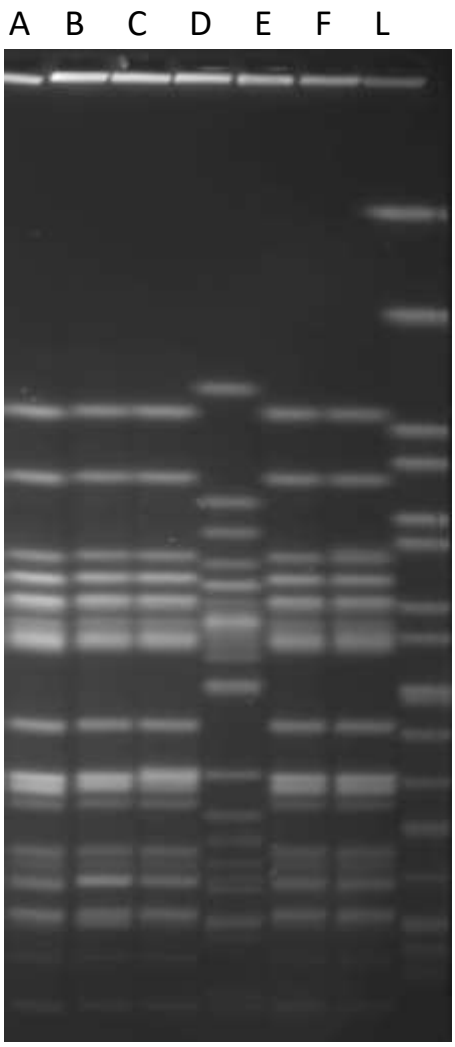
CLINICAL AND LABORATORY STANDARDS INSTITUTE http://www.clsi.org/

Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Approved Standard—Third Edition (M 31-A3)

Disk No.	Antibiotic disk		Ammount of antibotic in the disk (µg)	Size of inhibition zone		
	Abbreviation	Active compound		R resistant	I intermediate	S sensitive
1	AMP	ampicillin	10	≤ 13	14 - 16	≥ 17
2	S	streptomycin	10	≤ 11	12 - 14	≥ 15
3	S3	sulphonamides cp.	300	≤ 12	13 - 16	≥ 17
4	TE	tetracycline	30	≤ 14	15 - 18	≥ 19
5	SXT	trimethoprim-sulfamethoxazole	1,25/23,7	≤ 10	11 - 15	≥ 16
6	C	chloramphenicol	30	≤ 12	13 - 17	≥ 18
7	KF (KZ)	cephalothin	30	≤ 14	15 - 17	≥ 18
8	NA	nalidixic acid	30	≤ 13	14 - 18	≥ 19
9	CAZ	ceftazidime	30	≤ 14	15 - 17	≥ 18
10	GN (CN)	gentamicin	10	≤ 12	13 - 14	≥ 15
11	AMC	amoxicillin-clavulanic acid	30 (20+10)	≤ 13	14 - 17	≥ 18
12	CIP	ciprofloxacin	5	≤ 16	17 - 22	≥ 23

Restriction analysis of bacterial genomic DNA



PFGE profile

A…chicken steak sample 1

B…tomato salad sample 2

C…raw chicken meat sample 1

D… raw chicken meat sample 3

E…patient 1

F…patient 2

L…ladder

Result sheet:

Strain name	Source	Species identification (16S RNA)	Susceptibility to 12 antibiotics (zone in mm into the first line and R-resistant/S-susceptible/I-intermediate into the second line)												ESBL (+/-)	Restriction analysis of genomic DNA (PFGE pattern)
			AMP	S	S3	TE	STX	C	KF (KZ)	NA	CAZ	GN (CN)	AMC	CIP		
STRAIN A																
STRAIN B																
STRAIN C																
STRAIN D																
STRAIN E																

Conclusion:

Is your strain resistant to antibiotics and producing ESBL?

Did we find phenotypically and genotypically similar isolates in food and patient samples?

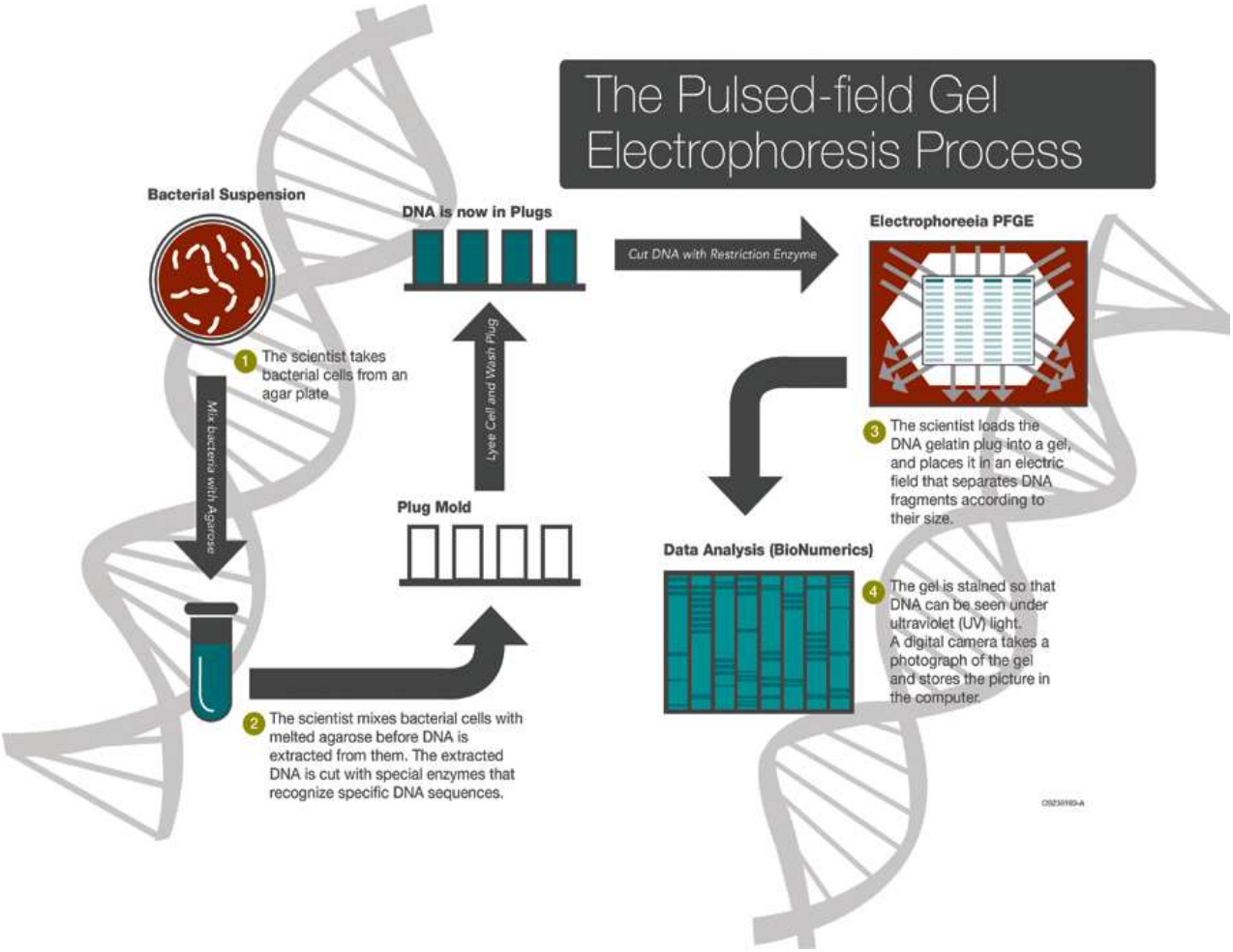
What was the sources of the infections in patients?

Pulsed field gel electrophoresis

Principle:
Pulsed field gel electrophoresis (PFGE) is a technique used for the separation of large deoxyribonucleic acid (DNA) molecules by applying to a gel matrix an electric field that periodically changes direction.

Use:
PFGE may be used for genotyping or genetic fingerprinting. It is commonly considered a gold standard in epidemiological studies of pathogenic organisms. Subtyping has made it easier to discriminate among strains and thus to link environmental or food isolates with clinical infections.

Procedure:
PFGE involves embedding organisms in agarose, lysing the organisms in situ, and digesting the chromosomal DNA with restriction endonucleases that cleave infrequently. Slices of agarose containing the chromosomal DNA fragments are inserted into the wells of an agarose gel, and the restriction fragments are resolved into a pattern of discrete bands in the gel by an apparatus that switches the direction of current according to a predetermined pattern. The DNA restriction patterns of the isolates are then compared with one another to determine their relatedness. If strains show same or very similar restriction pattern they are related.



Literature: Tenover FC et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol