Antimicrobial resistance determinants in silage

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Animal products may play a role in developing and spreading antimicrobial resistance in several ways. On the one hand, residues of antibiotics not adequately used in animal farming can enter the human body via food. However, resistant bacteria may also be present in animal products, which can transfer the antimicrobial resistance genes (ARG) to the bacteria in the consumer’s body by horizontal gene transfer. As previous studies have shown that fermented foods have a meaningful ARG content, it is indicated that such genes may also be present in silage used as mass feed in the cattle sector. In our study, we aspired to answer what ARGs occur in silage and what mobility characteristics they have? For this purpose, we have analyzed bioinformatically 52 freely available deep sequenced silage samples from shotgun metagenome next-generation sequencing. A total of 16 perfect matched ARGs occurred 54 times in the samples. More than half of these ARGs are mobile because they can be linked to integrative mobile genetic elements, prophages or plasmids. Our results point to a neglected but substantial ARG source in the food chain.

In intensive cattle farming, silage is an essential component of feed. An average dairy cow consumes 25–27 kg of this forage a day, reaching up to a silage consumption of 12,500 kg per lactation1,2. Silage is most commonly produced from maize or legume plants by the process of anaerobic fermentation. Throughout the fermentation process, fermenting microorganisms, including bacteria, multiply. As a result of this biochemical transformation, the silage is enriched with beneficial nutrients. If bacteria that are involved in the process harbor antimicrobial resistance genes (ARGs), the amount of these genes in the silage will increase in parallel with the bacterial counts. Consequently, silage, as a mass feed may continuously supply the gastrointestinal tract of animals with bacteria carrying ARGs. Bacteria entering the digestive system may come into contact with the host microbiota that facilitates the exchange of bacterial genes (e.g. ARGs) by horizontal gene transfer (HGT). HGT may take place as a result of three different processes: conjugation, transduction and transformation. Except for transformation, by which a bacterium can take up any gene from its environment, the routes of HGT require particular active delivery processes. By conjugation, cell-to-cell contact provides the opportunity for a copy of a plasmid to translocate to a recipient bacterium3. Transduction negates the condition of cell-to-cell contact, as in this case, bacteriophages act as a conduit for shuttling genes among bacteria4. The genetic environment of the genes involved in the transfer significantly influences the efficacy of the latter two HGT processes, i.e., the genes’ mobility. The reason why the mobility characteristics of ARGs involved in silage are worth taking into consideration is the following. If ARGs from silage are transmitted to pathogenic bacteria within an animal’s body, efficacy of antibiotic (AB) treatment may be reduced on the consequent bacterial diseases. In addition, in case of the gut colonization of silage-borne bacteria that carry ARGs, the appearance and enrichment of bacterial ARGs may take place in the animals’ environment after defecation. Decreased efficacy of AB treatments may result in economic loss, and the increased environmental ARG level may have additional veterinary and human health consequences. It is proven in former publications that the number of ARGs in fermented dairy products can increase due to the multiplication of fermenting bacteria5. Nevertheless, the description of this phenomenon cannot be found for silage in the literature. Our study aimed to examine the diversity, bacterial relatedness and mobility potential of ARGs deriving from silage. For this purpose, freely available next-generation sequencing (NGS) shotgun metagenome datasets were analyzed by a unified bioinformatics pipeline.

Results
Based on the taxon classification performed on a database containing complete reference genomes of plants, the most dominant plants in the silage belong to the Medicago genus and most likely to the alfalfa (M. sativa) species. Further results of the analysis of the 52 shotgun metagenomic sequenced samples (Table 2) are summarized in the following sections. After presenting the bacteriome and the identified AGRs (resistome), predictions

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regarding the mobility potential of ARGs were also resumed based on genetic characteristics that may play a significant role in HGT.

**Bacteriome.** By taxon classification, the number of reads aligning to bacterial genomes varied by samples (median: $2.06 \times 10^6$, IQR: $2.9 \times 10^6$). The relative abundances of genera that achieved more than 1% of the bacterial hits in any of the samples are shown in Fig. 1.

The dominant bacterial genera (with mean abundance) in descending order were Weissella (45.7%), Pantoea (18.5%), Lactiplantibacillus (13.5%), Pediciococcus (6.7%), Lacticaseibacillus (1.3%), Enterococcus (1.2%), Lactobacillus (1.0%), Kosakonia (0.8%), Staphylococcus (0.6%), Enterobacter (0.5%), Lactobacillus (0.5%), Bacillus (0.4%), Listeria (0.4%), Pseudomonas (0.4%), Leclercia (0.2%), Mammallibacillus (0.2%), Agrobacterium (0.1%).

**Resistome.** The median length of the filtered contigs harboring ARGs constructed by de novo assembly was 4,204 bp (IQR: 2,832). The number of ARGs found on the contigs ranged from 1 to 2. The identified 16 ARG types appeared 54 times in 20 of the analyzed 52 samples. These ARGs were the following: aadA2, antid-6-Ia, antid-9-Ia, aph(3’)-IIa, dfrG, erm(44)v, lmrD, lsaE, poxtA, qnrD1, qnrS1, sul1, sul2, tet(K), vatE (Fig. 2). The resistance mechanism of identified ARGs was the antibiotic inactivation (48.1%), antibiotic target protection (20.3%), antibiotic efflux (13.0%), antibiotic target alteration (9.3%), and antibiotic target replacement (9.3%) in descending order of frequency. Table 1 shows the bacterial species to which the ARG harboring contigs were assigned based by the taxon classification. In addition, the table also presents which drug classes are affected by the ARGs.

**Mobilome.** We found a total of 53 ARGs that are assumably mobile. Ten of these ARGs are linked to integrative mobile genetic elements (iMGE). A further two ARGs were detected in prophages and forty-one on plasmids. The frequencies of ARGs associated with iMGEs, phages and plasmids are summarized in Fig. 3 by bacterial species of origin.

Following the distance method proposed by Johansson et al., integrated mobile genetic element associated ARGs were detected in five samples (30, 45, 46, 48, 52) and five species (B. subtilis, E. hormaechei, E. faecium, L. paracasei, L. plantarum). B. subtilis associated aph(3’)-IIa in sample 30, and poxtA of E. faecium in sample 45, of L. plantarum in sample 52, and of L. paracasei in sample 46 were detected as iMGE linked gene. sul1 and aadA2 were detected in E. hormaechei co-existed with integrated mobile element in sample 48. Two prophage-linked ARGs were identified, the contig harboring erm(44)v classified to S. pseudoxylosus by VirSorter2 was found to be of dsDNA phage origin while the contig of lsaE from S. suis was predicted as ssDNA derived. These phage associated ARGs were detected in sample 37 and sample 45 respectively. Contigs with ARGs were predicted to belong to plasmids in 19 samples (Nrs. 11, 12, 17, 30, 32, 36, 37, 40, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52).
Discussion

Throughout our study, numerous perfect ARG matches were identified in the metagenome of *Medicago* silage samples. All but group A of the analyzed subsets had at least one sample containing one or more ARG. Among the PRJNA495415 Bioproject samples, the highest number of ARGs were found in group D. The interpretation of this finding is limited due to the lack of detailed information on the samples. Interestingly, all but one of the PRJNA764355 bioproject samples contained ARGs. Due to the lack of metadata, it is hard to find any reason...
The occurrence frequency of the ARGs flanked by IMGE, positioned in a plasmid or a phage.

For this high ARG level. However, one possible cause might be that the PRJNA764355 samples were sequenced deeper and thus contained approximately 1.3 times more reads than the PRJNA495415 samples. It is known from previous studies that deeper sequencing leads to the generation of more complex genes by the de novo assembly.

In the following, our results will be interpreted from a perspective of bacteriological significance, genomic relevance and furthermore, antimicrobial stewardship and possible clinical aspects.

Taking the microbiome into consideration, bacteria that were predicted to harbor the identified ARGs can be classified according to their presence in silage. In the literature, the following bacteria are mentioned to be characteristic for silage: B. subtilis, E. faecium, L. plantarum, L. lactis, L. brevis, L. acidipiscis, W. paramesenteroides. The genera of these species dominate the bacteriome of the samples. The identified Cronobacter sp. JZ38 may be of plant origin. However, it can be assumed that other species may be present as contaminants of the silage: A. amylophilus, E. hormaechei, E. faecalis, Gracilibacillus sp. SCU50, L. manihotivorans, L. paracasei, P. rettgeri, S. aureus, S. carnosus, S. pseudoxyllosus, S. saprophyticus, S. suis, T. halophilus. Nevertheless, some of these bacteria are members of the Lactobacillaceae family, the Leuconostoc or Enterobacter genera. Numerous species of these groups are typical for fermented food and feed components.

From a genomic point of view, the following was found in the literature regarding the co-occurrence of the ARGs identified in our study and the bacteria carrying them. AadA2 encoding an aminoglycoside nucleotidyldiphosphotransferase has been described in A. baumannii in former publications, ant(6)-Ia, that is an aminoglycoside nucleotidyldiphosphotransferase gene, appears in many species, including Lactobacillus spp. Its species-specific association with A. amylophilus has not been described in any former publications. aph(3')-IIa, an aminoglycoside phosphotransferase, to our knowledge, has not been detected in B. subtilis up until now. QnrS1 encoding a quinolone resistance protein was originally identified in Shigella flexneri. In line with our results, this gene has recently been mentioned to appear in Cronobacter spp. in a case report. E. hormaechei deriving aadA2 and sul1, a sulfonamide resistant dihydropteroate synthase gene that is described to appear in Gram-negative bacteria have been reported to appear in the genome of Enterobacter spp. and E. hormaechei, respectively in former publications as well.

Within the Enterococcus genus, two perfect ARG matches were identified, namely aph(3')-IIIa in E. faecalis and poxT in E. faecium. aph(3')-IIIa is an aminoglycoside phosphotransferase that normally appears in S. aureus and Enterococcus spp., while poxT is a gene encoding an ABC-F subfamily (ATP-binding cassette-F) protein that facilitates resistance to tetracycline, phenicol, and oxazolidinone via modification of the bacterial ribosome. First detection of poxT took place in a methicillin-resistant S. aureus strain, followed by other bacterial species, including E. faecium. Sul2, a sulfonamide resistant dihydropteroate synthase of Gram-negative bacteria is commonly described in E. coli. DfrG is a plasmid-encoded dihydrofolate reductase that, to our knowledge, has not been described in Gracilibacillus spp. until now, but has already appeared in the Bacillaceae family, ant(6)-Ia, an aminoglycoside nucleotidyldiphosphotransferase gene appears in many species, including Lactobacillus spp. Its species-specific association with L. manihotivorans has not been described in any publications. PoxT that was detected in L. paracasei, L. plantarum and L. brevis in the silage samples, has been described to appear in Lactobacillaceae, namely L. acidophilus, but not in these very species.
species that was detected harboring \( \text{aph}(3')-\text{IIIa} \) in the silage samples was \( \text{L. plantarum} \). This finding is in line with the ARG-species match results mentioned in former publications\(^1\). Furthermore, \( \text{L. plantarum} \) was also associated with \( \text{vatE} \) that encodes an acetylttransferase confering resistance against streptogramins\(^2\). \( \text{VatE} \) was originally found in \( \text{E. faecium} \)\(^3\) and has since then been identified in \( \text{Lactobacillaceae} \)\(^4\), but not specifically in \( \text{L. plantarum} \). \( \text{L. acidipiscis} \) \( \text{ant(9)-Ia} \) at an aminoglycoside nucleotidyltransferase gene\(^5\) was associated with this genus for first within this study. Gene \( \text{qnrD1} \) encoding a quinolone resistance protein that is normally detected in \( \text{Salmonella enterica} \)\(^6\), has already been found in \( \text{Providencia} \) spp\(^7\) and was attached to \( \text{P. rettgeri} \) in our study as well. \( \text{S. aureus} \) could have been associated with two ARGs, \( \text{ant(6)-Ia} \) and \( \text{tetK} \) encoding a tetracycline efflux protein, that are both common findings in \( \text{Staphylococcus} \) spp\(^8,9\). Although, \( \text{erm(44)v} \) was first detected in the \( \text{S. saprophyticus} \)\(^10\), no literature could be found about the appearance of this gene in \( \text{S. carnosus} \) or in \( \text{S. pseudoxylosus} \) species. \( \text{IsaE} \) encoding another ABC-F subfamily protein conferring resistance to pleuromutilin, lincosamide, and streptogramin A is a common finding in \( \text{Streptococcus} \) spp\(^11\) and has also been associated with \( \text{S. suis} \) in previous publications\(^12\). Besides the bacterial species mentioned above, \( \text{aph}(3')-\text{IIIa} \) was also detected in \( \text{T. halophilus} \). This ARG is often appears in \( \text{Enterococcus} \)\(^13\) but has not yet been written down in this species. Furthermore, to our knowledge, \( \text{W. paramesenteroides} \) associated \( \text{ant(6)-Ia} \) has first been detected in this study.

Throughout our study, several ARGs were predicted to be co-occurring with genetic attributes facilitating mobility. The bioinformatic analysis of the mobility characteristics relied upon the identification of three major mobility determination groups, namely iMGEs, phages and plasmids. We found \( \text{aph}(3')-\text{IIIa} \) linked to an integrated mobile genetic element in \( \text{B. subtilis} \) that is in line with similar findings of \( \text{E. coli} \)\(^1\). While \( \text{aadA2} \) and \( \text{sul1} \) have both been described to appear on plasmids in \( \text{E. hormaechei} \)\(^14\), we found them associated with iMGEs. Our finding on iMGE flanked \( \text{poxtA} \) in \( \text{E. hormaechei} \) is in line with the current literature\(^15\). We found the same co-occurrence, namely \( \text{poxtA} \) and an iMGE, in \( \text{L. paracasei} \). This phenomenon has not been published in that species to the best of our knowledge. Gene \( \text{erm(44)v} \) was found in \( \text{S. saprophyticus} \) and \( \text{S. suis} \). While a similar linkage can be found in the literature in connection with \( \text{erm(44)v} \)\(^16\), no details of other mobility characteristics are mentioned in a recent report of the latter gene\(^17\). All other mobile ARGs were detected on contigs that were predicted to derive from plasmids. In case of \( \text{aadA2} \) in \( \text{A. baumannii} \)\(^18\), \( \text{aadA2} \) and \( \text{sul1} \) in \( \text{E. hormaechei} \)\(^1\); \( \text{aph}(3')-\text{IIIa} \) in \( \text{E. faecalis} \)\(^19\); \( \text{sul2} \) in \( \text{E. coli} \)\(^1\); \( \text{aph}(3')-\text{IIIa} \) in \( \text{L. plantarum} \)\(^2\); \( \text{qnrS1} \) in \( \text{P. rettgeri} \)\(^2\); \( \text{ant(6)-Ia} \) in \( \text{S. aureus} \)\(^2\) and \( \text{erm(44)v} \) in \( \text{S. saprophyticus} \) plasmid associations have been formerly described in the literature. To our knowledge, no publications have yet been released on the plasmid occurrence of \( \text{aph}(3')-\text{IIIa} \) in \( \text{B. subtilis} \), \( \text{dfrG} \) in \( \text{Graclibacillus sp.} \) \( \text{SCU50} \), \( \text{ant(9)-Ia} \) in \( \text{L. acidipiscis} \), \( \text{IsaE} \) in \( \text{S. suis} \), \( \text{qnrS1} \) in \( \text{Cronobacter sp.} \) \( \text{JJ38} \), \( \text{erm(44)v} \) in \( \text{S. carnosus} \) and \( \text{S. pseudoxylosus} \). Hao et al. described \( \text{poxtA} \) embedded in a multi-resistance plasmid with mobile elements flanking in \( \text{E. fecalis} \). This gene has been found in a number of Gram-positive bacteria, including enterococci as well, but it has neither been identified in \( \text{L. plantarum} \) nor \( \text{L. brevis} \)\(^2\). Previous findings confirm the occurrence of \( \text{vat(E)} \) on plasmids\(^2\). Nevertheless, in spite of its frequent presence in enterococci\(^3\) there is no evidence of its former plasmid-associated appearance in \( \text{L. plantarum} \). We found that gene \( \text{aph}(3')-\text{IIIa} \) of \( \text{T. halophilus} \) was encoded on a plasmid that is consistent with the fact that \( \text{aph}(3')-\text{IIIa} \) is often identified on high molecular weight plasmids and chromosomes of the enterococcal species\(^2\). Nonetheless, to the best of our knowledge, a description of the \( \text{aph}(3')-\text{IIIa} \) gene in \( \text{T. halophilus} \) is a pioneering finding.

The mobility characteristics of the ARGs may not only provide us with information regarding the public health risk that may be associated with the samples, but also point to the possible origins of the genes. Regardless of human intervention, ARGs are present in the microbial communities\(^2\). However, antimicrobial use and abuse intensifies the horizontal transfer of ARGs and thus contributes to the spread of AMR. In the animal production sector, the use of antibiotics is common, thus bacteria appearing in the feces and in the surroundings of the animals (e.g. in farm air, on tools, vehicles or other settings related to animals) often harbor bacteria with an advanced ARG set. Silage may get in direct physical contact with these bacteria at the farms and thus get contaminated with a few ARGs. Consequently, the presence of ARGs in the silage samples was well-expected, but the abundance of resistance genes and MGEs may increase due to the application of antibiotics.

Examining further aspects of antimicrobial stewardship and possible clinical relevance, phenotypical manifestations and public health considerations associated with the detected ARGs are both important. Intense antimicrobial use (AMU) can be associated with the headway of AMR, as antibiotic pressure selects for bacteria carrying ARGs that facilitate bacterial survival. Quantitatively, the majority of AMU around the globe occurs in agricultural settings\(^4\).\(^9\). Intensive farming, that serves to fulfill the high global demand for animal proteins relies on an antibiotic infrastructure to treat and prevent disease and occasionally, to increase feed efficacy. In order to maximize economic gains, few countries still apply regulations that facilitate the use of low doses of antibiotics as growth promoters\(^9\), while other regions, like the U.S. or Europe, have banned this practice. Nevertheless, besides the treatment of symptomatic infectious diseases, antibiotics are still widely used in the livestock sector for metaphylactic and prophylactic purposes in higher doses\(^11,12\). Even though, compared to the poultry and pig production sector, average antibiotic usage has relatively lower rates by\(^13\), antimicrobial compounds are often chosen in this species as well. In cattle farming, mastitis is the most predominant reason for the administration of antibiotics by adult cattle, while enteritis and pneumonia is the most common reason for calves\(^4,5\). According to various reports and studies from around the world\(^4,6,7\), tetracyclines are of inevitable significance in the medication of cattle, while beta-lactams, macrolides, sulfonamides, lincosamides and ionophore antibiotics are also very widely used. Of the European Medicines Agency (EMA) Highest-Priority Critically Important Antibiotics (HPCIAs), namely third and fourth generation cephalosporines, fluoroquinolones and polymyxins, polymixins and fluoroquinolones are the most applied, although their sales rates are still far below the most frequently administered antibiotic groups by livestock species\(^7\). In our samples \( \text{E. faecium} \), \( \text{L. paracasei} \), \( \text{L. plantarum} \), \( \text{L. brevis} \), \( \text{S. aureus} \) and \( \text{S. suis} \) harbored genes, namely \( \text{poxtA} \) and \( \text{IsaE} \) that may confer resistance against multiple antibiotic groups, including tetracyclines. Moreover, \( \text{poxtA} \) was detected in the proximity of iMGEs in \( \text{L. paracasei} \) and \( \text{E. faecium} \) and harbored on a plasmid in \( \text{L. brevis} \). In line with our findings, \( \text{Enterococcus} \) species related
Antimicrobial resistance is an emerging global threat to public health that both affects agriculture and the healthcare sector. The usage of antibiotics in livestock species exceeds the rate of human applications. Antibiotic use in food animal medicine is also considered a risk as it may provide an indirect transfer route of antibiotic residue through the food chain. Even though antimicrobials administered for veterinary use, may exert an undesired effect on the food chain, the presence of ARGs in dairy cattle nutrition research is still under-represented in the literature. According to our results, microbial mass contained in fermented feeds have other medical risks than transmitting contagious diseases, like listeriosis. The bacterial content of these mass feeds, that is either, required for the fermentation processes or collected from various sources of contamination on the farms, could play an essential role in the ARG shift through the food chain.

Materials and methods

Data. We searched appropriate datasets in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) repository. In December 2021, only two shotgun metagenomic BioProjects (PRJNA495415, PRJNA764355) could have been found that had adequate depth for the de novo assembly that our study is based on. The median raw count (interquartile range, IQR) of the samples was 26,5 × 10⁶ (3.0 × 10⁶) and 34.7 × 10⁶ (1.5 × 10⁷) in datasets PRJNA495415 and PRJNA764355, respectively. There is limited metadata available of the samples in the NCBI SRA database (Table 2). Nevertheless, it can be assumed from the metadata that the samples of PRJNA495415 were taken at different fermentation periods. Samples were taken on days 0, 7, 14 and 28 were classified in groups A, B, C and D, respectively. Based on metadata of PRJNA764355 samples, no such stratification was possible, so all samples were classified as group E.

Bioinformatic analysis. Quality based filtering and trimming of the raw short reads was performed with TrimGalore (v.0.6.6, https://github.com/FelixKrueger/TrimGalore), setting 20 as a quality threshold. Only reads longer than 50 bp were retained and taxonomically classified using Kraken2 (v2.1.1) and a database created from the NCBI RefSeq complete archaeal, bacterial, viral and plant genomes. For this taxon assignment, the -confidence 0.5 parameter was used to obtain more precise species level hits. The taxon classification data was managed in R using functions of the packages phyloseq and microbiome. The preprocessed reads
were assembled to contigs with MEGAHIT (v1.2.9)\(^8\) using default settings. The contigs were also classified taxonomically with Kraken2 with the same database as above. From the contigs all possible open reading frames (ORFs) were gathered with Prodigal (v2.6.3)\(^8\). The protein translated ORFs were aligned to the ARG sequences taxonomically with Kraken2 with the same database as above. From the contigs all possible open reading frames associated. In the MobileElementFinder database (v1.0.2) for each bacterial species, only those with a distance threshold defined within iMGEs and ARGs were considered of the Comprehensive Antibiotic Resistance Database (CARD, v.3.1.3)\(^2\)\(^\text{21,82}\) by Resistance Gene Identifier (RGI, 1.1)\(^\text{84}\). The phage content of the assembled contigs was predicted by VirSorter2 (v2.2.3)\(^\text{85}\). The findings were filtered for dsDNAphages and ssDNAs. All data management procedures, analyses and plots were performed in R environment (v4.1.0)\(^7\)\(^7\).

**Table 2.** Analyzed samples. The samples of dataset PRJNA495415 were taken on days 0, 7, 14 and 28 were classified in groups A, B, C and D, respectively. All samples from BioProject PRJNA764355 are assigned to group E. Column Run contains the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) run identifiers.

| BioProject | PRJNA495415 | PRJNA764355 |
|------------|-------------|-------------|
| Group A    | B           | C           | D           | E           |
| Id Run     | Id Run      | Id Run      | Id Run      | Id Run      |
| 1 SRR7990583 | 11 SRR7990585 | 21 SRR7990580 | 31 SRR7990581 | 41 SRR16036389 |
| 2 SRR7990587 | 12 SRR7990586 | 22 SRR7990585 | 32 SRR7990584 | 42 SRR16036390 |
| 3 SRR7990591 | 13 SRR7990590 | 23 SRR7990589 | 33 SRR7990588 | 43 SRR16036391 |
| 4 SRR7990592 | 14 SRR7990593 | 24 SRR7990594 | 34 SRR7990595 | 44 SRR16036392 |
| 5 SRR7990598 | 15 SRR7990599 | 25 SRR7990596 | 35 SRR7990597 | 45 SRR16036393 |
| 6 SRR7990604 | 16 SRR7990605 | 26 SRR7990600 | 36 SRR7990601 | 46 SRR16036394 |
| 7 SRR7990608 | 17 SRR7990609 | 27 SRR7990602 | 37 SRR7990603 | 47 SRR16036395 |
| 8 SRR7990610 | 18 SRR7990611 | 28 SRR7990606 | 38 SRR7990607 | 48 SRR16036396 |
| 9 SRR7990612 | 19 SRR7990613 | 29 SRR7990614 | 39 SRR7990615 | 49 SRR16036397 |
| 10 SRR7990616 | 20 SRR7990617 | 30 SRR7990618 | 40 SRR7990619 | 50 SRR16036398 |

**Data availability**

The datasets analysed in the current study are available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) repository and can be accessed through the PRJNA495415 and PRJNA764355 BioProject identifiers.

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Author contributions
N.S. takes responsibility for the integrity of the data and the accuracy of the data analysis. A.G.T., M.P., N.S. and S.Á.N. conceived the concept of the study. A.G.T., M.P., N.S. and S.Á.N. participated in the bioinformatic analysis. A.G.T., K.S., M.P., N.S. and S.Á.N. participated in the drafting of the manuscript. A.G.T., K.S., M.P., N.S. and S.Á.N. carried out the critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript.

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