

Salmonella enterica serovar Schwarzengrund: Distribution, Virulence and Antimicrobial Resistance

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ABSTRACT

Introduction. The numbers of *Salmonella enterica* serovar Schwarzengrund infections have been increasing globally in recent years. This serotype has been isolated from both poultry, retail meat and other foods, leading to multiple outbreaks. Alongside the increase in infections, there are concerns about the increasing levels of antimicrobial resistance (AMR) among *S. Schwarzengrund* strains. **Purpose.** To better understand the genetic factors possibly contributing to the growing prevalence of *S. Schwarzengrund*, the sequences of 2,058 isolates from human patients (N=313) and food and animal-associated sources including chicken (N=1,145), turkey (N=300), and pork (N=132) were analyzed. **Methods.** The data were extracted from GenBank and evaluated for their AMR genes with AMRFinder. Additionally, putative virulence genes and plasmid transfer genes were assessed using the Virulence and AMR Plasmid Transfer Factor Database. **Results.** AMR genes were common among the isolates, with 1269 (61.7%) carrying at least one AMR gene. The most commonly observed resistance genes included *aph(3'')-Ib* (aminoglycoside; N=969, 47.1%), *tet(A)* (tetracycline; N=190, 9.2%) and *sul2* (sulfonamide; N=150, 7.3%) (Figure 2). There were two major AMR gene profiles, as shown by the large groups in Figure 3. One of these includes the group without any detected AMR genes (upper ball in Figure 3), while the other are the strains carrying only *aph(3'')-Ib*. The latter group is predominantly from chickens.

METHODS

To conduct the analyses of *S. Schwarzengrund* genetics, sequenced isolates were identified using the NCBI Pathogen Detection Isolate Browser (Figure 1; panel A). WGS data (B) and metadata (C) from 2,058 isolates from human patients (N=313) and food and animal-associated sources including chicken (N=1,145), turkey (N=300), and pork (N=132) and environmental sources (N=21) were extracted from GenBank and Isolate Browser and evaluated for their AMR genes with AMRFinder, either using GalaxyTrakr (D) or extracting from the Isolate Browser. Putative virulence genes and plasmid transfer genes were assessed using the Virulence and AMR Plasmid Transfer Factor Database (<https://virulence.fda.gov>; Algarni et al, 2023) E). Metadata, AMR, virulence and plasmid transfer gene data were imported into Excel (Microsoft) and BioNumerics (Applied Maths) for further analyses (F). Analyses with Excel included descriptive statistics of gene prevalence, while BioNumerics was used for phylogenetics based on presence/absence of genes among the different metadata categories.



Figure 1. Methods used to conduct the bioinformatics analyses of the *S. Schwarzengrund* strains included in the study. The details of each step are outlined in the paragraph above.

RESULTS

AMR genes were common among the isolates, with 1269 (61.7%) carrying at least one AMR gene. The most commonly observed resistance genes included *aph(3'')-Ib* (aminoglycoside; N=969, 47.1%), *tet(A)* (tetracycline; N=190, 9.2%) and *sul2* (sulfonamide; N=150, 7.3%) (Figure 2). There were two major AMR gene profiles, as shown by the large groups in Figure 3. One of these includes the group without any detected AMR genes (upper ball in Figure 3), while the other are the strains carrying only *aph(3'')-Ib*. The latter group is predominantly from chickens.

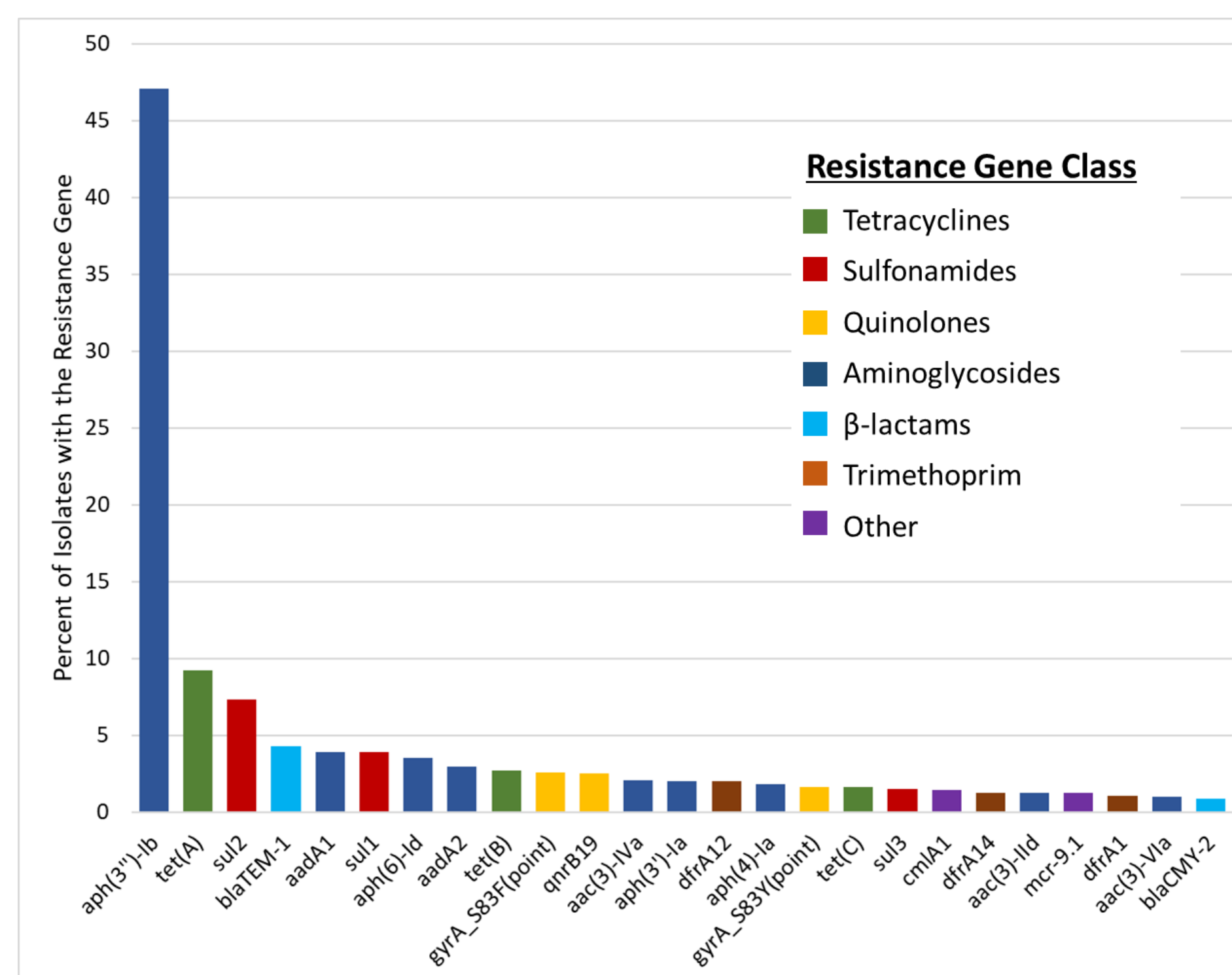


Figure 2. Percent of *S. Schwarzengrund* strains in the study that carried the respective AMR genes. The bars are color coded based on antimicrobial classes.

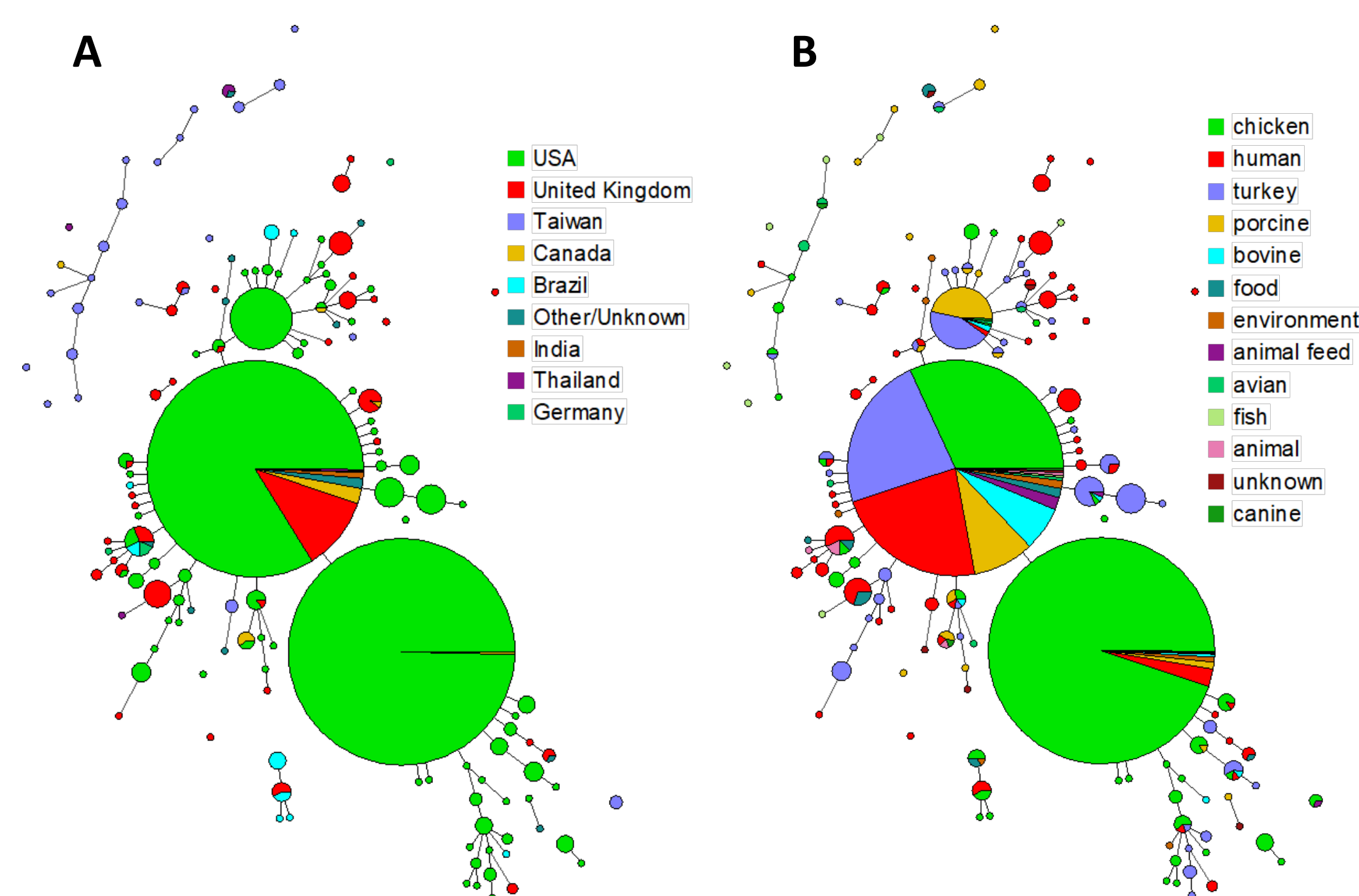


Figure 3. Minimum spanning tree analyses based on the AMR gene profiles of the *S. Schwarzengrund* strains included in the study. The trees are either color coded based on country of origin (panel A) or isolation source (panel B). The relative size of the circles are proportional to the group size.

Among the identified plasmid types, approximately 1,060 (51.5%) carried multiple transfer genes associated with IncFIB-FIC plasmids. Additionally, IncI1 (N=101, 4.9%), IncHI2 (N=62, 3.0%), or IncHI1 (N=24, 1.2%) genes were detected in at least 1% of the strains. Many of the strains carrying the IncFIB-FIC plasmids also are positive for *aph(3'')-Ib*. Strains lacking detected plasmid sequences typically did not carry any identified AMR genes.

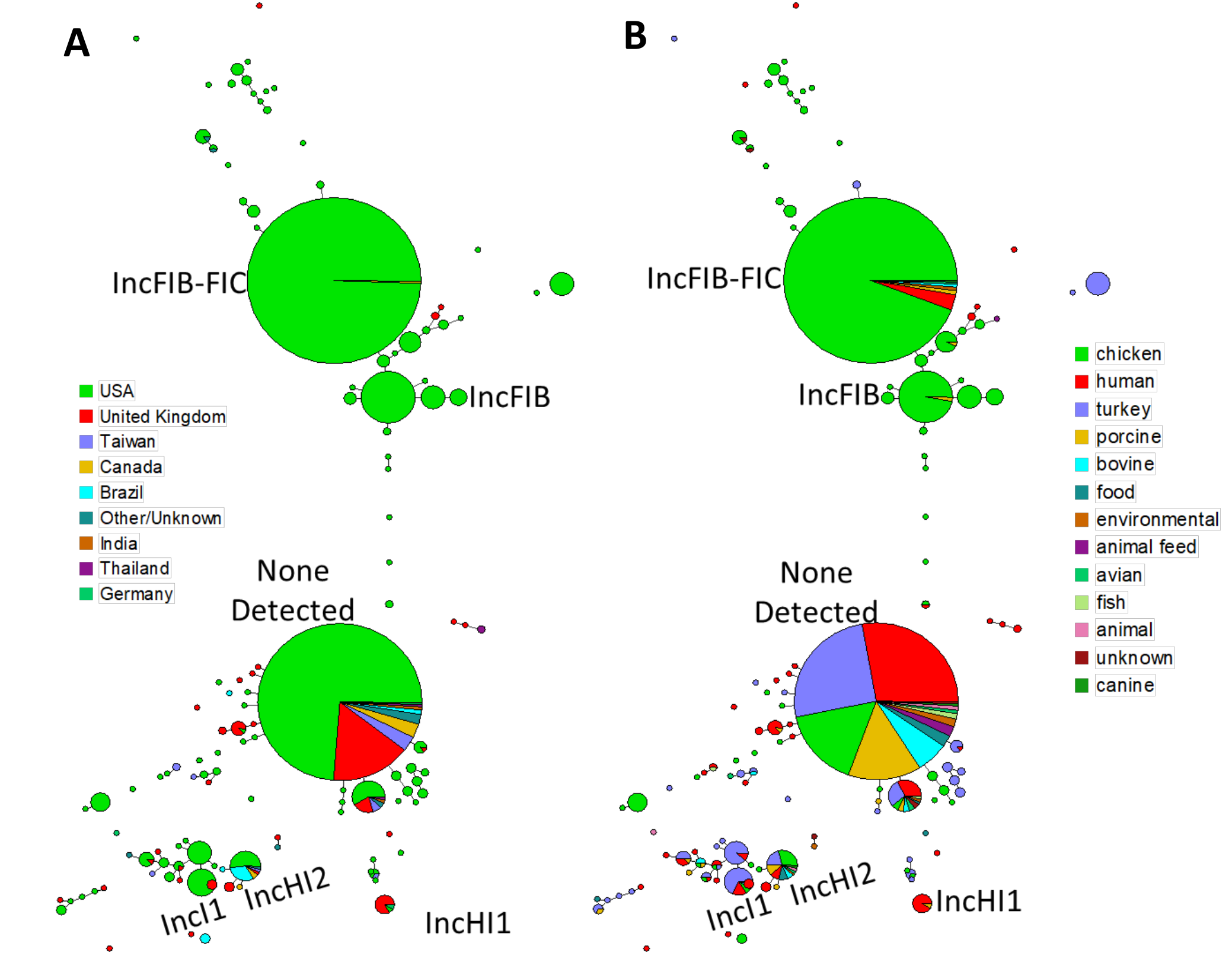


Figure 4. Minimum spanning tree analyses based on the plasmid transfer gene profiles of the *S. Schwarzengrund* strains included in the study. The trees are either color coded based on country of origin (panel A) or isolation source (panel B). The relative size of the circles are proportional to the group size.

RESULTS Continued

The virulence gene profiles of isolates from human patients exhibited diversity, yet they typically overlapped with profiles from different food sources. The aerobactin iron acquisition genes were prevalent among chicken isolates (N=1019, 89.0%), but less frequent among isolates from other sources (N=65, 7.2%). In addition, some of the strains had different combinations of fimbrial operons (*stb*, *stc*, *ste*, *stf*, *stk* and *stj* operons), but like the *sta* operon that was common in each of the other strains examined.

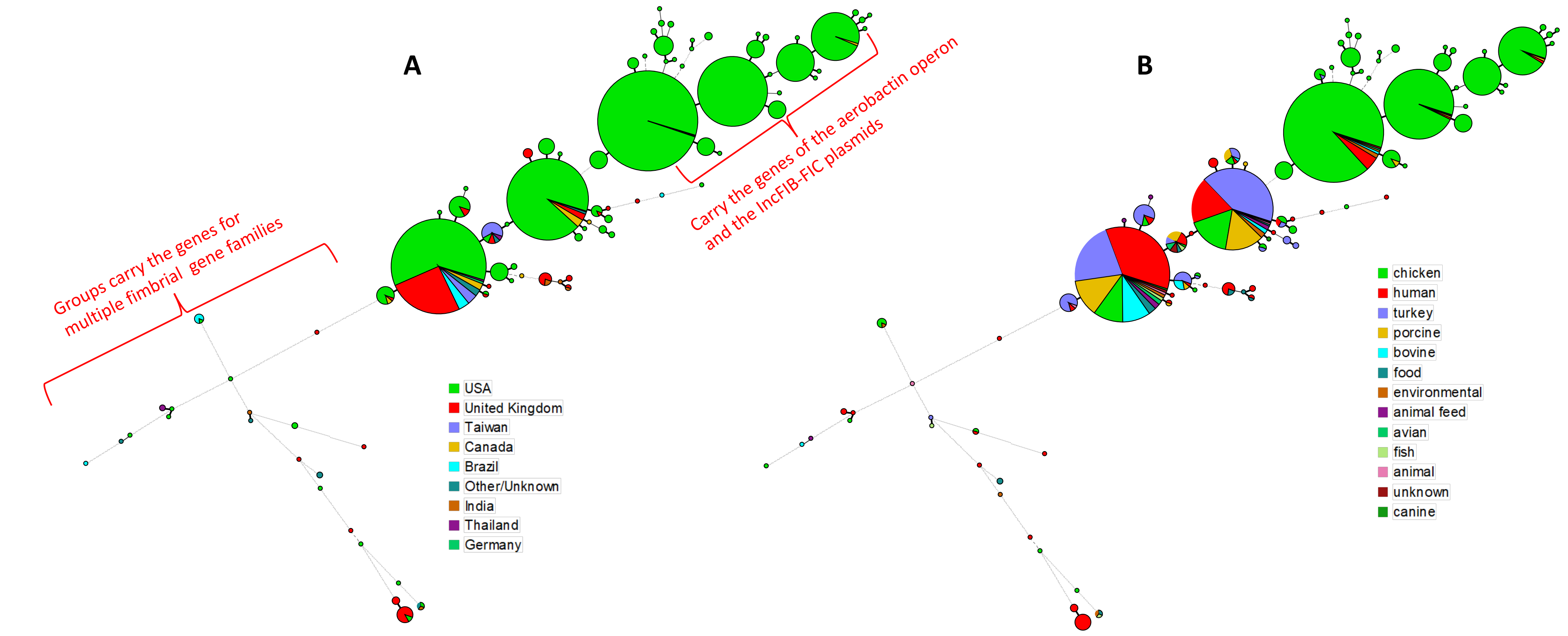


Figure 5. Minimum spanning tree analyses based on the phylogenetically relevant virulence genes of the *S. Schwarzengrund* strains included in the study. The trees are either color coded based on country of origin (panel A) or isolation source (panel B). The relative size of the circles are proportional to the group size.

Table 1. Percentage of *S. Schwarzengrund* strains carrying representative virulence genes based on their isolation source.

Source	<i>cdtB</i>	<i>lplA</i>	<i>pagO</i>	<i>plfA</i>	<i>sofA</i>	<i>sopE</i>	<i>sseK1</i>	<i>stx</i>	<i>stbC</i>	<i>stcC</i>	<i>stcE</i>	<i>stfC</i>	<i>stkC</i>	<i>stjC</i>	<i>tfpC</i>	<i>trtA</i>	<i>scfI</i>	<i>envF</i>	<i>hlyE</i>	<i>icmF</i>	<i>SGI-1</i>	<i>lucA</i>	<i>luta</i>
animal	88%	13%	100%	88%	100%	25%	0%	88%	100%	13%	13%	13%	0%	0%	100%	0%	100%	13%	88%	100%	38%	0%	0%
animal feed	94%	0%	100%	94%	35%	0%	94%	100%	0%	0%	6%	0%	6%	94%	6%	100%	6%	88%	100%	41%	0%	0%	
avian	100%	0%	100%	100%	54%	0%	100%	100%	0%	0%	0%	0%	0%	100%	23%	100%	0%	100%	100%	31%	23%	23%	
bovine	98%	0%	100%	98%	18%	0%	98%	100%	0%	2%	0%	2%	98%	8%	100%	2%	98%	100%	18%	8%	8%	8%	
canine	100%	0%	100%	100%	50%	0%	100%	100%	0%	0%	0%	0%	100%	17%	100%	0%	100%	100%	50%	17%	17%	17%	
chicken	99%	1%	100%	99%	77%	0%	99%	97%	1%	1%	1%	1%	99%	86%	99%	1%	99%	100%	77%	88%	88%		
environment	86%	14%	90%	86%	90%	67%	10%	86%	100%	14%	14%	14%	10%	14%	95%	29%	95%	14%	86%	95%	67%	29%	29%
fish	80%	20%	90%	80%	90%	20%	10%	80%	100%	20%	20%	20%	10%	10%	100%	0%	90%	20%	80%	90%	0%	0%	0%
food	87%	13%	87%	87%	22%	13%	87%	100%	13%	13%	13%	4%	13%	83%	0%	74%	13%	87%	74%	17%	0%	0%	
human	92%	2%	94%	92%	32%	8%	92%	100%	8%	7%	8%	6%	2%	88%	12%	96%	9%	92%	96%	34%	9%	9%	
porcine	100%	0%	100%	100%	53%	0%	100%	100%	0%	0%	0%	0%	0%	100%	7%	100%	0%	100%	100%	48%	7%	7%	
turkey	100%	0%	100%	100%	53%	0%	100%	100%	0%	0%	0%	0%	0%	100%	8%	100%	0%	100%	100%	52%	1%	1%	
unknown	100%	0%	100%	100%	63%	0%	100%	100%	0%	0%	0%	0%	0%	100%	25%	100%	0%	100%	100%	25%	25%	25%	

SUMMARY & KEY TAKEAWAYS

- Salmonella* serovar Schwarzengrund infections have been increasing globally over the last several years; however, the majority of strains that have undergone whole genome sequences are from the United States (Aarestrup et al, 2007; Duc et al, 2020).
- Over half (~62%) of the strains carried at least one AMR gene, with *aph(3'')-Ib* being the most common
- Many of these strains carrying *aph(3'')-Ib* have IncFIB-FIC plasmids, which is consistent with our earlier finding that many of these plasmid carry the gene (Felix et al, 2024)
- This fusion plasmid also commonly harboring the aerobactin operon, were highly prevalent among isolates from chicken-related sources and approximately 10% from human patients.
- Among the strains collected from human patients, there were diverse virulence gene profiles were observed, suggesting that multiple factors may contribute to increased virulence in *S. Schwarzengrund*.
 - Some diversity in the fimbrial genes among many strains.
- Even there was diversity among the virulence gene profiles of isolates from human patients; in the majority of the cases their virulence gene profiles typically overlapped with profiles from different food sources.

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