Journal of Global Antimicrobial Resistance 26 (2021) 317–322

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Plasmid-associated antimicrobial resistance and virulence genes in *Escherichia coli* in a high arctic reindeer subspecies

Marianne Sunde[a], Silje N. Ramstad[b], Knut Rudi[b], Davide Porcellato[b], Anuradha Ravi[c], Jane Ludvigsen[b], Carlos G. das Neves[a,d], Morten Tryland[d], Erik Ropstad[b], Jannice S. Slettemeås[a], Amar A. Telke[a,*]

[a] *Norwegian Veterinary Institute, Oslo, Norway*
[b] *Norwegian University of Life Sciences, Ås, Norway*
[c] *Norwegian University of Science and Technology, Trondheim, Norway*
[d] *UiT The Arctic University of Norway, Tromsø, Norway*

ARTICLE INFO

Article history:
Received 20 January 2021
Revised 11 May 2021
Accepted 1 June 2021
Available online 30 June 2021

Editor: Dr Jon Hobman

Keywords:
Wild reindeer
Svalbard
Antimicrobial resistance
Escherichia coli
IncF plasmid

ABSTRACT

Objectives: In extreme environments, such as the Arctic region, the anthropogenic influence is low and the presence of antimicrobial-resistant bacteria is unexpected. In this study, we screened wild reindeer (*Rangifer tarandus platyrhynchus*) from the Svalbard High Arctic Archipelago for antimicrobial-resistant *Escherichia coli* and performed in-depth strain characterisation.
Methods: Using selective culturing of faecal samples from 55 animals, resistant *E. coli* were isolated and subjected to minimum inhibitory concentration (MIC) determination, conjugation experiments and whole-genome sequencing.
Results: Twelve animals carried antimicrobial-resistant *E. coli*. Genomic analysis showed IncF plasmids as vectors both for resistance and virulence genes in most strains. Plasmid-associated genes encoding resistance to ampicillin, sulfonamides, streptomycin and trimethoprim were found in addition to virulence genes typical for colicin V (ColV)-producing plasmids. Comparison with previously reported IncF ColV plasmids from human and animal hosts showed high genetic similarity. The plasmids were detected in *E. coli* sequence types (STs) previously described as hosts for such plasmids, such as ST58, ST88 and ST131.
Conclusion: Antimicrobial-resistant *E. coli* were detected from Svalbard reindeer. Our findings show that successful hybrid antimicrobial resistance–ColV plasmids and their host strains are widely distributed also occurring in extreme environmental niches such as arctic ecosystems. Possible introduction routes of resistant bacterial strains and plasmids into Svalbard ecosystems may be through migrating birds, marine fish or mammals, arctic fox (*Vulpes lagopus*) or via human anthropogenic activities such as tourism.

1. Introduction

Recent studies have shown that antimicrobial-resistant bacteria can disseminate and persist in complete absence of selection pressure from antimicrobial agents, as exemplified by the expansion of third-generation cephalosporin resistance among *Escherichia coli* in Nordic broiler production where use of antimicrobials is almost absent [1–3]. The reason why resistant bacteria can be successful in the absence of selective pressure is not fully understood, but plasmids containing both antimicrobial resistance genes and plasmid addiction systems are considered important contributors. Acquisition of resistance genes by bacterial clones with increased fitness and host adaption may also enable the spread and persistence of antimicrobial resistance [4].

The microbial communities of most environmental reservoirs are usually not directly exposed to selection pressure from antimicrobial agents. However, many studies have documented antimicrobial-resistant bacteria in different ecological niches, such as wildlife populations, soil and water [5,6]. Some of these studies have demonstrated a link between anthropogenic activity and increased occurrence of antimicrobial-resistant bacteria [7–9]. In extreme environments, such as the Arctic region, the anthropogenic influence is minimal and the presence of antimicrobial-resistant

* Corresponding author. Mailing address: Norwegian Veterinary Institute, Section for Food Safety and Animal Health Research, Pb 750 Sentrum, 0106 Oslo, Norway. Tel.: +47 912 92993.
E-mail address: amar.anandrao.telke@vetinst.no (A.A. Telke).

https://doi.org/10.1016/j.jgar.2021.06.003

bacteria is unexpected. However, a recent study found genes encoding antimicrobial resistance from soil samples taken at the Svalbard High Arctic Archipelago [10].

Svalbard, which is Norwegian territory, is an archipelago located between the Norwegian mainland and the North Pole. These islands consist of large areas with untouched nature, arctic climate and very low human population density, restricted to a few smaller villages. Svalbard reindeer (*Rangifer tarandus platyrhynchus*) is one of the seven remaining subspecies of reindeer, which after the last Pleistocene glaciations spread over all the arctic and subarctic areas, locally adapting to these new locations and leading to the appearance of different subspecies [11]. Because of the geographical isolation of the Svalbard Archipelago, Svalbard reindeer have since had little or no contact with other reindeer and have well-recognised morphological and physiological singularities as a response to adapting to a high arctic environment. Svalbard reindeer remain the most isolated of the high arctic subspecies of reindeer [12,13]. Thus, the Svalbard reindeer could be a good sentinel species for monitoring changes in the arctic environment. The aim of this study was to investigate whether Svalbard reindeer in a remote 'antibiotic free' arctic environment can be carriers of antimicrobial-resistant *E. coli*. We performed in-depth sequence-based bacterial characterisation, with special focus on plasmid content, to evaluate possible source and routes of introduction for antimicrobial-resistant bacteria to these environments.

2. Materials and methods

2.1. Sampling and isolation of bacteria

Svalbard reindeer in Adventdalen and Reindalen, both defined as national parks on Svalbard, were in 2010 and 2011 immobilised in connection with field studies focusing on virus infections and parasites. We used the same animals for our study and collected faecal samples from a total of 55 apparently healthy animals. To obtain faecal samples, 16 animals from Adventdalen were chemically immobilised in 2010 [14] and 39 animals from Reindalen were physically restrained in 2011. All immobilisations and sampling were carried out according to national regulations on the use of animals for scientific experiments and with permits from the competent Norwegian authorities. Approval from the Governor of Svalbard was also obtained, and the projects linked to this sampling were registered at the Research in Svalbard Database (www.researchinsvalbard.no) under project numbers RIS-ID 3753 and RIS-ID 10892. Samples were stored at –20°C until analysis. Faecal material was investigated by selective methodology for isolation of antimicrobial-resistant *E. coli*. Faecal material from each animal was plated directly on seven different MacConkey agar plates (Becton Dickinson & Co., Le Pont-de-Claix, France) supplemented with different antimicrobial agents. Agar plates contained the following antimicrobials and concentrations: ampicillin 8 mg/L; tetracycline 8 mg/L; nalidixic acid 16 mg/L; cefotaxime 1 mg/L; sulfamethoxazole 256 mg/L; and streptomycin 16 mg/L and 32 mg/L. Plates were incubated in an aerobic atmosphere at 37°C for 24–48 h. Typical colonies were subcultured on blood agar plates, confirmed as *E. coli* by standard bacteriological testing, and subjected to antimicrobial susceptibility testing. One resistant *E. coli* isolate from each animal was chosen and included for further investigations.

2.2. Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) of 14 antimicrobial agents were determined by the broth microdilution method (VetMIC™ GN-mo; National Veterinary Institute, Uppsala, Sweden) following the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (www.eucast.org). Classification of isolates as susceptible or resistant was based on epidemiological cut-off values (ECOFFs) defined by EUCAST. *Escherichia coli* ATCC 25922 was included on a regular basis for quality control.

2.3. Conjugation and confirmation of transconjugants

Conjugation by liquid mating with *E. coli* DH5α as recipient strain was carried out as described previously [15]. Selection of transconjugants was performed by plating dilutions of the mating solutions onto Mueller–Hinton agar plates with 20 mg/L nalidixic acid and applying disks containing relevant antimicrobial agents (corresponding to the resistance profile of the donor) as previously described [15]. Colonies of presumptive transconjugants were selected from growth within the inhibition zones and were subcultured. The colony morphology of the transconjugants was inspected after growth on blood agar and on lactose–saccharose–bromthymol blue agar (*E. coli* DH5α has small characteristic colonies and is not a lactose-fermenter). The resistance profile of the transconjugants was determined by disk diffusion as described by EUCAST.

2.4. Whole-genome sequencing (WGS) and genomic analysis

One isolate per animal positive for carriage of antimicrobial-resistant *E. coli* ($n = 12$) and two transconjugant strains (obtained from the most multiresistant strains, 2010-01-5562-str and 2011-01-8208-4-str) were subjected to WGS and further genomic analysis. Genomic DNA was extracted using a Wizard® Genomic Purification Kit (Promega Corp., USA). Sequencing libraries were prepared using a Nextera XT DNA Lib Prep Kit (Illumina Inc., San Diego, CA, USA). Isolates were sequenced on an Illumina MiSeq platform (Illumina Inc.). Genome sequence data were submitted to the NCBI Sequence Read Archive (SRA) under accession no. **PRJNA673093**. Quality control of the Illumina raw reads was done using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and multiQC [16] was used to merge the results. Furthermore, Trimmomatic [17] was used to trim the reads to remove duplicate reads, ILLUMINACLIP was used to remove the adaptors, and bbduk v.38.86 was used to remove PhiX. Illumina reads were assembled using SPAdes v.3.12 with default settings [18]. The online tools ResFinder 3.2, SeroTypeFinder 2.0, MLST 2.0, FimTyper 1.0, pMLST 2.0, PlasmidFinder 2.1 and PointFinder available online from the Centre for Genomic Epidemiology (https://cge.cbs.dtu.dk/services/) were used for the respective genomic analyses. The complete Virulence Factor Database (VFDB) was available from web server at http://www.mgc.ac.cn/VFs/ [19]. Virulence genes were detected using NCBI BLASTn and the VFDB updated on 20 July 2020, where BLAST hits with e-value $\leq 1e^{-10}$, query coverage $\geq$90% and nucleotide identity $\geq$90% were considered as positive hits.

For the phylogenetic analyses, raw reads of sequence type 58 (ST58) genomes were retrieved from EnteroBase (https://enterobase.warwick.ac.uk/) and assembled using Unicycler v.0.4.7 with default settings. Parsnp from the Harvest Tools suite [20] was used to identify single nucleotide polymorphisms (SNPs) in the core genomes of three ST58 strains from this study by aligning the genomes with the most closely related complete reference genomes from EnteroBase. The flag x was implemented to filter out recombination events, which resulted in alignment of >77% of the three genomes.

2.5. Plasmid sequence reconstruction

Plasmid sequences were assembled from the WGS assemblies using MOB-suite v.3.0.1 [21]. NCBI BLASTn was used to select

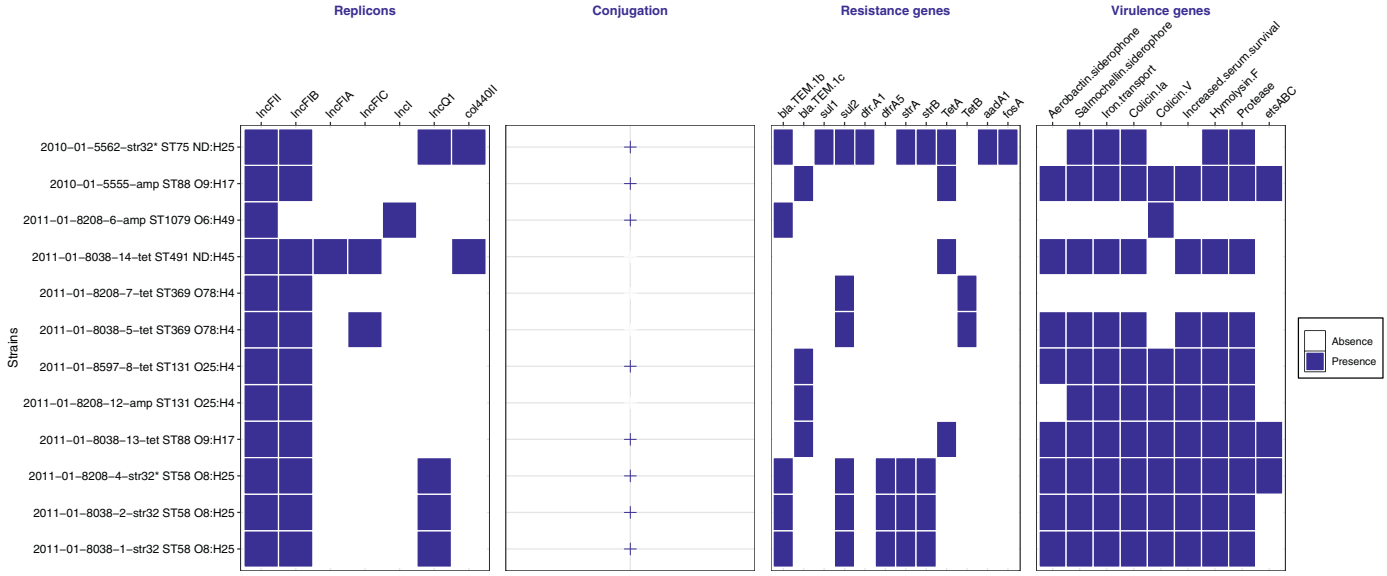

Fig. 1. Characteristics of antimicrobial-resistant *Escherichia coli* isolates from Svalbard reindeer, including plasmid-associated virulence genes. Blue squares represent the presence of a particular replicon, resistance gene or virulence gene (column) in a particular strain (rows). ST, sequence type; ND, not detected; *cia*, colicin Ia; *cva*, colicin V, *etsABC*, putative ABC transport system; *hylF*, haemolysin F; *iss*, increased serum survival protein; *iroBCDEN*, salmochelin siderophore; *iucABCD*, aerobactin siderophore; *ompT*, protease; *sitABC*, iron transport proteins. * Transconjugants obtained from these donors were also included for plasmid sequence analysis.

closely related complete plasmids for comparative analysis. Plasmid sequences were also confirmed by mapping trimmed Illumina reads against a reference plasmid sequence. Bowtie v.2.3.4.2 was used for mapping of trimmed Illumina reads [22]. Plasmid sequences were annotated using Prokka v.1.14.5 with default settings [23]. Annotations were manually curated in Artemis Comparison Tool (ACT) [24] and CLC Main Workbench v.8 (CLC bio, QIAGEN, Aarhus, Denmark). BLAST comparison of plasmids was created in BLAST Ring Image Generator (BRIG) v.0.95-dev.0004 [25].

3. Results

3.1. Phenotypic and molecular characteristics of antimicrobial-resistant Escherichia coli isolates

Antimicrobial-resistant *E. coli* were detected from 12 of 55 animals (Fig. 1). Resistance to ampicillin, streptomycin, sulfonamides and tetracycline was most commonly observed. None of the isolates were resistant to quinolones or produced extended-spectrum β-lactamases (ESBLs) or plasmid-mediated AmpC β-lactamases. Genes responsible for antimicrobial resistance were the same as those commonly occurring in isolates from humans and animals, such as the $bla_{\text{TEM-1}}$ gene encoding ampicillin resistance, *sul1* and *sul2* mediating resistance to sulfonamides, *tetA* and *tetB* encoding tetracycline resistance, *strA–strB* and *aadA* encoding streptomycin resistance, and the integron-associated gene cassettes *drfA1* and *dfrA5* encoding resistance to trimethoprim. Two isolates had ceftazidime MICs of 1 mg/L, one step above the ECOFF at 0.5 mg/L. However, no genes or mutations were found that could explain the slightly elevated ceftazidime MIC in these isolates. In addition, one strain was resistant to tetracycline but a gene responsible for tetracycline resistance could not be identified. An overview of the antimicrobial resistance genes detected in the isolates is shown in Fig. 1. The MIC distributions of the resistant isolates can be found in Table 1.

Virulence genes typical for colicin V (ColV)-producing IncF plasmids were present in the majority of isolates. These included genes for aerobactin biosynthesis (*iucABCD*), the receptor for aerobactin (*iutA*) and salmochelin biosynthesis (*iroBCDEN*) in addition to iron transporter genes (*sitABC*), haemolysin (*hlyF*), putative type 1 secretion system (*etsABC*) and colicin V (*cva*). The *ompT* gene encoding outer membrane protease and *iss* for increased serum survival were also present. In addition, genes encoding adhesion were found. Fig. 1 gives an overview of plasmid-associated virulence genes in the isolates, whereas all the of the virulence genes for the respective isolates are available in Supplementary Table S1. Conjugation experiments showed that most of the strains carried resistance genes on self-conjugative plasmids (Fig. 1).

The isolates grouped into seven different multilocus sequence typing (MLST) sequence types (STs). Four STs were represented by more than one isolate, namely ST58 ($n = 3$), ST131 ($n = 2$), ST88 ($n = 2$) and ST369 ($n = 2$) (Fig. 1). The ST131 isolates were categorised as O25:H4 serotype, with *fimH*22 type and were susceptible to fluoroquinolones. They therefore do not belong to the globally distributed *E. coli H*30Rx sublineage of ST131. The two ST88 isolates were recovered in different years and from animals in different geographic areas, whereas the three ST58 strains originated from animals in Reindalen sampled in 2011.

Phylogenetic analysis of the three ST58 isolates from this study and a selection of ST58 downloaded from EnteroBase demonstrated a close genetic relationship between our isolates and a group of previously sequenced ST58 isolates. Sequences of the ST58 strains originated from humans, animals and different environmental niches and were isolated in different countries and years (Supplementary Fig. S1). The closest relative to strain 2011-01-8208-4-str32 was strain SCK30-22, a serotype O8:H25 *E. coli* isolated from an undisclosed human infection from the Netherlands. Strain SCK30-22 was used to extract the core genome of 2011-01-8208-4-str32 and to identify SNPs between them. We found 164 SNP counts for 2011-01-8208-4-str32 compared with SCK30-22. Only 64 SNP count differences were found between 2011-01-8038-1-str32 and 2011-01-8038-2-str32.

3.2. Plasmid sequence analysis

Analysis using PlasmidFinder indicated that all isolates harboured IncF plasmids, while a single strain also contained an IncI plasmid. Seven IncF plasmids had pMLST profile F2:A-:B1. They were present in ST58, ST88 and ST131 strains, whereas the re-

Table 1
Distribution of minimum inhibitory concentrations (MICs) and antimicrobial resistance in *Escherichia* coli isolated from Svalbard reindeer.

Substance	Distribution (%) of MIC values (mg/L)*															
	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512
Ampicillin							1		2				1	1	7	
Ciprofloxacin	1	3	8													
Nalidixic acid							1	3	7	1						
Gentamicin						4	8									
Streptomycin									4	4				1	1	2
Tetracycline							4	1		1	1	2	3			
Florphenicol									11	1						
Colistin						7	5									
Sulfamethoxazole										5		1				6
Trimethoprim				6	1	1						4				
Chloramphenicol								9	2	1						
Kanamycin										12						
Cefotaxime			3	7	2											
Ceftazidime					4	6	2									

*White fields indicate range of dilutions tested. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration are given as the lowest concentration tested. Vertical lines denote epidemiological cut-off values for resistance.

maining IncF plasmids had unique pMLST profiles (Supplementary Table S1).

From one strain (2011-01-8208-4-str), a nearly complete IncF plasmid sequence was determined (plasmid p8208-4). The nucleotide sequence of the plasmid is available in NCBI GenBank with accession no. **MW228449**. This plasmid was subjected to detailed investigation. The approximate size was 148 200 bp with an average GC content of 51%. A total of 167 open reading frames (ORFs) were predicted and annotated. A typical IncF plasmid carrying toxin–antitoxin (TA)-based addiction system (*yacAB*) and plasmid maintenance proteins (PsiA–PsiB, plasmid SOS inhibition proteins; ParAB/SopAB, plasmid-partitioning proteins) was present. Besides, the complete genetic region encoding the transfer component (*tra*) was present, spanning ~32 kb of the plasmid. Plasmid p8208-4 contained many insertion sequence (IS) elements such as IS*2*, IS*4*, IS*6*, IS*26* and IS*110*, which play a key role in bacterial genome organisation and evolution. Antimicrobial resistance genes were clustered in a characteristic resistance gene locus containing a deleted class 1 integron with *dfrA5* and only 24 bp of the 3′-conserved segment (CS). A cluster with $bla_{\text{TEM-1b}}$–IS*26*–*repA*–*sul2*–*strA*–*strB* flanked by direct copies of IS*26*, corresponding to the composite transposon Tn*6029*, was found adjacent to the partial 3′-CS (Fig. 2). Virulence genes typical for IncF ColV plasmids were located on p8208-4, such as *iucABCD, iutA, iroBCDEN, sitABC, hlyF, etsABC, cva, ompT* and *iss*. Conjugation experiments showed that the plasmid was self-transferrable. Sequence data of the transconjugant strain confirmed the presence of plasmid-specific regions such as replicon sequences and resistance genes.

Comparison of ColV plasmids from GenBank closely related to p8208-4 is shown in Fig. 2. Plasmid p8208-4 shared >99% nucleotide identity and >99% coverage/length with previously characterised plasmids, namely pG749_1 (GenBank accession no. **CP014489**), pSF-088-1 (GenBank accession no. **CP012636**) and pDB4277 (GenBank accession no. **KP398867**). The core backbones of plasmids derived from ST88 and ST131 strains were found to be similar to p8208-4, but were different in resistance gene content. The characteristics of the remaining IncF plasmids from the other strains in this study indicated the presence of hybrid antimicrobial resistance–virulence (ColV) plasmids in most of the strains, as shown in Fig. 1. BLAST comparison of plasmids with FAB formula F2:A-:B1 from this study, including p8208-4, is shown in Supplementary Fig. S2.

4. Discussion

In this study, we demonstrated that wild reindeer in a remote high arctic ecosystem harboured antimicrobial-resistant *E. coli*. The resistance genes and most of the virulence determinants were associated with plasmids. The virulence determinants were similar to those found on IncF ColV plasmids [26]. The majority of the resistant *E. coli* contained IncF ColV plasmids and these plasmids are known to carry a 'battery' of virulence genes. They are strongly associated with avian pathogenic *E. coli* (APEC) but are also reported from clinical and intestinal carrier isolates of human origin [26]. Other studies have reported IncF ColV plasmids in *E. coli* STs also found as hosts for such plasmids in this study, such as ST58, ST88 and ST131 [26,27]. This may indicate successful host and plasmid combinations. Their presence in arctic environments further underlines the success of these STs and their associated plasmids. ST131 with IncF plasmids of type F2:A-:B1 have also been detected from Antarctic pinnipeds in a previous study [28].

Plasmid p8208-4 showed high genetic similarity to previous ColV plasmids. The virulence genes and plasmid backbones were almost identical to the corresponding regions in previously characterised plasmids. Furthermore, we observed that the antimicrobial resistance gene locus, which is regarded as the less conserved part of ColV plasmids, was identical to the corresponding region of other ColV plasmids (Fig. 1). This included a specific genetic signature, consisting of the *dfrA5* gene cassette and 24 bp of the 3′CS. This signature has been shown to be present in highly related IncF ColV plasmids obtained both from animal and human hosts from different geographic locations [26]. The finding of IncF ColV plasmids in multiple isolates in this study, with a high genetic similarity to previously described IncF ColV plasmids, suggests that they originate from ecological compartments where such plasmids are commonly circulating, such as poultry and/or humans [26,29]. Most of our strains contained self-mobilisable plasmids, however the conjugation experiments failed to demonstrate transmission of plasmids for a subset of the strains. Possible explanations could be incapability of the method used or that genes needed for conjugation were interrupted, such as the presence of an IS element in crucial genes of the conjugation machinery.

The majority of STs and plasmids detected have been reported in previous studies [26,27,30,31]. Phylogenetic analyses of ST58 demonstrated that our strains grouped with previously sequenced strains. This indicates that the strains from reindeer have an external source and that they have been introduced to Svalbard. The introduction routes of resistant bacteria to wild fauna in Svalbard are largely unknown. A possible theory is that migratory birds can introduce such bacteria to remote locations such as Svalbard. Several bird species that breed on Svalbard overwinter in densely populated parts of central Europe, where they are exposed to resident microbiota. As an example, the pink-footed goose (*Anser brachyrhynchus*) migrates to Svalbard from mainland Norway, Den-

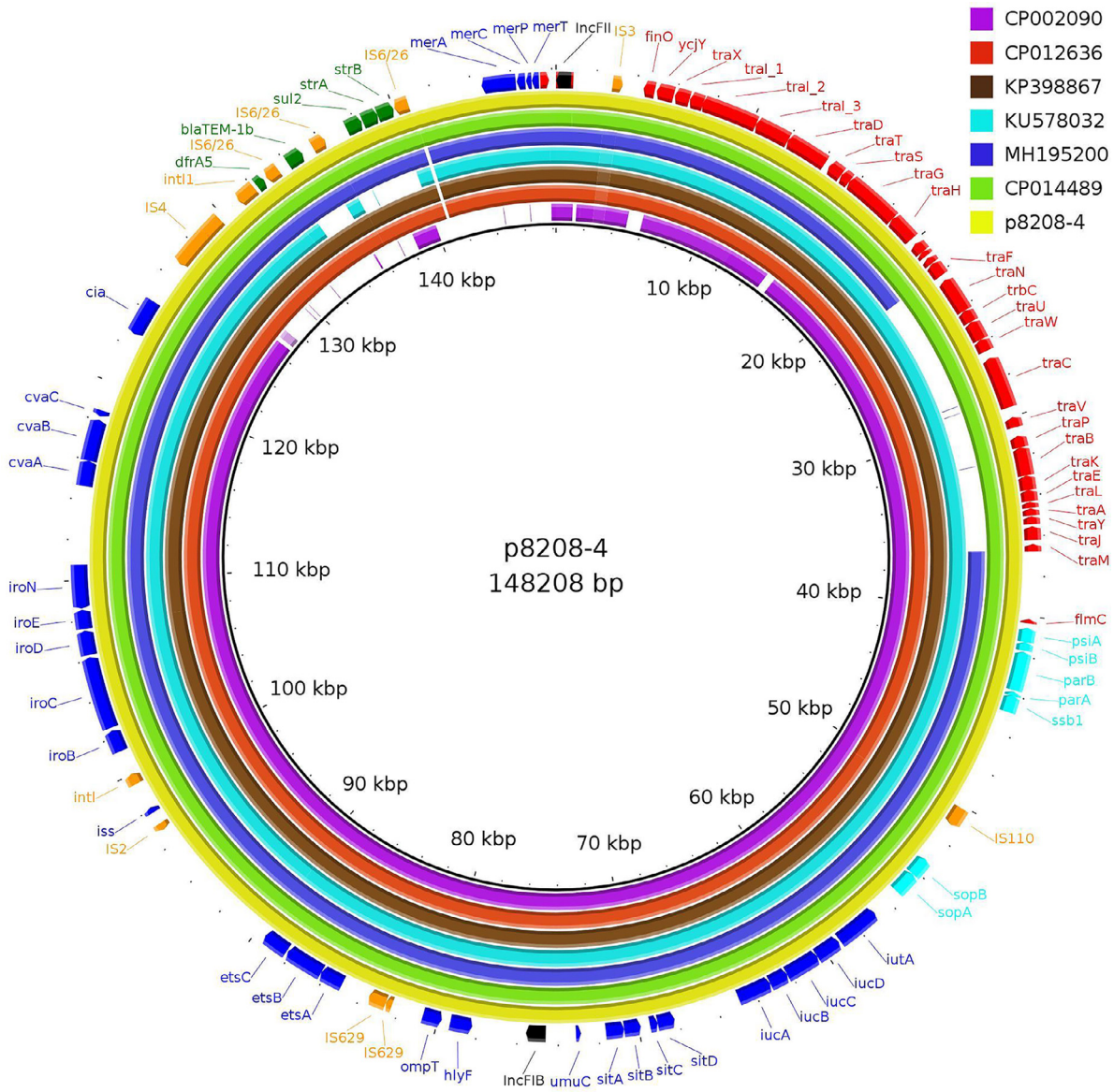

Fig. 2. BLAST comparison of closely related ColV plasmids from GenBank to p8208-4. Coloured arrows represent genes and their functions. The outer ring shows the gene content of p8208-4 along different reading frames that correlate with regions in plasmids that have been detected in the BLAST analysis.

mark, the Netherlands and Belgium [32], whereas the barnacle goose (*Branta leucopsis*) that visits Svalbard in summer migrates from Scotland [33]. Compared with Svalbard, all of these European locations represent ecosystems more exposed to anthropogenic activity, agriculture and antimicrobial compounds, and with a higher environmental load of antimicrobial-resistant bacteria. A former study has also reported antimicrobial-resistant *E. coli* in arctic birds sampled in Siberia and Greenland, indicating migrating birds as potential vectors for resistant *E. coli* [34]. However, Svalbard is also a popular tourist attraction, especially during the summer months, with more than 100 cruise boats docking in the archipelago every year. One should therefore not rule out that the large number of people visiting the archipelago could lead to the introduction of resistant bacteria. Intensified livestock production cannot be directly blamed for the presence of resistant bacteria on Svalbard, since no such production exists on the archipelago. There are no other indigenous ruminant species in Svalbard, however cows and sheep have been introduced in the past, both at Longyearbyen and at the Russian mining stations of Barentsburg and Pyramiden, but they are no longer present.

The culture-based approach used in this study may not be the optimal method to define the overall resistome within a given environment. Metagenomic DNA sequencing could produce valuable additional information. Also, we included only one (randomly chosen) isolate per animal for phenotypic and genotypic analyses, which limits further exploration of ST diversity within the same host. Polyclonal carriage of resistant *E. coli* could be possible, however this was not investigated further in this study. The culture-based screening used in this study was designed to detect resistance to relevant antimicrobial agents for human and animal use. Our strategy included screening for resistance to 'older' types of antimicrobials, such as streptomycin and sulfonamides. We believed this would increase the chance of detecting resistant isolates, as resistance to newer and broad-spectrum antimicrobials, such as extended-spectrum cephalosporins and fluoroquinolones, was considered more unlikely to be present due to a presumably lower prevalence.

Monitoring antimicrobial resistance within pristine ecosystems such as the Arctic and at their interface with human-influenced areas could enable more insight into the frequency with which resis-

tant bacteria and genes encoding resistance are exchanged in microbial communities. We therefore argue that wild reindeer, and likely other indigenous and migrant species, can function as important host reservoirs and potential vectors for the spread of resistant bacteria and genetic determinants responsible for resistance. In pristine areas such as the Arctic, this might highlight the importance of wildlife species as sentinels for monitoring the spread of antimicrobial resistance.

In conclusion, environmental dissemination of antimicrobial resistance appears to have reached the most remote Arctic regions such as Svalbard, supporting previous studies. Furthermore, our findings demonstrate that successful plasmids, such as IncF ColV plasmids, and their host strains are widely distributed, occurring also in remote and high arctic environmental niches. Sequence-based analyses support an external source for the plasmids and strains. In the future, a combination of well-designed monitoring programmes, preferably global ones, and advanced sequencing technology will probably contribute to better understanding of the epidemiology of antimicrobial resistance also comprising environmental reservoirs.

Acknowledgments

The authors thank Aina Steihaug Barstad, Ronny Aanes, Marit Madderom, Alina L. Evans, Jon Martin Arnemo and Brage Bremset Hansen for their contribution to the laboratory work, field work and sampling. The authors are grateful to the Public Health Institute, Norway, for sequencing of two transconjugant strains. Bioinformatics work was performed on the Saga computing cluster, owned by the University of Oslo and Uninett/Sigma2, and operated by the Department for Research Computing at the University of Oslo IT Department (http://www.usit.uio.no/).

Funding: This work was supported by a grant from the Svalbard Science Forum registered under ID RIS 3753 at the Research in Svalbard Database (www.researchinsvalbard.no) and by a parallel project ran by the Norwegian University of Life Sciences registered at the same database under ID RIS 2909. The study was also supported by the Norwegian Veterinary Institute and the Norwegian University of Life Science.

Competing interests: None declared.

Ethical approval: Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2021.06.003.

References

[1] Borjesson S, Bengtsson B, Jernberg C, Englund S. Spread of extended-spectrum β-lactamase producing *Escherichia coli* isolates in Swedish broilers mediated by an Incl plasmid carrying $bla_{CTX\text{-}M\text{-}1}$. Acta Vet Scand 2013;55:3.
[2] Mo SS, Norstrom M, Slettemeas JS, Lovland A, Urdahl AM, Sunde M. Emergence of AmpC-producing *Escherichia coli* in the broiler production chain in a country with a low antimicrobial usage profile. Vet Microbiol 2014;171:315–20.
[3] Myrenas M, Slettemeas JS, Thorsteinsdottir TR, Bengtsson B, Borjesson S, Nilsson O, et al. Clonal spread of *Escherichia coli* resistant to cephalosporins and quinolones in the Nordic broiler production. Vet Microbiol 2018;213:123–8.
[4] Pitout JD, DeVinney R. *Escherichia coli* ST131: a multidrug-resistant clone primed for global domination. F1000Res 2017;6:F1000.
[5] Dolejska M, Literak I. Wildlife is overlooked in the epidemiology of medically important antibiotic-resistant bacteria. Antimicrob Agents Chemother 2019;63 e01167-19.
[6] Vittecoq M, Godreuil S, Prugnolle F, Durand P, Brazier L, Renaud N, et al. Antimicrobial resistance in wildlife. Ecol Evol 2016;53:519–29.
[7] Arnold KE, Williams NJ, Bennett M. Disperse abroad in the land': the role of wildlife in the dissemination of antimicrobial resistance. Biol Lett 2016;12:20160137.
[8] Skurnik D, Ruimy R, Andremont A, Amorin C, Rouquet P, Picard B, et al. Effect of human vicinity on antimicrobial resistance and integrons in animal faecal *Escherichia coli*. J Antimicrob Chemother 2006;57:1215–19.
[9] Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. Call of the wild: antibiotic resistance genes in natural environments. Nat Rev Microbiol 2010;8:251–9.
[10] McCann CM, Christgen B, Roberts JA, Su JQ, Arnold KE, Gray ND, et al. Understanding drivers of antibiotic resistance genes in High Arctic soil ecosystems. Environ Int 2019;125:497–504.
[11] Røed KH, Côté S, Yannic G. *Rangifer tarandus*: classification and genetic variation. Reindeer and caribou: health and disease. Tryvan M, Kutz SJ, editors editors. Boca Raton, FL: CRC Press; 2019.
[12] Røed KH. Comparison of the genetic variation in Svalbard and Norwegian reindeer. Can J Zool 1985;63:2038–42.
[13] Cote SD, Dallas JF, Marshall F, Irvine RJ, Langvatn R, Albon SD. Microsatellite DNA evidence for genetic drift and philopatry in Svalbard reindeer. Mol Ecol 2002;11:1923–30.
[14] Evans AL, Lian M, das Neves CG, Os O, Andersen R, Aanes R, et al. Physiologic evaluation of medetomidine–ketamine anesthesia in free-ranging Svalbard (*Rangifer tarandus platyrhynchus*) and wild Norwegian reindeer (*Rangifer tarandus tarandus*). J Wildl Dis 2013;49:1037–41.
[15] Sunde M, Norstrom M. The prevalence of, associations between and conjugal transfer of antibiotic resistance genes in *Escherichia coli* isolated from Norwegian meat and meat products. J Antimicrob Chemother 2006;58:741–7.
[16] Ewels P, Magnusson M, Lundin S, Kaller M. MultiQC: summarize analysis results for multiple tools and samples in a single report. Bioinformatics 2016;32:3047–8.
[17] Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 2014;30:2114–20.
[18] Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012;19:455–77.
[19] Liu B, Zheng DD, Jin Q, Chen LH, Yang J. VFDB 2019: a comparative pathogenomic platform with an interactive web interface. Nucleic Acids Res 2019;47:D687–92.
[20] Treangen TJ, Ondov BD, Koren S, Phillippy AM. The Harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. Genome Biol 2014;15:524.
[21] Robertson J, Nash JHE. MOB-suite: software tools for clustering, reconstruction and typing of plasmids from draft assemblies. Microb Genom 2018;4:e000206.
[22] Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol 2009;10:R25.
[23] Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics 2014;30:2068–9.
[24] Carver TJ, Rutherford KM, Berriman M, Rajandream MA, Barrell BG, Parkhill J. ACT: the Artemis Comparison Tool. Bioinformatics 2005;21:3422–3.
[25] Alikhan NF, Petty NK, Ben Zakour NL, Beatson SA. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics 2011;12:402.
[26] McKinnon J, Roy Chowdhury P, Djordjevic SP. Molecular analysis of an IncF ColV-like plasmid lineage that carries a complex resistance locus with a trackable genetic signature. Microb Drug Resist 2020;26:787–93.
[27] Reid CJ, McKinnon J, Djordjevic SP. Clonal ST131-*H22* *Escherichia coli* strains from a healthy pig and a human urinary tract infection carry highly similar resistance and virulence plasmids. Microb Genom 2019;5:e000295.
[28] Mora A, Garcia-Pena FJ, Alonso MP, Pedraza-Diaz S, Ortega-Mora LM, Garcia-Parraga D, et al. Impact of human-associated *Escherichia coli* clonal groups in Antarctic pinnipeds: presence of ST73, ST95, ST141 and ST131. Sci Rep 2018;8:4678.
[29] de Oliveira AL, Rocha DA, Finkler F, de Moraes LB, Barbieri NL, Pavanelo DB, et al. Prevalence of ColV plasmid-linked genes and in vivo pathogenicity of avian strains of *Escherichia coli*. Foodborne Pathog Dis 2015;12:679–85.
[30] McKinnon J, Roy Chowdhury P, Djordjevic SP. Genomic analysis of multidrug-resistant *Escherichia coli* ST58 causing urosepsis. Int J Antimicrob Agents 2018;52:430–5.
[31] Reid CJ, Blau K, Jechalke S, Smalla K, Djordjevic SP. Whole genome sequencing of *Escherichia coli* from store-bought produce. Front Microbiol 2019;10:3050.
[32] Madsen J, Williams JH, Johnson FA, Tombre IM, Dereliev S, Kuijken E. Implementation of the first adaptive management plan for a European migratory waterbird population: the case of the Svalbard pink-footed goose *Anser brachyrhynchus*. Ambio 2017;46:275–89.
[33] Kolzsch A, Bauer S, de Boer R, Griffin L, Cabot D, Exo KM, et al. Forecasting spring from afar? Timing of migration and predictability of phenology along different migration routes of an avian herbivore. J Anim Ecol 2015;84:272–83.
[34] Sjolund M, Bonnedahl J, Hernandez J, Bengtsson S, Cederbrant G, Pinhassi J, et al. Dissemination of multidrug-resistant bacteria into the Arctic. Emerg Infect Dis 2008;14:70–2.