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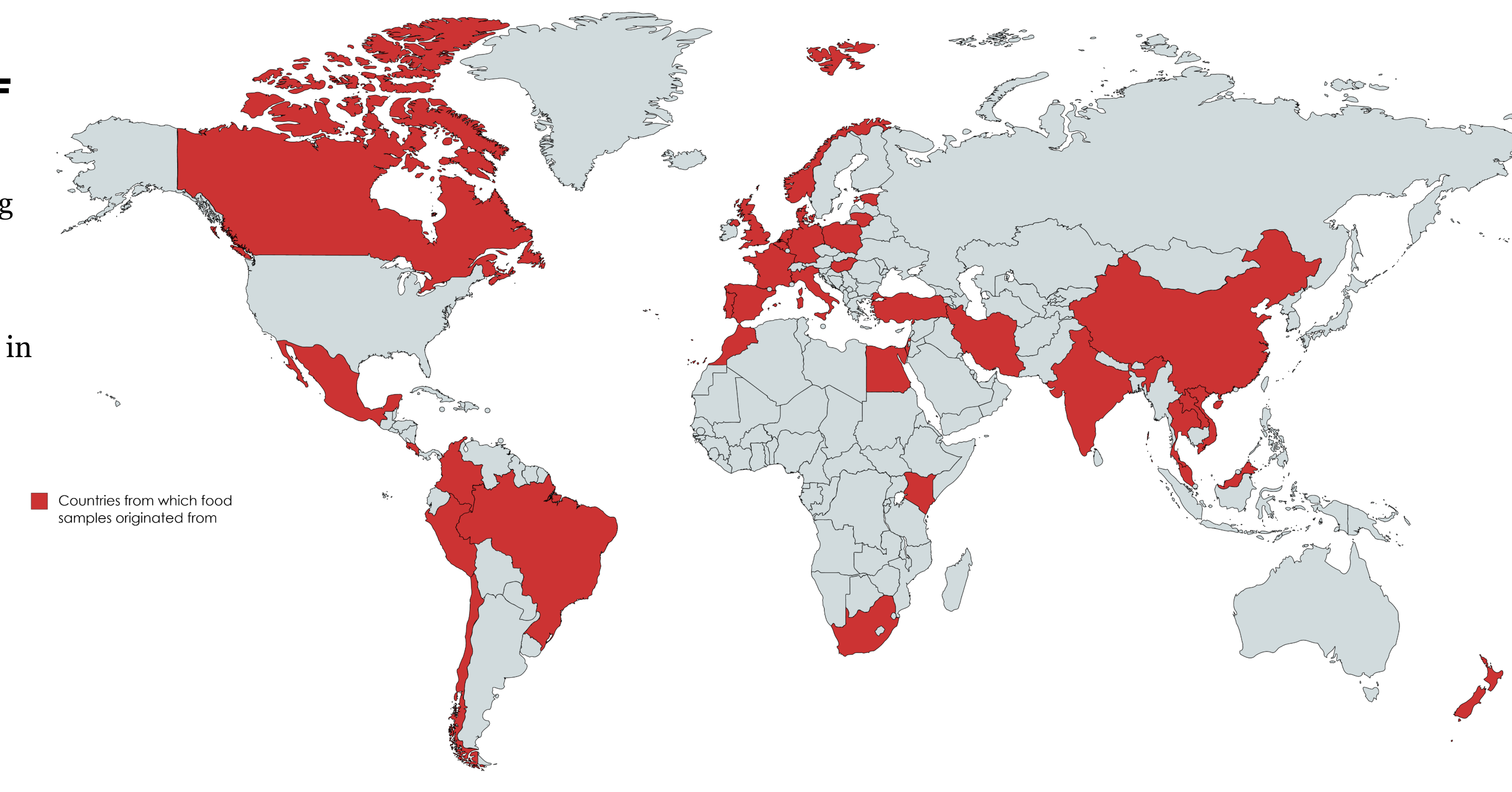
IMPORTED FOODS AS A TRANSMISSION ROUTE OF ESBL/AMPC-PRODUCING ENTEROBACTERIACEAE

BACKGROUND

THE SPREAD OF ANTIMICROBIAL RESISTANCE (AMR) IS OF GROWING CONCERN GLOBALLY

Extended-spectrum beta-lactamases (ESBL) and plasmidic AmpC (pAmpC) producing Enterobacteriaceae are a major concern for public health worldwide. ESBL/pAmpC-producing Enterobacteriaceae have been isolated from different food products and especially broiler meat has been recognized as having a high prevalence of ESBL/pAmpC-producing bacteria. As sanitation and antibiotic usage can vary greatly in different countries, imported foods may pose an increased risk of AMR.



MATERIALS & METHODS

Vegetables (n=60), fruits and berries (n=50), meat (n=60, including n=10 raw broiler meat samples) and seafood products (n=30) were sampled. Additionally, four subsamples of each raw broiler meat sample were taken, for a total of n=40 subsamples. Samples included raw, ready-to-eat, frozen and cooked products from 35 different countries and were obtained from nine different retail stores in the Helsinki region. Country of origin was unknown in 16 % (32/200) samples, and one sample originated from the Indian Ocean and nine from the Pacific Ocean. Samples were enriched and streaked onto selective agar plates supplemented with third generation cephalosporin. From one to three colonies from each plate with suspected Enterobacteriaceae growth were subjected to further investigation. Species identification was performed with MALDI-TOF, susceptibility testing to determine ESBL/AmpC phenotype with disc diffusion according to EUCAST recommendations and whole genome sequencing (WGS) with Illumina NovaSeq 6000 with paired-end-reads and 2x100 bp read length.

WGS analysis was performed with servers by Center for Genomic Epidemiology, DTU, Denmark. WGS was performed for each sample phenotypically positive for ESBL/AmpC-producing *Escherichia coli* and/or *Klebsiella pneumoniae*, excluding subsamples. If a sample yielded phenotypically different isolates, each different isolate was subjected to WGS.

RESULTS

Phenotypic results revealed ESBL/AmpC-producing *E. coli* and *K. pneumoniae* in 3 % (2/60) and 2 % (1/60) in vegetable samples, respectively, originating from chili (*K. pneumoniae*) and coriander (*E. coli*). ESBL/AmpC-producing *E. coli* was found in 17 % (10/60) of meat samples, all originating from raw broiler meat. In addition, ESBL/AmpC-producing *K. pneumoniae* was recovered from 10 % (6/60) of meat samples, originating from the same raw broiler meat (5/6) and also frozen turkey ligament (1/6). Subsampling of raw broiler meat revealed ESBL/AmpC-producing *E. coli* and *K. pneumoniae* in 88 % (35/40) of subsamples.

Altogether 21 isolates were subjected to WGS, consisting of 17 *E. coli* and four *K. pneumoniae* isolates, originating from one chili pepper, two coriander, one turkey and 10 broiler meat samples. Genotypic results are presented in Table 1.

CONCLUSIONS

Broiler meat serves as a reservoir for ESBL/AmpC-producing bacteria. The finding of resistant bacteria in the vegetable samples, however, strengthens the importance of monitoring samples from a wide spectrum of food products, as vegetables are commonly eaten without heating.

The finding of resistance genes commonly linked to human sources ($bla_{\text{CTX-M-15}}$, $bla_{\text{SHV-12}}$, $bla_{\text{OXA-1}}$) highlights the potential transmission route of AMR via food products and emphasizes the importance of hygiene measures. AmpC-type resistance gene $bla_{\text{CMY-2}}$ and ESBL-type $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-55}}$ and $bla_{\text{CTX-M-65}}$ are associated with food-producing animals, especially poultry, and were recovered from the broiler meat samples in our study. Multidrug resistance among the sequenced isolates was common. Plasmids linked to the spread of AMR, particularly IncI1, were detected in the isolates. Isolates consisted of a variety of ST types, some of which have been described from human isolates (i.e. ST37, ST307, ST38).

WGS is a powerful tool in assessing the genetic relatedness of globally spreading bacteria and should be implemented widely to assess the risks of AMR in food.

ACKNOWLEDGEMENTS

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Table 1. Genomic analyses of food isolates (n=21).

							Resistance genes									
Isolate	**Product**	**Origin**	**Species**[a]	**MLST**[b]	**Plasmids**	**Phenotype**[c]	Aminoglycoside	Beta-lactam	Fluoroquinolone	Fosfomycin	Macrolide, Lincosamide, Streptogramin B	Phenicol	Rifampicin	Sulphonamide	Tetracycline	Trimethoprim
A35-1	Chili pepper	Malesia	*K. pneumoniae*	ST307	*IncFIB(K), IncFII(K)*	ESBL	*aac(3)-IIe, aac(6')-Ib-cr, aph(3'')-Ib, aph(6)-Id*	$bla_{\text{CTXM-15}}$, $bla_{\text{OXA-1}}$, $bla_{\text{SHV-100}}$, $bla_{\text{TEM-1B}}$	*oqxA, oqxB, qnrB1*	*fosA*		*catB3*		*sul2*	*tet*(A)	*dfrA14*
A35.2-2	Chili pepper	Malesia	*K. pneumoniae*	ST101	*IncFIB(K), IncFII*	AmpC		$bla_{\text{DHA-1}}$, $bla_{\text{SHV-100}}$	*oqxA, oqxB, qnrS1*	*fosA*				*sul1*	*tet*(A)	*dfrA1*
A40.2-1	Coriander	Malesia	*E. coli*	ST155	*IncFIB(AP001918), IncFIC(FII)*	ESBL	*aph(3')-Ia, aph(3'')-Ib, aph(6)-Id*	$bla_{\text{CTX-M-55}}$, $bla_{\text{TEM-1B}}$			*mdf*(A)	*floR*	*ARR-2*	*sul2*	*tet*(A)	*dfrA14*
A41.2-1	Coriander	Malesia	*E. coli*	ST479*	*IncFIB(AP001918), p0111*	ESBL	*aac(3)-IV, aadA5, aph(4)-Ia*	$bla_{\text{CTX-M-65}}$	*oqxA, oqxB*		*mdf*(A)	*floR*		*sul1, sul2*	*tet*(A)	*dfrA17*
C32.1-2	Frozen turkey ligament	Poland	*K. pneumoniae*	ST307	*IncFIB(K), IncFII(K)*	ESBL	*aac(3)-IIa, aac(6')-Ib-cr, aph(6)-Id, aph(3'')-Ib*	$bla_{\text{CTXM-15}}$, $bla_{\text{OXA-1}}$, $bla_{\text{SHV-100}}$, $bla_{\text{TEM-1B}}$	*oqxA, oqxB, qnrB1*	*fosA*		*catB3*		*sul2*	*tet*(A)	*dfrA14*
C51-1	Raw broiler meat	Lithuania	*E. coli*	ST189	*IncI1*	ESBL	*aadA1, aadA2*	$bla_{\text{SHV-12}}$			*mdf*(A)	*cmlA1*		*sul3*	*tet*(A)	
C51.2-2	Raw broiler meat	Lithuania	*E. fergusonii***	ST8330	*ColpVC, IncB/O/K/Z, IncFIB(AP001918), IncFII, IncI2, IncX1*	ESBL+AmpC	*aac(3)-IV, aadA1, ant(2'')-Ia, aph(3')-Ia, aph(3'')-Ib, aph(4)-Ia, aph(6)-Id*	$bla_{\text{CMY-2}}$, $bla_{\text{TEM-1B}}$				*catA1, floR*		*sul1, sul2*	*tet*(B)	
C56.1-1	Raw broiler meat	Lithuania	*E. coli*	ST4994	*Col156, Col8282, ColpVC, IncI1, IncI2*	ESBL		$bla_{\text{TEM-52C}}$			*mdf*(A)					
C61-1	Raw broiler meat	Lithuania	*E. coli*	ST1011	*IncFIB(AP001918), IncFII, IncI1*	ESBL+AmpC	*aadA2, aph(3'')-Ib, aph(6)-Id*	$bla_{\text{CMY-2}}$, $bla_{\text{TEM-1B}}$			*mdf*(A)			*sul2*	*tet*(A)	*dfrA12*
C66-1	Raw broiler meat	Lithuania	*E. coli*	ST423	*IncFIB(pLF82), IncFII(pSE11), IncI1*	ESBL	*aadA1*	$bla_{\text{CARB-2}}$, $bla_{\text{SHV-12}}$			*mdf*(A)	*cmlA1*		*sul3*	*tet*(A)	*dfrA16*
C71.1-1	Raw broiler meat	Lithuania	*E. coli*	ST1485	*IncB/O/K/Z, IncFIA, IncFIB(AP001918), IncFIC(FII), IncHI2, IncHI2A, p0111*	ESBL	*aadA1, aadA3, aph(3'')-Ib, aph(6)-Id*	$bla_{\text{CMY-2}}$, $bla_{\text{TEM-1B}}$			*mdf*(A)	*cmlA1*		*sul2, sul3*	*tet*(A)	*dfrA14*
C76.1-1	Raw broiler meat	Lithuania	*E. coli*	ST201	*IncFIB(AP001918), IncFII(pCoo), IncI1*	ESBL+AmpC	*aadA1*	$bla_{\text{CMY-2}}$, $bla_{\text{TEM-135}}$	*qnrS1*		*mdf*(A)	*cmlA1*		*sul3*	*tet*(A)	*dfrA15*
C76.1-2	Raw broiler meat	Lithuania	*E. coli*	ST83*	*IncFIA, IncFIB(AP001918), IncI1*	ESBL	*aadA1, aadA2*	$bla_{\text{SHV-12}}$			*mdf*(A)	*cmlA1*		*sul3*	*tet*(A)	
C81.1-1	Raw broiler meat	Lithuania	*E. coli*	ST38	*Col156, IncFII(29), IncI1*	ESBL	*aadA5*	$bla_{\text{CTX-M-1}}$			*mdf*(A)			*sul2*		*dfrA17*
C81.3-1	Raw broiler meat	Lithuania	*E. coli*	ST1638	*IncFIB(AP001918), IncFIC(FII), IncFII(pHN7A8), IncI1, IncX1*	AmpC	*aadA1, aadA2, aph(3'')-Ib, aph(6)-Id*	$bla_{\text{CMY-2}}$, $bla_{\text{TEM-1B}}$	*qnrB19*		*mdf*(A)	*cmlA1*		*sul3*	*tet*(B)	*dfrA8*
C81.1-2	Raw broiler meat	Lithuania	*E. coli*	ST641	*IncFIB(AP001918), IncFIB(pLF82), IncFII(pSE11), IncX1*	ESBL+AmpC	*aadA1, aadA2*	$bla_{\text{CARB-2}}$, $bla_{\text{CMY-2}}$, $bla_{\text{TEM-1B}}$	*qnrS1*		*mdf*(A)	*cmlA1*		*sul3*	*tet*(A)	*dfrA16*
C86.1-1	Raw broiler meat	Lithuania	*E. coli*	ST641	*IncFIB(AP001918), IncFIB(pLF82), IncFII(29), IncFII(pSE11), IncX1*	ESBL+AmpC	*aadA1, aadA3*	$bla_{\text{CARB-2}}$, $bla_{\text{CMY-2}}$, $bla_{\text{TEM-1B}}$	*qnrS1*		*mdf*(A)	*cmlA1*		*sul3*	*tet*(A)	*dfrA16*
C86.1-2	Raw broiler meat	Lithuania	*E. coli*	ST38	*Col156, IncFII(29), IncI1*	ESBL	*aadA1, aadA5, aph(3'')-Ib, aph(6)-Id*	$bla_{\text{CTX-M-1}}$			*mdf*(A)	*floR*		*sul1, sul2*	*tet*(A)	*dfrA17*
C86.3-1	Raw broiler meat	Lithuania	*K. pneumoniae*	ST37	*IncFIA(HI1), IncR*	ESBL	*aac(3)-IId, aph(3'')-Ib, aph(6)-Id*	$bla_{\text{CTX-M-15}}$, $bla_{\text{SHV-81}}$, $bla_{\text{TEM-1B}}$	*oqxA, oqxB*	*fosA*				*sul2*		*dfrA14*
C91-2	Raw broiler meat	Lithuania	*E. coli*	ST117	*ColpVC, IncB/O/K/Z, IncFIB(AP001918), IncFIC(FII), IncFII(29)*	AmpC	*aadA1, aph(3'')-Ib, aph(6)-Id*	$bla_{\text{CMY-2}}$, $bla_{\text{TEM-1B}}$			*mdf*(A)			*sul1, sul2*	*tet*(A)	*dfrA8*
C96-1	Raw broiler meat	Lithuania	*E. coli*	ST88	*IncFII(29), IncI1*	AmpC	*aadA1, aph(3'')-Ib, aph(6)-Id*	$bla_{\text{CMY-2}}$, $bla_{\text{TEM-1B}}$			*mdf*(A), *mph*(B)	*catA1*		*sul1, sul2*		*dfrA1, dfrA8*

[a] Analysis tools by Center for Genomic Epidemiology: SPAdes 3.9 for assembly, KmerFinder 3.1 for species determination (** Identified as *E. coli* with SpeciesFinder 1.2), MLST 2.0 for multilocus sequence type (* Scheme *Escherichia coli* #1 used first, if ST unidentified then #2 used), PlasmidFinder 2.0 for plasmid replicons, KmerResistance 2.2 for acquired resistance genes. [b] MLST = multilocus sequence type. [c] Based on phenotypic tests.