbin, 2009) and variant sites were identified using SAMtools (Li et al., 2009). Mixed culture was inferred when 5% or more variant sites with >5% heterozygosity (i.e. <95% of reads as either reference or alternate allele) were found in a single isolate. Isolates with inferred mixed culture were excluded from further analyses.

Reads were assembled de novo using default parameters in Unicycler (Wick et al., 2017) and contigs were annotated using Prokka (Seemann, 2014). Core genes were identified and a multiple sequence alignment was generated with Roary (Page et al., 2015). Single nucleotide polymorphisms from the core genome alignment were ex-

tracted using SNP-sites (Page et al., 2016) and phylogenetic trees were generated using FastTree (Price et al., 2009).

Multilocus sequence typing (MLST) was performed in silico using SRST2 (v0.2.0) (Inouye et al., 2014) and the *Escherichia coli* #1 database retrieved from pubmlst.org. Isolates with novel locus alleles were confirmed as *E. coli* using BLAST (Johnson et al., 2008) and then as a novel allele sequence by inspecting the read alignments in Geneious (Kearse et al., 2012). Isolates not confirmed as *E. coli* were excluded from further analyses (Fig. S2). Resfinder (Zankari et al., 2012) and Pointfinder (Zankari et al., 2017) were used to identify acquired genes and point mutations predicted to confer antimicrobial resistance, respectively. The integron/integrase gene *intI1* was detected with seqpoet (v0.3.4) using previously described primer sequences (Orman et al., 2002). Heavy metal and biocide resistance genes were identified using BacMet and its associated experimentally confirmed resistance gene database (Pal et al., 2014).

We retained genetic data from multiple isolates per sample, when applicable, in order to capture additional diversity of *E. coli* strains; however, to minimize overrepresentation of a single clone, only genomic information for one isolate was included in statistical analyses if multiple isolates from the same sample had identical sequence types and AMR gene profiles. This occurred in four samples; thus, a total of 141 isolates were included in downstream statistical analyses (see Fig. S2).

Fisher's exact tests with a Bonferroni correction were performed to test whether antibiotic resistance classes and individual genes were statistically overrepresented in the combined northern, northwestern, and western regions compared to the southcentral region of Alaska, using the package exact2x2 (Fay, 2010) in R (R Core Team, 2016). Only genes found in four or more isolates were tested. Rarefaction curves were generated based on the number of *E. coli* sequence types isolated from each location (for locations with >5 AMR *E. coli* isolates) and each type of enrichment using iNEXT (Hsieh et al., 2016) in R.

2.4. Satellite telemetry

We investigated the migratory movements of individuals attending sampling sites using remote animal telemetry (Kays et al., 2015). Specifically, we captured gulls and equipped them with GPS satellite platform transmitter terminals (PTTs). Permission for capture and deployment of PTTs was granted by the Alaska Department of Fish and Game (permit #16-109), the U.S. Fish and Wildlife (permit #MB789758-5), and the U.S. Geological Survey Alaska Science Center Animal Care and Use Committee (approval #2016-6). A total of 42 adult glaucous-winged gulls, herring gulls, glaucous-winged/herring gull hybrids, and glaucous gulls, the same species from which feces were sampled, were live-captured at five Alaska locations: the Central Peninsula landfill (referred to as Soldotna landfill), located approximately four kilometers south of Soldotna, Alaska, in May 2016 (n = 7) and June 2017 (n = 10); the Bethel landfill, in August 2017 (n = 10); the city dock in Cold Bay, in August 2017 (n = 1); the Utqiaġvik Municipal Solid Waste Landfill, in August 2018 (n = 12); and the Nome Municipal Landfill, in September 2018 (n = 2). Capture of gulls was attempted at the coast adjacent to Adak, but efforts were not successful. The single individual marked at Cold Bay was apparently depredated by a bald eagle (*Haliaeetus leucocephalus*) within 48 h of transmitter deployment, and thus excluded from the analyses. Due to management practices at the Anchorage Regional Landfill (e.g. active hazing of birds), capture of gulls was not attempted.

Gulls were captured using baited noose carpets and fitted with 22 g solar-powered GPS PTTs built by GeoTrak, Inc. (Apex, NC, USA). PTTs were affixed dorsally onto each gull with a 6.35 mm Teflon ribbon backpack harness (Hupp et al., 2011) and programmed to acquire GPS locations every 3 h during local daytime hours (5:00 am–10:00 pm), and one GPS location during the night (0:00 am–4:00 am;

seven total GPS locations per day) from the date of deployment through 31 October. Beginning on 1 November, PTTs were programmed to acquire two GPS locations during local daylight hours (9:00 am–3:00 pm) and one GPS location during night (11:00 pm–3:00 am). PTTs were programmed to communicate GPS data to the Argos System (Fancy et al., 1998) (http://www.argos-system.org/) every two days during a five-hour transmission period. Argos receivers onboard six polar-orbiting satellites opportunistically intercepted PTT transmissions depending on their orbital schedules, hence GPS data were redundantly transmitted many times to increase the probability of capturing a complete tracking record.

2.5. Predicted spatial use by gulls

We used gull GPS locations and environmental data to estimate resource selection within each of two general migratory corridors used by our sample of marked birds (i.e., gulls marked in Bethel, Nome, and Utqiaġvik migrated across the Bering Strait, whereas gulls captured in Soldotna dispersed southeast along the west coast of North America). Resource selection functions (RSF) model the relative probability of selection of resources by an individual or individuals. We therefore used RSF to estimate the relative likelihood of gull use at any given location within the two migratory corridors during the first 30 days of autumn migration, which corresponded to the maximum period of shedding of AMR *E. coli* reported for ring-billed gulls (*Larus delawarensis*) (Franklin et al., 2020). Specifically, we fitted a logistic mixed-effects regression to model the relative probability of gull use as a function of environmental predictors. We subsequently predicted the relative probability of gull use throughout the migratory corridor for 30 days post-dispersal from landfill locations.

We selected three environmental predictors for our RSF: distance to coastline (in km), compound topographic or wetness index (integrates flow accumulation and terrain slope), and human population count (Table S5). Each environmental predictor was resampled to a predefined grid in the Alaska Albers equal-area map projection and a cell size of 5 km × 5 km. We calculated the distance of each grid cell to each of the four deployment locations as an additional RSF predictor.

We defined all gull GPS locations during the autumn dispersal period (i.e. the first 30 days of migration) above land as occupied or 'used' locations, and excluded off-shore locations (n = 1346) from subsequent analyses as we presumed bacteria deposited into the marine environment were unlikely to significantly contribute to dispersal risk. In total, 2108 GPS locations were recorded above land during the selected period (Bethel: n = 341, Nome: n = 143, Utqiaġvik: n = 626, Soldotna: n = 908). We split individuals in two groups, with gulls from Bethel, Nome, and Utqiaġvik grouped as "NW Alaska", and birds from Soldotna as "Soldotna" according to their use of different migratory corridors. We sampled pseudo-absence locations randomly from the study area. Prior to sampling, we determined maximum distance from the coast for locations occupied by satellite tracked gulls (93 km, rounded to 100 km), and restricted random sampling of pseudo-absence locations to within this distance (100 km) from the coast as to avoid an artificially strong effect of this predictor. The number of pseudo-absence locations was chosen to match the number of available presence locations (n = 2108). We randomly assigned deployment locations and individual identifiers to pseudo-absences, and annotated all presence and pseudo-absence locations with all environmental predictors and the respective distance to deployment location.

We derived the RSFs using a logistic mixed-effects regression. We modeled the relative probability of gull habitat use using presence (1) and pseudo-absence (0) as a dependent variable, and, as described above, the three environmental variables and distance to the respective deployment location as independent predictors. To account for differences in available habitat along migratory corridors, we allowed for different slopes in the response by including grouping according to mi-

gration corridor as an interaction term for the variables human population size and wetness index. Additionally, we accounted for variation between individuals by including individual as a random effect. We initially fitted a model with the full dataset, and evaluated the model using residual diagnostics for hierarchical regression models (Hartig, 2017) (Fig. S8), which showed no or only slight deviation from expectations. The full model exhibited a slightly heavy-tailed distribution of residuals not uncommon for logistic regression, but was still able to predict presences and pseudo-absences for all deployment locations well (see Figs. S9 and S10). We assessed goodness-of-fit using McFadden's pseudo-R^2 (McFadden, 1974), which for the full model was $R^2 = 0.73$. We subsequently applied a random sub-sample cross-validation (100 sub-samples of n = 750 locations each) to avoid overfitting. We maintained a 50:50 ratio between presences and pseudo-absences in the sub-sampled datasets. We averaged resulting models into a single final model with coefficients averaged over all 100 replicate models (Bartoń, 2019) (Fig. S11).

We used the averaged model to predict relative probability of gull use along the entire migratory corridors. Considering each landfill deployment location separately, we applied the RSF to predict model response for each cell in the gridded environmental data. This resulted in spatial grids representing relative probability of gull use for gulls from the four deployment locations for the entire respective migratory corridor. We then combined the relative probability of gull use predicted by the RSF with an indication of accessibility of locations within the migratory corridor to gulls during the autumn dispersal period. We determined accessibility of any given site along the migratory corridors using the estimates of migration speed during the autumn dispersal period. We defined concentric rings for each day of the dispersal period, centered on deployment locations. A band's inner and outer radius respectively corresponded to the minimum and maximum observed (tracking) migration distance for that day. We considered only increases in radius, thus ignoring days where the dispersal distance was shorter than on previous days. To restrict these theoretical areas of accessibility to the migratory corridors, we intersected each daily ring with the respective migration corridors. The remaining areas indicated the area accessible to migratory gulls within their migratory corridor during any given day of the autumn dispersal period. We finally used these areas to mask the prediction of relative probability of gull use, and set the probability of gull use in areas outside the area for a given day of the autumn dispersal period to 0. This allowed us to assess the relative probability of any given location within the migratory corridors to be used by migratory gulls from the tagged populations.

2.6. Relative AMR dispersal risk

In the absence of a standardized measure of risk for AMR genes (Berendonk et al., 2015; Martínez et al., 2015), we developed our own "AMR Risk Metric" that was assigned to all four landfill locations at which gulls were marked. Our AMR Risk Metric incorporated information on the frequency of four categories of AMR genes as inferred from whole genome sequencing of *E. coli* isolates obtained from selectively enriched gull fecal samples (i.e., C3G plates). In developing these four categories of risk, we considered existing literature (e.g., Centers for Disease Control and Prevention, 2019; Kadri et al., 2018; Magiorakos et al., 2012; World Health Organisation, 2019), and applied similar criteria to establish our metric. In particular, difficult-to-treat resistance (DTR) was defined by Kadri et al. (2018) as phenotypic resistance to at least one carbapenem, extended-spectrum cephalosporin, and fluoroquinolone. Here, we modified this definition by excluding carbapenem resistance, as carbapenem-resistant Enterobacteriaceae are classified as urgent threats by the Centers for Disease Control and Prevention (Centers for Disease Control and Prevention, 2019) and was considered a critically important antimicrobial. Each isolate was assigned a single category in the following order of

inclusion: 1) presence of genes conferring resistance to critically important antimicrobials, defined here as colistin and carbapenems (P_{cr}), 2) presence of genes associated with resistance to extended-spectrum cephalosporins and fluoroquinolones (P_{DTR}), 3) presence of genes associated with resistance to ≥3 antibiotic classes (i.e., multidrug resistant; P_{MDR}), and 4) presence of genes associated with resistance to 1–2 antibiotic classes (P_{1-2}). We then determined frequency of isolates by dividing the number of isolates in each category by the total number of samples collected at each location. To derive the AMR Risk Metric for each location, we then weighted the frequency of each category according to perceived dissemination concern, based on the authors' collective experience in the field and clinic and based on published literature (Centers for Disease Control and Prevention, 2019; Martínez et al., 2015; World Health Organisation, 2019). The AMR Risk Metric for each location was defined as:

$$10P_{cr} + 6P_{DTR} + 3P_{MDR} + P_{1-2}$$

where P is the frequency of each category of isolates from each location. The minimum and maximum AMR Risk Metric value is 0 and 10.

Finally, we combined the AMR Risk Metric, predictions of gull spatial use during the first 30 days of migration, and a model describing shedding of colistin-resistant *E. coli* by ring-billed gulls over the first 30 days post-infection from a challenge experiment (Franklin et al., 2020). Here, shedding in a flock of infected gulls was best described as a 3-parameter log-normal function (Fig. S12A) with an amplitude of 43.35 (95% CI: 24.74, 61.95), a scale parameter of 0.37 (95% CI: 0.21, 0.53), and a location parameter of 14.95 days (95% CI: 12.06, 17.84). We scaled the function so that intensity of shedding would range from 0 to 1. For each location and cell in the spatial predictions of gull use, we defined the relative dispersal risk as:

$$R_d(j) = R_{AMR}(j) * \sum_{d=0}^{d_{max}} \frac{p_o(d,j) * i_s(d)}{d_{max}},$$

where $R_d(j)$ is the relative dispersal risk of any cell for location j, R_{AMR} the AMR Risk Metric for location j, d_{max} the maximum number of days for which the shedding curve is defined (30 days), $p_o(d,j)$ the predicted use by gulls for location j at any day d during the dispersal period, and $i_s(d)$ the normalized shedding intensity from the shedding model. As R_{AMR} theoretically ranges from 0 to 10, and the second term from 0 to 1, the relative dispersal risk R_d can range from 0 (no risk) to 10 (extremely high risk for critically important AMR genes).

3. Results and discussion

We phenotypically screened gull fecal samples collected from seven locations in Alaska (Fig. S1) during 2016–2017 for AMR *E. coli* using selective and non-selective enrichment approaches. We identified at least one AMR *E. coli* isolate from 16% (126/773) of the samples collected and more than one isolate from 4% of samples (30/773; Table S1; Fig. S2). The apparent frequency of AMR *E. coli* in gull feces varied among locations in Alaska (Figs. 1A, S3) and was strongly correlated with human population size of the community nearest to the sampling location (Figs. 1B, S3), ranging from 0% (0/108 and 0/107, respectively) in Cold Bay (population 65) and Adak (population 309) to 31% (33/107) in Anchorage (population 298,965) based on selective enrichment and subsequent phenotypic screening.

We characterized the genetic diversity of 141 AMR *E. coli* isolates recovered from 121 gull fecal samples using whole genome sequencing (Fig. S2). A total of 72 different multilocus sequence types were identified, the majority of which were represented by a single *E. coli* isolate (Table S2). The highest sequence type diversity was detected in gull feces from Anchorage and Bethel and through non-selective enrichment; however, rarefaction analyses provide evidence that higher diversity may have been obtained at other locations, such as Utqiaġvik, if additional sampling efforts were employed (Fig. S4). A phylogenetic tree
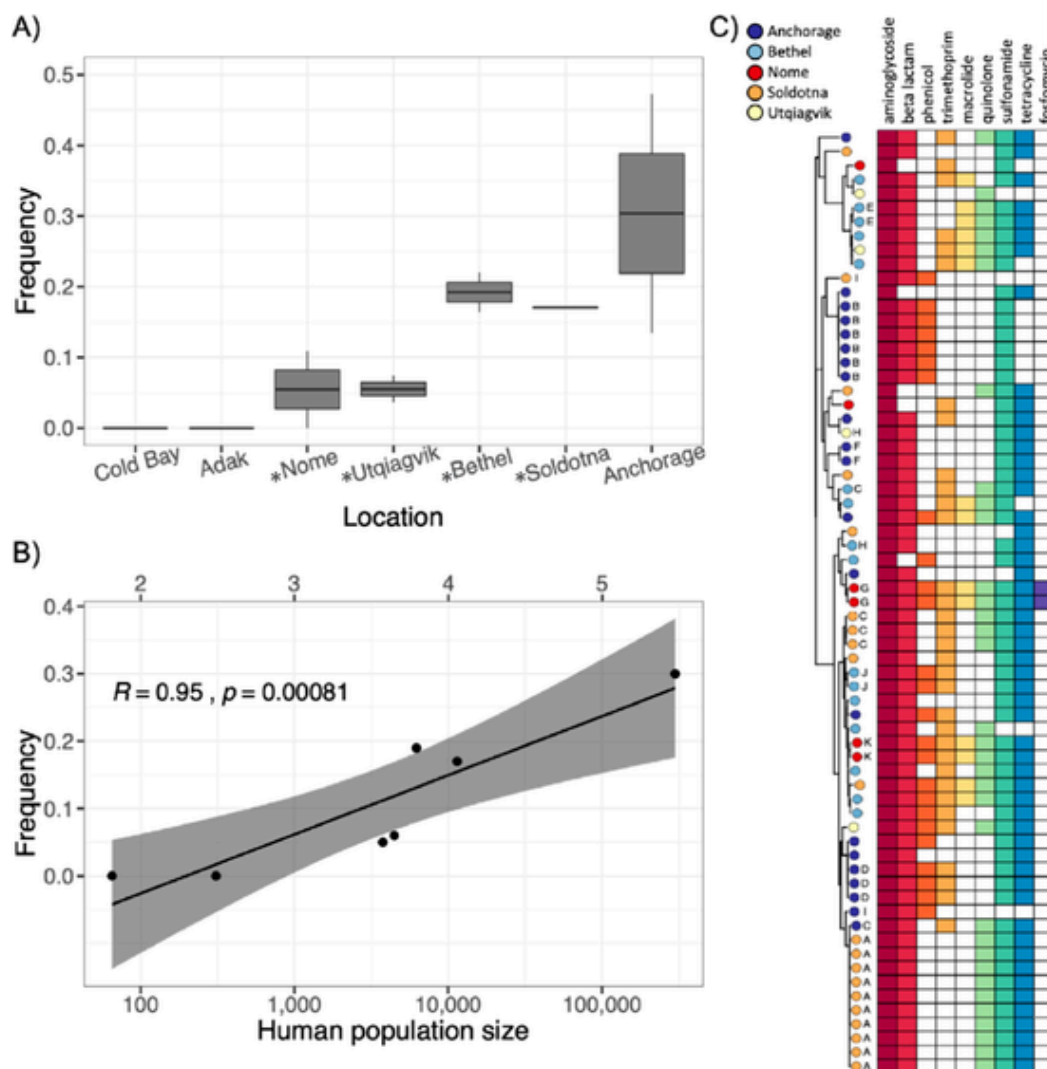
Fig. 1. Frequency and genetic relationship of antimicrobial resistant *E. coli* from seven locations in Alaska. A) Boxplot representing frequency (based on selective enrichment) of AMR *E. coli* (i.e. phenotypically resistant to ≥1 of 13 antibiotics) in gull fecal samples originating from seven locations (ordered by increasing population size) throughout two sampling periods. Asterisks represent locations from which gull dispersal was tracked using satellite telemetry. B) Scatter plot of the frequency of AMR *E. coli* (based on selective enrichment) and human population size of the sampling location on a log scale with base 10. A linear regression line, 95% confidence interval (grey shading), Spearman rank correlation coefficient (R), and *p*-value are displayed. C) Midpoint rooted core genome phylogenetic tree of multidrug resistant *E. coli* isolates. Colored circles indicate sampling location where isolates originated. Presence of at least one resistance gene in the corresponding antibiotic class is indicated by a colored square in the matrix. Identical AMR gene profiles, as defined by presence of each of 58 resistance genes, is indicated by the same letter (A–K). No letter signifies a unique gene profile. Full resistance gene profiles of all antimicrobial resistant *E. coli* isolates are provided in Fig. S5. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

based on the core genome of all isolates revealed extensive genetic diversity and limited dissemination of clonal isolates both within and among sampling locations (Fig. S5).

A total of 58 different AMR genes associated with resistance to nine different antibiotic classes were identified among the sequenced *E. coli* isolates (Fig. S5). Five genes, *aph(3″)-Ib*, *aph(6)-Id*, *tet(A)*, *sul2*, and *bla*$_{TEM-1B}$, were each found in >30% of isolates (n = 46–51 out of 141 isolates), and commonly co-occurred in the same isolate (Fig. S6). Macrolide resistance, and specifically the *mph(A)* gene, was significantly overrepresented (adjusted *p* = 0.01) among isolates originating from the combined northern, northwestern, and western sampling locations (i.e. Bethel, Cold Bay, Nome, Utqiaġvik) (Table S3). We also identified a total of 96 biocide and heavy metal resistance genes among the 141 sequenced isolates (Fig. S7). Antimicrobial resistance genes were not identified in ten isolates that exhibited phenotypic resistance to either extended spectrum beta-lactams, gentamicin, or mecillinam (Ahlstrom et al., 2020). This could reflect limitations in short read

whole genome sequencing or these isolates may harbor novel resistance genes.

The integron/integrase gene *intI1*, a proxy for anthropogenic pollution (Gillings et al., 2015), was detected in 23% (33/141) of isolates and was positively associated with multidrug resistance (MDR; i.e. harbored genes associated with resistance to three or more antibiotic classes (Schwarz et al., 2010); *p* = 0.0005. Overall, 48% (67/141) of isolates exhibited MDR, though the proportion of isolates with MDR varied between locations, sampling times (May/June vs. August), and enrichments. Two isolates from Nome harbored genes associated with resistance to all nine antibiotic classes to which resistance was detected in this study (Fig. 1C). No isolates harboring *intI1* or exhibiting MDR were identified from samples collected at either Cold Bay or Adak, the communities with the smallest human populations sampled as part of this study and where residents incinerate their trash (Table S4).

Through tracking of 41 glaucous, glaucous-winged, herring, or hybrid gulls from four landfill locations, we were able to document the autumn dispersal of gulls from landfills in northern (Utqiaġvik), north-

western (Nome), western (Bethel) and southcentral (Soldotna) Alaska (Fig. 2). Of the 41 gulls marked at landfill locations, 27 individuals were tracked for at least 14 days following departure from landfill sites as part of autumn migration, providing 3454 GPS locations throughout a defined post-dispersal period of 30 days (Ramey et al., 2020). Among the 17 individuals dispersing from landfills in Bethel, Nome, and Utqiaġvik, all gulls dispersed generally southward or westward, with most birds crossing the Bering Strait. Gulls that were tracked throughout the pre-defined 30-day post-dispersal period migrated to lands or coastal waters (i.e., within 100 km of shore) adjacent to Chukotka (n = 8) and Kamchatka, Russia (n = 7). Of the 10 gulls dispersing from the Soldotna landfill, all generally dispersed to the south and east with three birds reaching as far as southern San Francisco Bay, California (Fig. 2A).

Recorded GPS locations for gulls on or above land were used to develop a RSF to generalize habitat use of gulls during migration. We used logistic regression to model habitat use based on environmental covariates quantified over a spatial domain that was constrained to regions consistent with the observed dispersal vectors of birds marked at each of the four landfill locations (Figs. S8–S9, Table S5). After evaluating that the RSF modeled habitat use of gulls successfully (Figs. S10–S11), it was applied to predict a relative probability of habitat use by gulls migrating from the Bethel, Nome, and Utqiaġvik landfills to the northern, northwestern, and western coasts of Alaska and eastern coasts of Chukotka and Kamchatka, Russia (Fig. 2B). Similarly, the RSF was applied to predict habitat use along the southern and southeastern coasts of Alaska and western coasts of British Columbia, Washington, Oregon, and California by gulls departing from the landfill in Soldotna (Fig. 2C).

By combining information on the 1) frequency of AMR, including DTR and MDR, excreted by gulls in their feces at four Alaska landfills, 2) probable habitat use by and migration speed of gulls upon initiation of autumn migration, and 3) shedding patterns of colistin-resistant bacteria exhibited by closely-related ring-billed gulls experimentally inoculated in a previous challenge study (Franklin et al., 2020), we were able to model the relative dispersal risk of clinically relevant AMR bacteria by gulls across the landscape (Fig. 3). Among the four Alaska landfills we studied, our model estimated the highest relative dispersal risk to be from the landfill in Soldotna to areas within and adjacent to southcentral Alaska; Vancouver, British Columbia; Seattle, Washington; and San Francisco, California. More moderate relative dispersal risk was predicted for gulls departing from the Bethel and Nome landfills to areas in northern, northwestern, and western Alaska and to areas along the Chukotka and northeastern Kamchatka coasts of Russia. The relative dispersal risk of clinically relevant AMR by gulls from the Utqiaġvik landfill was found to be comparatively lower than for gulls departing from landfills in Bethel and Nome across the same general dispersal areas (Fig. 3). Overall, the estimated relative risk of Alaska landfill-foraging gulls dispersing clinically relevant AMR bacteria across the landscape was low, given that our highest relative dispersal risk value was 0.35 out of a maximum possible value of 10. The highest relative dispersal risk value would only be attributed to areas predicted to be occupied daily by gulls shedding bacteria with critically important AMR genes upon landfill departure.

Previous studies have found that AMR bacteria harbored by wildlife is generally correlated with anthropogenic inputs into the environment (Atterby et al., 2016; Skurnik et al., 2006); however, limited re-
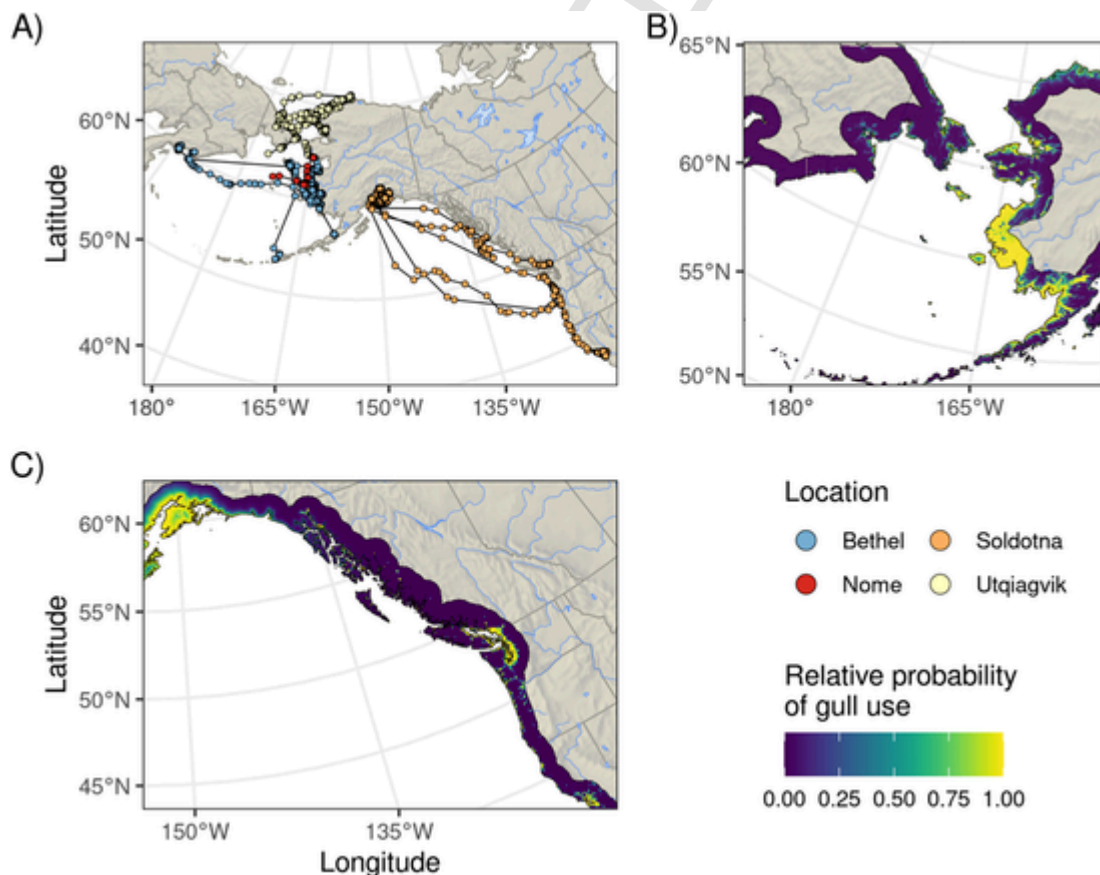

Fig. 2. Dispersal locations and relative probability of habitat use by Alaska gulls. A) Post-dispersal locations of gulls marked at the Bethel (blue), Nome (red), Soldotna (orange), and Utqiaġvik (yellow) landfills in Alaska for 30 days following the inferred initiation of autumn migration. B) Predicted relative probability of habitat selection use by gulls marked at Bethel, Nome, and Utqiaġvik landfills and C) at the Soldotna landfill for 30 days after initiation of autumn migration. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
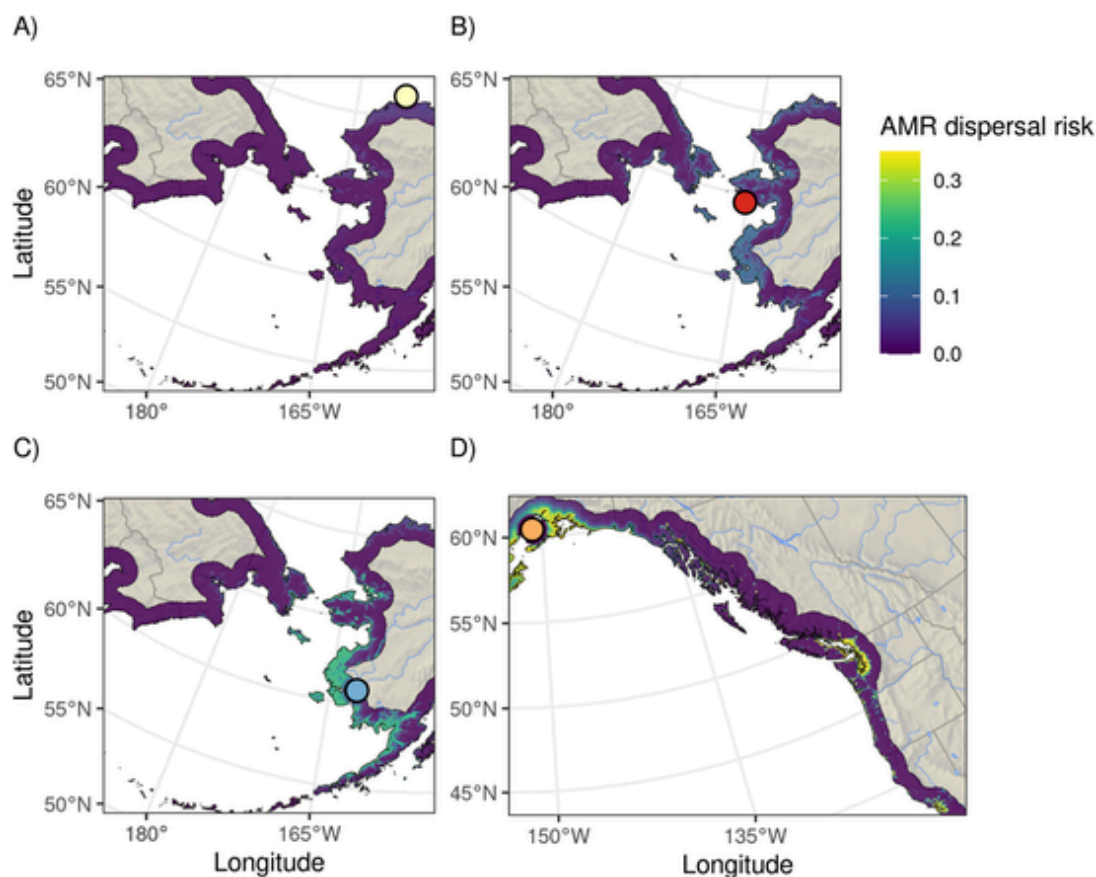
Fig. 3. Relative dispersal risk of clinically relevant antimicrobial resistant bacteria by gulls during autumn migration from four Alaska landfills. A) Utqiaġvik (yellow), B) Nome (red), C) Bethel (blue), and D) Soldotna (orange). Relative dispersal risk is based on the frequency and weighted clinical relevance of resistance genes identified among fecal bacteria detected at each location, estimated shedding patterns for gulls from an experimental challenge study, and patterns of predicted habitat use during a 30-day dispersal period inferred from satellite tracking and habitat modeling. Landfill marking locations including areas of inferred pre-migratory local movement (colored circles) are indicated. The relative AMR dispersal risk can range from 0 (no risk; inferred probability of occupancy by gulls shedding AMR bacteria is 0.00) to 10 (extremely high risk; full inferred probability of occupancy by gulls shedding bacteria with critically important AMR genes for 30 consecutive days following onset of autumn migration is 1.00). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

search has been conducted to assess this correlation using data systematically obtained from areas with discrete and relatively well-defined anthropogenic point sources over a wide geographical area. Using data from gulls inhabiting communities isolated by tens or hundreds of kilometers and having little to no commercial livestock, we found the frequency of *E. coli* exhibiting AMR in gull feces was strongly correlated with the local human population size. Furthermore, no isolates exhibiting MDR or those harboring *int1* were identified from gulls inhabiting areas adjacent to the smallest communities we visited (population ≤ 309). As such, we infer that gulls indeed serve as indicators of anthropogenic AMR in the local environment.

We identified diverse *E. coli* strains and resistance genes in gull feces collected at seven sampling locations and found relatively few clonal isolates. These results suggest that analysis of a higher number of samples and/or isolates may have yielded additional genetic diversity not captured in our study. Although non-selective enrichment captured more strain diversity, selective enrichment, employed to specifically enrich for cephalosporin-resistant *E. coli*, was four times more likely to capture MDR isolates. Therefore, future studies may benefit by using this information to optimize sample collection and/or enrichment protocols to address specific research questions (Döpfer et al., 2008).

Landfill-foraging gulls have been found to harbor clinically relevant AMR bacteria, including resistance to critically important antibiotics (Ahlstrom et al., 2019b; Dolejska et al., 2015). Our satellite tracking data provide evidence that gulls may disperse such bacteria across long distances over relatively short time periods upon the initia-

tion of autumn migration from landfills. Such long range, short duration migration periods are well within reported shedding periods for AMR bacteria exhibited by experimentally challenged gulls and mallards in laboratory settings (Franklin et al., 2020; Sandegren et al., 2018). For example, gulls flew to Chukotka, Russia within as few as two days of departure from the landfill in Utqiaġvik, Alaska and to southern San Francisco Bay within nine days of departure from the Soldotna landfill (Fig. S12). For perspective, ring-billed gulls were estimated to shed infectious doses of colistin-resistant bacteria for at least 16 days following experimental inoculation (Franklin et al., 2020). Thus, we conclude that gulls are capable of dispersing clinically relevant AMR bacteria across western North America and to East Asia from landfills in Alaska. Our finding of identical resistance gene profiles among *E. coli* isolates isolated from gull feces collected hundreds of kilometers apart could be a function of such long-distance dispersal of AMR bacteria by gulls; however, similar anthropogenic inputs into the environment at disparate locations within Alaska and/or transport of resistance determinants between locations by humans or other wildlife could also explain such findings.

Although samples from Nome had relatively low overall frequency of AMR *E. coli*, some gulls inhabiting this location harbored *E. coli* isolates that were highly drug resistant (e.g., resistant to 8 or 9 antibiotic classes). Nome is the closest community to East Asia that we sampled, and one of two U.S. marine vessel ports operating in the Bering Strait region. Additional sampling is needed to ascertain whether gulls in Nome consistently harbor highly drug resistant *E. coli* isolates and

where such high levels of resistance may originate. We also detected a higher frequency of macrolide resistance at sample locations in northern, northwestern, and western Alaska – those closest to East Asia. Thus, information on the occurrence of macrolide resistance among bacteria from Eastern Russia could be useful for assessing potential sources and dissemination routes (Bevan et al., 2017; Mughini-Gras et al., 2019).

The flexible modeling framework presented here provides novel insights into where clinically relevant AMR bacteria may be dispersed by gulls foraging at Alaska landfills upon the onset of autumn migration. Our results, and those from potential future modeling efforts using a similar approach, may be useful for optimizing surveillance of AMR in the environment and in identifying mitigation strategies to minimize spread through environmentally mediated pathways. Carbapenem resistant *E. coli*, for example, were recently reported from gulls in southcentral Alaska, including birds sampled at the Soldotna landfill (Ahlstrom et al., 2019b). Regional surveillance programs targeting the detection of critically important AMR (e.g., carbapenem resistance) may therefore consider prioritizing coordinated sampling efforts at urban coastal locations in southcentral Alaska, throughout the Pacific Northwest, and in central California, given that these locations shared relatively high dispersal risk values by gulls departing the Soldotna landfill. More generally, improved management of solid waste (e.g., incineration or more rapid coverage at landfills) and sewage (e.g., incorporation of low-energy anaerobic–aerobic treatment or enhanced disinfection processes) or tactical management of wildlife (e.g. hazing or blockage), might be considered at key habitats where birds or other free-ranging animals are predicted to have elevated risk of acquiring or disseminating clinically relevant AMR bacteria. Such actions may be especially prudent where habitat use by synanthropic wildlife overlaps with areas where people and domestic animals are likely to come into contact with deposited feces (e.g., municipal parklands, agricultural fields, or beaches). Alternatively, such actions may be deemed unnecessary at areas where AMR acquisition and dissemination risks are predicted to be comparatively low.

We recognize that the seven Alaska communities and four landfills where we focused gull fecal sampling and marking efforts are unlikely to represent the most important point sources of AMR into the environment at national or international scales. We also appreciate that other organisms or pathogens maintained by wildlife may be of higher priority elsewhere. Our modeling framework was therefore developed such that it may be adapted to estimate relative dispersal risk of a variety of pathogens maintained in birds or other wildlife across diverse landscapes. For example, our modeling approach could be readily adapted to evaluate the relative dispersal risk of avian influenza viruses by wild waterfowl across poultry-dense regions of Asia, Europe, and North America. Thus, we encourage others to apply or modify this model to assess the relative dispersal risk of diverse bacterial, parasitic, and viral pathogens by migratory birds and other wildlife.

4. Conclusions

Using a combination of phenotypic, genomic, and animal telemetry approaches, we demonstrate that gulls acquire AMR bacteria from anthropogenic sources, which they may subsequently disperse across and between continents via migratory movements. The frequency of detection of AMR *E. coli* in gulls was strongly correlated with human population size of the local community. Satellite telemetry results of gulls inhabiting Alaska landfills demonstrated autumn migration to Russia, Canada, and California within the demonstrated shedding period of AMR bacteria by gulls. Our flexible modeling framework estimates the relative dispersal risk of pathogens by wildlife through space and time and can therefore be adapted to identify high and low risk locations that can be targeted for intervention or future sampling regimes.

Abbreviations

AMR
  antimicrobial resistance and antimicrobial resistant
MDR
  multidrug resistant
PTT
  platform transmitter terminals
RSF
  resource selection function
MLST
  multilocus sequencing typing
DTR
  difficult-to-treat resistance

  Ahlstrom et al., 2020

Declaration of competing interest

The authors have no competing interests to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.144551.

References

Ackerman, J.T., Peterson, S.H., Tsao, D.C., Takekawa, J.Y., 2018. California gull (*Larus californicus*) space use and timing of movements in relation to landfills and breeding colonies. Waterbirds 41, 384–400. doi:10.1675/063.041.0402.

Ahlstrom, C.A., Bonnedahl, J., Woksepp, H., Hernandez, J., Olsen, B., Ramey, A.M., 2018. Acquisition and dissemination of cephalosporin-resistant *E. coli* in migratory birds sampled at an Alaska landfill as inferred through genomic analysis. Sci. Rep. 8, 7361. doi:10.1038/s41598-018-25474-w.

Ahlstrom, Christina A., Bonnedahl, J., Woksepp, H., Hernandez, J., Reed, J.A., Tibbitts, L., Olsen, B., Douglas, D.C., Ramey, A.M., 2019a. Satellite tracking of gulls and genomic characterization of faecal bacteria reveals environmentally mediated acquisition and dispersal of antimicrobial-resistant *Escherichia coli* on the Kenai Peninsula, Alaska. Mol. Ecol. 28, 2531–2545. doi:10.1111/mec.15101.

Ahlstrom, Christina A., Ramey, A.M., Woksepp, H., Bonnedahl, J., 2019b. Repeated detection of carbapenemase-producing *Escherichia coli* in gulls inhabiting Alaska. Antimicrob. Agents Chemother. 63, e00758-19. doi:10.1128/AAC.00758-19.

Ahlstrom, C.A., van Toor, M.L., Woksepp, H., Chandler, J.C., Reed, J.A., Reeves, A.B., Waldenström, J., Franklin, A.B., Douglas, D.C., Bonnedahl, J., Ramey, A.M., 2020. Data for Continental-scale Dispersal of Antimicrobial Resistant Bacteria by Alaska Landfill-foraging Gulls. doi:10.5066/P9D72Q3W.

Allen, H.K., Donato, J., Wang, H.H., Cloud-Hansen, K., Davies, J., Handelsman, J., 2010. Call of the wild: antibiotic resistance genes in natural environments. Nat. Rev. Microbiol. 8, 251–259. doi:10.1038/nrmicro2312.

Alm, E.W., Daniels-Witt, Q.R., Learman, D.R., Ryu, H., Jordan, D.W., Gehring, T.M., Santo Domingo, J., 2018. Potential for gulls to transport bacteria from human waste sites to beaches. Sci. Total Environ. 615, 123–130. doi:10.1016/j.scitotenv.2017.09.232.

Atterby, C., Ramey, A.M., Hall, G.G., Jarhult, J., Borjesson, S., Bonnedahl, J., 2016. Increased prevalence of antibiotic-resistant *E. coli* in gulls sampled in Southcentral Alaska is associated with urban environments. Infect. Ecol. Epidemiol. 6, 32334. doi:10.3402/iee.v6.32334.

Bartoń, K., 2019. MuMIn: Multi-Model Inference. R package.

Berendonk, T.U., Manaia, C.M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Bürgmann, H., Sørum, H., Norström, M., Pons, M.-N., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisand, V., Baquero, F., Martinez, J.L., 2015. Tackling antibiotic resistance: the environmental framework. Nat. Rev. Microbiol. 13, 310–317. doi:10.1038/nrmicro3439.

Bevan, E.R., Jones, A.M., Hawkey, P.M., 2017. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. J. Antimicrob. Chemother. 72, 2145–2155. doi:10.1093/jac/dkx146.

Bond, A.L., 2016. Diet changes in breeding herring gulls (*Larus argentatus*) in Witless Bay, Newfoundland and Labrador, Canada, over 40 years. Waterbirds 39, 152–158. doi:10.1675/063.039.sp115.

Cassini, A., Högberg, L.D., Plachouras, D., Quattrocchi, A., Hoxha, A., Simonsen, G.S., Colomb-Cotinat, M., Kretzschmar, M.E., Devleesschauwer, B., Cecchini, M., Ouakrim, D.A., Oliveira, T.C., Struelens, M.J., Suetens, C., Monnet, D.L., Strauss, R., Mertens, K., Struyf, T., Catry, B., Latour, K., Ivanov, I.N., Dobreva, E.G., Tambic Andrašević, A., Soprek, S., Budimir, A., Paphitou, N., Žemličková, H., Schytte Olsen, S., Wolff Sönksen, U., Märtin, P., Ivanova, M., Lyytikäinen, O., Jalava, J., Coignard, B., Eckmanns, T., Abu Sin, M., Haller, S., Daikos, G.L., Gikas, A., Tsiodras, S., Kontopidou, F., Tóth, Á., Hajdu, Á., Guólaugsson, Ó., Kristinsson, K.G., Murchan, S., Burns, K., Pezzotti, P., Gagliotti, C., Dumpis, U., Liumiene, A., Perrin, M., Borg, M.A., de Greeff, S.C., Monen, J.C., Koek, M.B., Elstrøm, P., Zabicka, D., Deptula, A., Hryniewicz, W., Caniça, M., Nogueira, P.J., Fernandes, P.A., Manageiro, V., Popescu, G.A., Serban, R.I., Schréterová, E., Litvová, S., Štefkovicová, M., Kolman, J., Klavs, I., Korošec, A., Aracil, B., Asensio, A., Pérez-Vázquez, M., Billström, H., Larsson, S., Reilly, J.S., Johnson, A., Hopkins, S., 2019. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. Lancet Infect. Dis. 19, 56–66. doi:10.1016/S1473-3099(18)30605-4.

Centers for Disease Control and Prevention, 2019. Antibiotic Resistance Threats in the United States. U.S. Department of Health and Human Services, CDC, Atlanta, GA. doi:10.15620/cdc:82532.

Chamosa, L.S., Álvarez, V.E., Nardelli, M., Quiroga, M.P., Cassini, M.H., Centrón, D., 2017. Lateral antimicrobial resistance genetic transfer is active in the open environment. Sci. Rep. 7, 513. doi:10.1038/s41598-017-00600-2.

D'Costa, V.M., King, C.E., Kalan, L., Morar, M., Sung, W.W.L., Schwarz, C., Froese, D., Zazula, G., Calmels, F., Debruyne, R., Golding, G.B., Poinar, H.N., Wright, G.D., 2011. Antibiotic resistance is ancient. Nature 477, 457–461. doi:10.1038/nature10388.

Dolejska, M., Masarikova, M., Dobiasova, H., Jamborova, I., Karpiskova, R., Havlicek, M., Carlile, N., Priddel, D., Cizek, A., Literak, I., 2015. High prevalence of *Salmonella* and IMP-4-producing Enterobacteriaceae in the silver gull on Five Islands, Australia. J. Antimicrob. Chemother. 71, 63–70. doi:10.1093/jac/dkv306.

Döpfer, D., Buist, W., Soyer, Y., Munoz, M.A., Zadoks, R.N., Geue, L., Engel, B., 2008. Assessing genetic heterogeneity within bacterial species isolated from gastrointestinal and environmental samples: how many isolates does it take? Appl. Environ. Microbiol. 74, 3490–3496. doi:10.1128/AEM.02789-07.

Duhem, C., Roche, P., Vidal, E., Tatoni, T., 2008. Effects of anthropogenic food resources on yellow-legged gull colony size on Mediterranean islands. Popul. Ecol. 40, 91–100. doi:10.1007/s10144-007-0059-z.

EUCAST EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 1.0http://www.amcli.it/wp-content/uploads/2015/10/EUCAST_detection_resistance_mechanisms_V1.pdf2013s 20 October 2020

EUCAST Breakpoint tables for interpretation of MICs and zone diamters. Version 6.0http://www.eucast.org/2016s 16 November 2017

Fancy, S.G., Pank, L.F., Douglas, D.C., Curby, C.H., Garner, G.W., Amstrup, S.C., Regelin, W.L., 1998. Satellite Telemetry: A New Tool for Wildlife Research and Management. Resource Publication. Washington, D.C..

Fay, M.P., 2010. Confidence intervals that match Fisher's exact or Blaker's exact tests. Biostatistics 11, 373–374. doi:10.1093/biostatistics/kxp050.

Forsberg, K.J., Reyes, A., Wang, B., Selleck, E.M., Sommer, M.O.A., Dantas, G., 2012. The shared antibiotic resistome of soil bacteria and human pathogens. Science 337, 1107–1111. doi:10.1126/science.1220761.

Franklin, A.B., Ramey, A.M., Bentler, K.T., Barrett, N.L., McCurdy, L.M., Ahlstrom, C.A., Bonnedahl, J., Shriner, S.A., Chandler, J.C., 2020. Gulls as sources of environmental contamination by colistin-resistant bacteria. Sci. Rep. 10, 4408. doi:10.1038/s41598-020-61318-2.

Gillings, M.R., Gaze, W.H., Pruden, A., Smalla, K., Tiedje, J.M., Zhu, Y.G., 2015. Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. ISME J 9, 1269–1279. doi:10.1038/ismej.2014.226.

Giroux, J.F., Patenaude-Monette, M., Lagarde, F., Thiériot, E., Brousseau, P., Molina, P., 2016. The rise and fall of ring-billed gulls (*Larus delawarensis*) in Eastern North America. Waterbirds 39, 87–98. doi:10.1675/063.039.sp101.

Gomi, R., Matsuda, T., Matsumur, Y., Yamamoto, M., Tanaka, M., Ichiyam, S., Yoneda, M., 2017. Whole-genome analysis of antimicrobialresistant and extraintestinal pathogenic *Escherichia coli* in river water. Appl. Environ. Microbiol. 83, e02703–e02716. doi:10.1128/AEM.02703-16.

Guenther, S., Aschenbrenner, K., Stamm, I., Bethe, A., Semmler, T., Stubbe, A., Stubbe, M., Batsajkhan, N., Glupczynski, Y., Wieler, L.H., Ewers, C., 2012. Comparable high rates of extended-spectrum-beta-lactamase-producing *Escherichia coli* in birds of prey from Germany and Mongolia. PLoS One 7, e53039. doi:10.1371/journal.pone.0053039.

Gullberg, E., Albrecht, L.M., Karlsson, C., Sandegren, L., Andersson, D.I., 2014. Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. MBio 5, e01918-1. doi:10.1128/mBio.01918-14.

Hartig, F., 2017. DHARMa: Residual Diagnostics for Hierarchical (Multi-level/Mixed) Regression Models (R Package Version 0.1.5).

Hernández, J., Bonnedahl, J., Eliasson, I., Wallensten, A., Comstedt, P., Johansson, A., Granholm, S., Melhus, Å., Olsen, B., Drobni, M., 2010. Globally disseminated human pathogenic *Escherichia coli* of O25b-ST131 clone, harbouring bla$_{CTX-M-15}$, found in Glaucous-winged gull at remote Commander Islands, Russia. Environ. Microbiol. Rep. 2, 329–332. doi:10.1111/j.1758-2229.2010.00142.x.

Hsieh, T.C., Ma, K.H., Chao, A., 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). Methods Ecol. Evol. 7, 1451–1456. doi:10.1111/2041-210X.12613.

Hupp, J.W., Yamaguchi, N., Flint, P.L., Pearce, J.M., Tokita, K., Shimada, T., Ramey, A.M., Kharitonov, S., Higuchi, H., 2011. Variation in spring migration routes and breeding distribution of northern pintails *Anas acuta* that winter in Japan. J. Avian Biol. 42, 289–300. doi:10.1111/j.1600-048X.2011.05320.x.

Inouye, M., Dashnow, H., Raven, L.-A., Schultz, M.B., Pope, B.J., Tomita, T., Zobel, J., Holt, K.E., 2014. SRST2: rapid genomic surveillance for public health and hospital microbiology labs. Genome Med 6, 90. doi:10.1186/s13073-014-0090-6.

Johnson, M., Zaretskaya, I., Raytselis, Y., Merezhuk, Y., McGinnis, S., Madden, T.L., 2008. NCBI BLAST: a better web interface. Nucleic Acids Res. 36, W5–W9. doi:10.1093/nar/gkn201.

Kadri, S.S., Adjemian, J., Lai, Y.L., Spaulding, A.B., Ricotta, E., Rebecca Prevots, D., Palmore, T.N., Rhee, C., Klompas, M., Dekker, J.P., Powers, J.H., Suffredini, A.F., Hooper, D.C., Fridkin, S., Danner, R.L., 2018. Difficult-to-treat resistance in gram-negative bacteremia at 173 US hospitals: retrospective cohort analysis of prevalence, predictors, and outcome of resistance to all first-line agents. Clin. Infect. Dis. 67, 1803–1814. doi:10.1093/cid/ciy378.

Kays, R., Crofoot, M.C., Jetz, W., Wikelski, M., 2015. Terrestrial animal tracking as an eye on life and planet. Science 348, aaa2478. doi:10.1126/science.aaa2478.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649. doi:10.1093/bioinformatics/bts199.

Kronvall, G., Smith, P., 2016. Normalized resistance interpretation, the NRI method. APMIS 124, 1023–1030. doi:10.1111/apm.12624.

Li, H., Durbin, R., 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25, 1754–1760. doi:10.1093/bioinformatics/btp324.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., 1000 Genome Project Data Processing Subgroup, 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25, 2078–2079. doi:10.1093/bioinformatics/btp352.

Lin, Y., Dong, X., Wu, J., Rao, D., Zhang, L., Faraj, Y., Yang, K., 2020. Metadata analysis of *mcr-1*-bearing plasmids inspired by the sequencing evidence for horizontal transfer of antibiotic resistance genes between polluted river and wild birds. Front. Microbiol. 11, 352. doi:10.3389/fmicb.2020.00352.

Ma, L., Li, A.D., Yin, X. Le, Zhang, T., 2017. The prevalence of integrons as the carrier of antibiotic resistance genes in natural and man-made environments. Environ. Sci. Technol. 51, 5721–5728. doi:10.1021/acs.est.6b05887.

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A., Weber, J.T., Monnet, D.L., 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 18, 268–281. doi:10.1111/j.1469-0691.2011.03570.x.

Martínez, J.L., Coque, T.M., Baquero, F., 2015. What is a resistance gene? Ranking risk in resistomes. Nat. Rev. Microbiol. 13, 116–123. doi:10.1038/nrmicro3399.

McFadden, D., 1974. Conditional logit analysis of qualitative choice behavior. In: Frontiers in Econometrics. New York, Academic Press, pp. 105–142.

Mughini-Gras, L., Dorado-García, A., van Duijkeren, E., van den Bunt, G., Dierikx, C.M., Bonten, M.J.M., Bootsma, M.C.J., Schmitt, H., Hald, T., Evers, E.G., de Koeijer, A., van Pelt, W., Franz, E., Mevius, D.J., Heederik, D.J.J., 2019. Attributable sources of community-acquired carriage of *Escherichia coli* containing β-lactam antibiotic resistance genes: a population-based modelling study. Lancet Planet. Heal. 3, e357–e369. doi:10.1016/S2542-5196(19)30130-5.

Nevers, M.B., Byappanahalli, M.N., Shively, D., Buszka, P.M., Jackson, P.R., Phanikumar, M.S., 2018. Identifying and eliminating sources of recreational water quality degradation along an urban coast. J. Environ. Qual. 47, 1042–1050. doi:10.2134/jeq2017.11.0461.

Orman, B.E., Piñeiro, S.A., Arduino, S., Galas, M., Melano, R., Caffer, M.I., Sordelli, D.O., Centrón, D., 2002. Evolution of multiresistance in nontyphoid *Salmonella* serovars

from 1984 to 1998 in Argentina. Antimicrob. Agents Chemother. 46, 3963–3970. doi:10.1128/AAC.46.12.3963-3970.2002.

Page, A.J., Cummins, C.A., Hunt, M., Wong, V.K., Reuter, S., Holden, M.T.G., Fookes, M., Falush, D., Keane, J.A., Parkhill, J., 2015. Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics 31, 3691–3693. doi:10.1093/bioinformatics/btv421.

Page, A.J., Taylor, B., Delaney, A.J., Soares, J., Seemann, T., Keane, J.A., Harris, S.R., 2016. SNP-sites: rapid efficient extraction of SNPs from multi-FASTA alignments. Microb. genomics 2, e000056. doi:10.1099/mgen.0.000056.

Pal, C., Bengtsson-Palme, J., Rensing, C., Kristiansson, E., Larsson, D.G.J., 2014. BacMet: antibacterial biocide and metal resistance genes database. Nucleic Acids Res. 42, D737–D743. doi:10.1093/nar/gkt1252.

Price, M.N., Dehal, P.S., Arkin, A.P., 2009. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Mol. Biol. Evol. 26, 1641–1650. doi:10.1093/molbev/msp077.

R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Found. Stat. Comput, Vienna, Austria (doi:ISBN 3-900051-07-0).

Ramey, A.M., Ahlstrom, C.A., van Toor, M.L., Woksepp, H., Chandler, J.C., Reed, J.A., Reeves, A.B., Waldenström, J., Franklin, A.B., Bonnedahl, J., Douglas, D.C., 2020. Tracking data for three large-bodied gull species and hybrids (*Larus* spp.) (ver 1.0, June 2020). U.S. Geological Survey Data Release. doi:10.5066/P9FZ4OJW.

Sandegren, L., Stedt, J., Lustig, U., Bonnedahl, J., Andersson, D.I., Järhult, J.D., 2018. Long-term carriage and rapid transmission of extended spectrum beta-lactamase-producing *E. coli* within a flock of Mallards in the absence of antibiotic selection. Environ. Microbiol. Rep. 10, 576–582. doi:10.1111/1758-2229.12681.

Schwarz, S., Silley, P., Simjee, S., Woodford, N., van Duijkeren, E., Johnson, A.P., Gaastra, W., 2010. Editorial: assessing the antimicrobial susceptibility of bacteria obtained from animals. J. Antimicrob. Chemother. 65, 601–604. doi:10.1093/jac/dkq037.

Seemann, T., 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30, 2068–2069. doi:10.1093/bioinformatics/btu153.

Seng, P., Drancourt, M., Gouriet, F., La Scola, B., Fournier, P., Rolain, J.M., Raoult, D., 2009. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin. Infect. Dis. 49, 543–551. doi:10.1086/600885.

Sheppard, A.E., Stoesser, N., Wilson, D.J., Sebra, R., Kasarskis, A., Anson, L.W., Giess, A., Pankhurst, L.J., Vaughan, A., Grim, C.J., Cox, H.L., Yeh, A.J., Sifri, C.D., Walker, A.S., Peto, T.E., Crook, D.W., Mathers, A.J., Mathers, A.J., 2016. Nested Russian doll-like genetic mobility drives rapid dissemination of the carbapenem resistance gene bla_{KPC}. Antimicrob. Agents Chemother. 60, 3767–3778. doi:10.1128/AAC.00464-16.

Skurnik, D., Ruimy, R., Andremont, A., Amorin, C., Rouquet, P., Picard, B., Denamur, E., 2006. Effect of human vicinity on antimicrobial resistance and integrons in animal faecal *Escherichia coli*. J. Antimicrob. Chemother. 57, 1215–1219. doi:10.1093/jac/dkl122.

State of Alaska Department of Labor and Workforce Development Alaska population estimates 2016http://live.laborstats.alaska.gov/pop/index.cfms 12 December 2019

USDA 2017 Census of Agriculture, United Stateshttps://www.nass.usda.gov/Publications/AgCensus/2017/index.php#full_report2019s 12 December 2019

Varela, A.R., Manageiro, V., Ferreira, E., Guimarães, M.A., da Costa, P.M., Caniça, M., Manaia, C.M., 2015. Molecular evidence of the close relatedness of clinical, gull and wastewater isolates of quinolone-resistant *Escherichia coli*. J. Glob. Antimicrob. Resist. 3, 286–289. doi:10.1016/j.jgar.2015.07.008.

Vittecoq, M., Laurens, C., Brazier, L., Durand, P., Elguero, E., Arnal, A., Thomas, F., Aberkane, S., Renaud, N., Prugnolle, F., Solassol, J., Jean-Pierre, H., Godreuil, S., Renaud, F., 2017. VIM-1 carbapenemase-producing *Escherichia coli* in gulls from southern France. Ecol. Evol. 7, 1224–1232. doi:10.1002/ece3.2707.

Weiser, E.L., Powell, A.N., 2010. Does garbage in the diet improve reproductive output of Glaucous gulls? Condor 112, 530–538. doi:10.1525/cond.2010.100020.

Wick, R.R., Judd, L.M., Gorrie, C.L., Holt, K.E., 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput. Biol. 13, e1005595. doi:10.1371/journal.pcbi.1005595.

Woolhouse, M., Ward, M., van Bunnik, B., Farrar, J., 2015. Antimicrobial resistance in humans, livestock and the wider environment. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 370, 20140083. doi:10.1098/rstb.2014.0083.

World Health Organisation Critically important antimicrobials for human medicine 6th Revision 2018https://Apps.Who.Int/Iris/Bitstream/Handle/10665/312266/9789241515528-Eng.Pdf?Ua=12019

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, F.M., Larsen, M.V., 2012. Identification of acquired antimicrobial resistance genes. J. Antimicrob. Chemother. 67, 2640–2644. doi:10.1093/jac/dks261.

Zankari, E., Allesøe, R., Joensen, K.G., Cavaco, L.M., Lund, O., Aarestrup, F.M., 2017. PointFinder: a novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens. J. Antimicrob. Chemother. 72, 2764–2768. doi:10.1093/jac/dkx217.