Note: This is Online Appendix 1 of Seakamela, E.M., Diseko, L., Malatji, D., Makhando, L., Motau, M., Jambwa, K. et al., 2022, 'Characterisation and antibiotic resistance of Yersinia enterocolitica from various meat categories, South Africa', *Onderstepoort Journal of Veterinary Research* 89(1), a2006. https://doi.org/10.4102/ojvr.v89i1.2006

TABLE S1: Number of samples collected per category of retail outlets.

Categories of retail outlets	Number of sample collected
Small	4
Medium	8
Large	12
Chain	16

TABLE S2: Summary of data according to location, animal species and sample category.

Samples	Location/ Municipalities	Animal species	Sample category	Frequency	Percentage
1	Bronkhospruit	Beef	Raw intact meat	16	3%
2	Bronkhospruit	Beef	Raw processed meat	9	2%
3	Bronkhospruit	Beef	Ready to eat	4	1%
4	Bronkhospruit	Pork	Raw intact meat	3	1%
5	Bronkhospruit	Pork	Raw processed meat	6	1%
6	Bronkhospruit	Pork	Ready to eat	11	2%
7	Emalahleni	Beef	Raw intact meat	33	6%
8	Emalahleni	Beef	Raw processed meat	6	1%
9	Emalahleni	Pork	Raw intact meat	2	0%
10	Emalahleni	Pork	Raw processed meat	19	3%
11	Emalahleni	Pork	Ready to eat	7	1%
12	Pretoria	Beef	Raw intact meat	125	22%
13	Pretoria	Beef	Raw processed meat	34	6%
14	Pretoria	Beef	Ready to eat	15	3%
15	Pretoria	Pork	Raw intact meat	47	8%
16	Pretoria	Pork	Raw processed meat	50	9%
17	Pretoria	Pork	Ready to eat	44	8%
18	Rustenburg	Beef	Raw intact meat	38	7%
19	Rustenburg	Beef	Raw processed meat	25	4%
20	Rustenburg	Beef	Ready to eat	27	5%
21	Rustenburg	Pork	Raw intact meat	28	5%
22	Rustenburg	Pork	Raw processed meat	18	3%
23	Rustenburg	Pork	Ready to eat	14	2%
			Total	**581**	**100%**

TABLE S3: Meat products according to sample categories.

Category	**Meat**
Biltong	sticks, crumps, skeed
Bone or skeletal tissue	Ribs, T-bone, flip, beefsteak, loins-bone
Tripe	Tripe
Organs	Heart, kidney, lungs, liver, spleen
Processed	Mince, patties, sausage, wors, griller smokie, sosaties, snitzels, burger, cabanossi, russian, polony, vienna
Muscle	Chuck, goulash, blade, brisket, rump, shin, minutes-stick, stew, stir-fry

TABLE S4: Primer sequences, PCR preparation, and PCR condition used for sero-grouping and virotyping of *Y. enterocolitica in* the study.

PCR test	Targeted gene/s	Product size (bp)	Primer sequences (5′–3′)	PCR preparation	PCR condition	Reference
Confirmation of *Y. enterocolitica*	*16SrRNA*	328	F: ATACCGCATAACGTCTTCG R : TTCTTCTGCGAGTAACGTC	PCR was performed in total volume of 25 µL: 12.5µL Taq Polymerase Red Mastermix; 5.5µL nuclease free water, 5µL template DNA and 1µL of each primer	Initial denaturation step at 94°C for 3 min; 28 cycles of 95°C for 0.5 min, 55.7°C for 1 min and 72°C for 1 min; and the final extension of 72°C for 5min in a thermocycler	[28]
Serogrouping (mPCR-1)	*wbbU* (O:3), *per* (O:9), *wbcA* (O:8), *wzt* (O: 5,27)	463 837 269 662	*wbbU*-F: ACCTCGTATTTTTGAAGATGATCGC *wbbU*-R: GTACTCAATAACTTGCTGTTCGGA *per*-F: TCCTTCTCCAAATATATAGGTGCCA *per*-R: ATGCGGCATTAGATGAGATGGA *wbcA*-F: TGATGAACGAGGCGAGTTTGTT	PCR was performed in total volume of 50 µL: 25µL Taq Polymerase Red Mastermix; 7µL nuclease free water, 10µL template DNA and 1µL of each primer	45 cycles of 94°C for 1min, 60°C for 1min and 72°C for 1 min; and the final extension of 72°C for 5min in a thermocycler	[30]

			wbcA-R: TACTCCGTCTGTTATGCGGATTTAG *wzt*-F: GTTAGTTCCTGCATCTGATCGCC *wzt*-R: ATCCAGCATCCATGGCTCC			
Virulence genes (mPCR-2)	*yad A* *inv* *yst A* *virF*	849 570 145 590	yadA1: CTTCAGATACTGGTGTCGCTGT yadA2: ATGCCTGACTAGAGCGATATCC *inv*-F: CTGTGGGGAGAGTGGGGAAGTTTGG *inv*-R: GAACTGCTTGAATCCCTGAAAACCG *ystA*-F: AATGCTGTCTTCATTTGGAGCA *ystA*-R: ATCCCAATCACTACTGACTTC *virF*-F: TCATGGCAGAACAGCAGTCAG *virF*-R: ACTCATCTTACCATTAAGAAG	PCR was performed in total volume of 50 µL: 25µL Taq Polymerase Red Mastermix; 12µL nuclease free water, 5µL template DNA and 1µLof each primer	Initial denaturation step at 95°C for 10 min; 23 cycles of 95°C for 0.45 min, 60°C for 1min and 72°C for 1.10 min; and the final extension of 72 °C for 10min in a thermocycler	[10]
Virulence genes (mPCR-3)	*Fes* *fepA* *fepD*	561 438 381	*fes*-F:GCCGGCAGGCACAGCGTAAT *fes*-R:GGCCAACCCACCCAAAACTT *fepA*-F:TACGCCAAAATACCTTACGAT	PCR was performed in total volume of 25µL: 12.5µL Taq Polymerase Red Mastermix; 2µL	Initial denaturation step at 95°C for 10 min; 30 cycles of 94°C for 1min, 53°C for 1min and 72°C for 1min; and the final extension of 72	[31]

	myfA	272	*fepA*-R: TGTAAATACACCCCCACCTGA *fepD*-F:GTGTGATTGCCTTACTATTG *fepD*-R:CGGTCATCCTTTTATTACGG *myfA*-F: CAGATACACCTGCCTTCCATCT *myfA*- R:CTCGACATATTCCTCAACACGC	nuclease free water, 2.5µL template DNA and 1µLof each primer	°C for 10min in a thermocycler	
Virulence genes (Duplex)	*ystB* *ymoA*	180 330	*ystB*-F: TGTCAGCATTTATTCTCAACT *ystB*-R: GCCGATAATGTATCATCAAG *ymoA*-F: GACTTTTCTCAGGGGAATAC *ymoA*-R: GCTCAACGTTGTGTGTCT	PCR was performed in total volume of 20µL: 10µL Taq Polymerase Red Mastermix; 3µL nuclease free water, 3µL template DNA and 1µL of each primer	Initial denaturation step at 95°C for 5 min; 34 cycles of 94°C for 0.5 min, 50°C for 0.5min and 72°C for 1 min; and the final extension of 72 °C for 10min in a thermocycler	[32]
Virulence gene Single-plex	*Ail*	454	9A: TTATCAATTGCGTCTGTTAATGTGTA 10A: ATCGAGTTTGGAGTATTCATATGAAG	PCR was performed in total volume of 25µL: 12.5µL Taq Polymerase Red Mastermix; 3.5µL nuclease free water, 5µL template DNA and 2µL of each primer	Initial denaturation step at 94°C for 3 min; 28 cycles of 95°C for 0.5 min, 55.7°C for 1min and 72°C for 1 min; and the final extension of 72 °C for 5min in a thermocycler	[2]

TABLE S5: Primer sequences and PCR conditions for antimicrobial resistance genes used for in the study.

PCR test	Target gene	Sequence (5′–3′)	Product sizes (bp)	Annealing temperature (°C)*	Reference
Tetracycline	*tetA*	F: GGCGGTCTTCTT CATCATCATGC R: CGGCAGGCAGAGCAGTAGA	502	59	[33]
	tetB	F: CGCCCAGTGCTGTTGTTGTC R: CGCGTTGAGAAGAAGCTGAGGTG	173		
Trimethoprim	*Dfrl*	F: CGGTCGTAACACGTTCAAGT R: CTGGGGATTTCAGGAAAGTA	220	55.3	[34]
	DfrXII	F: AAATTCCGGGTGAGCAGAAG R: CCCGTTGACGGAATGGTTAG	429		
	DfrXIII	F: GCAGTCGCCCTAAAACAAAG R: GATACGTGTGACAGCGTTGA	294		
Sulphonamides	*sul1*	F: CGGCGTGGGCTACCTGAACG R: GCCGATCGCGTGAAGTTCCCG	433	63	[35]
	sul2	F: GCGCTCAAGGCAGATGGCATT R: GCGTTTGATACCGGCACCCGT	293		
	Sul3	F: CAACGGAAGTGGGCGTTGTGGA R: GCTGCACCAATTCGCTGAACG	244		
Phenicol	*cat1*	F: CTTGTCGCCTTGCGTATAAT R: ATCCCAATGGCATCGTAAAG	508		[34]

	Flo	F: CTGAGGGTGTCGTCATCTAC R: GCTCCGACAATGCTGACTAT	673	53.3	
	cmlA	F: CGCCACGGTGTTGTTGTTAT R: GCGACCTGCGTAAATGTCAC	394		
Beta lactams	*blaTEM*	F: TTAACTGGCGAACTACTTAC R: GTCTATTTCGTTCATCCATA	247	55.3	[35]
	blaCMY-2	F: GACAGCCTCTTTCTCCACA R: TGGACACGAAGGCTACGTA	1000		
	blaSHV	F: AGGATTGACTGCCTTTTTG R: ATTTGCTGATTTCGCTCG	393		
	blaPSE	F: TGCTTCGCAACTATGCTAC R: AGCCTGTGTTTGAGCTAGAT	438		
Quinolones	*qnrA*	F: TCAGCAAGAGGATTTCTCA R: GGCAGCACTATTACTCCCA	516	53	[36]
	qnrB	F:GATCGTGAAAGCCAGAAAGG R: ACGATGCCTGGTAGTTGTCC	469		
	qnrS	F:ACGACATTCGTCAACTGCAA R: TAAATTGGCACCCTGTAGGC	417		

* The PCR cycling conditions: initial denaturation at 94 ºC for 3 min, 30 cycles of denaturation at 94 ºC for 30 sec, extension at 72 ºC for 1 min followed by a final extension at 72 ºC for 10 min.

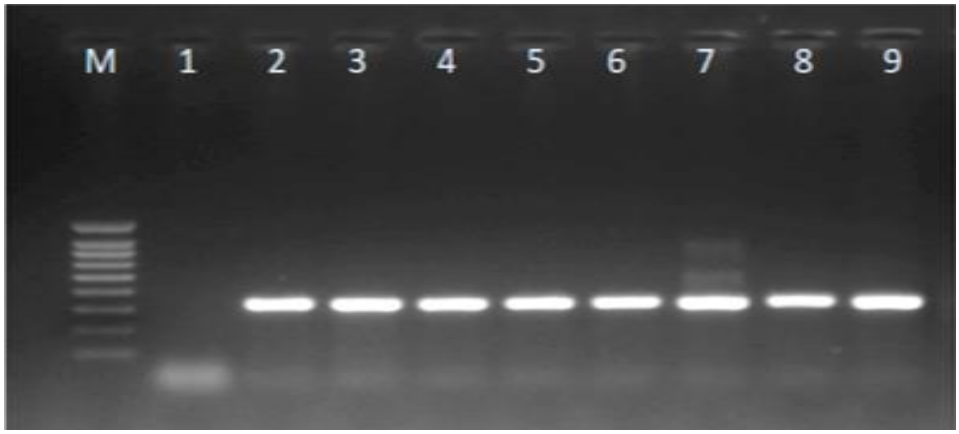

Figure S1: Confirmation of *Y. enterocolitica* by PCR (singleplex PCR amplification).

M-100bp ladder, lane 1-negative isolate; lane 2-positive control (328bp), Lane 3-9- positive isolates (328bp)

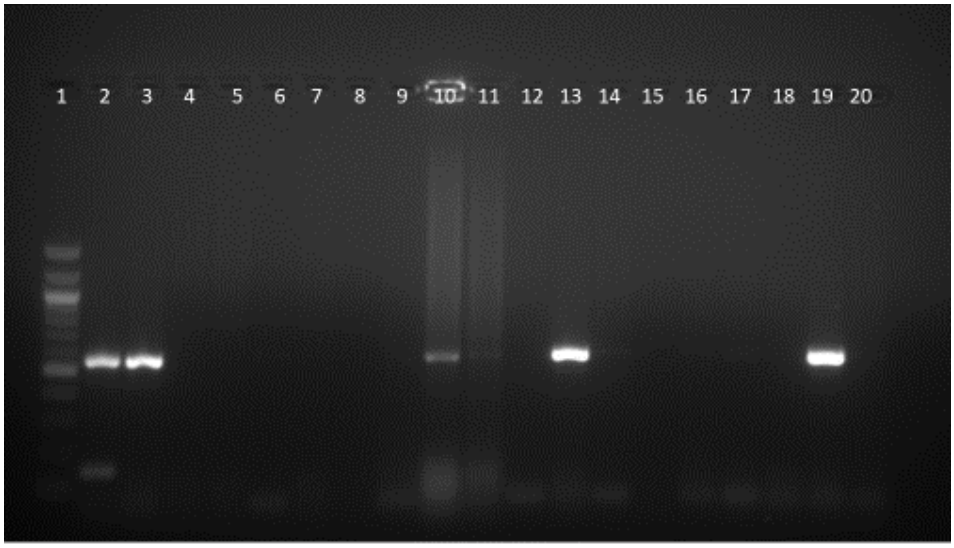

Figure S2: Virulence genes (multiplex PCR amplification).

Lane 1: 100bp ladder; Lane 20; Negative control; Lane 19: positive control; Lane 3: Positive *invA* isolate (570bp); Lane 4-9; Negative

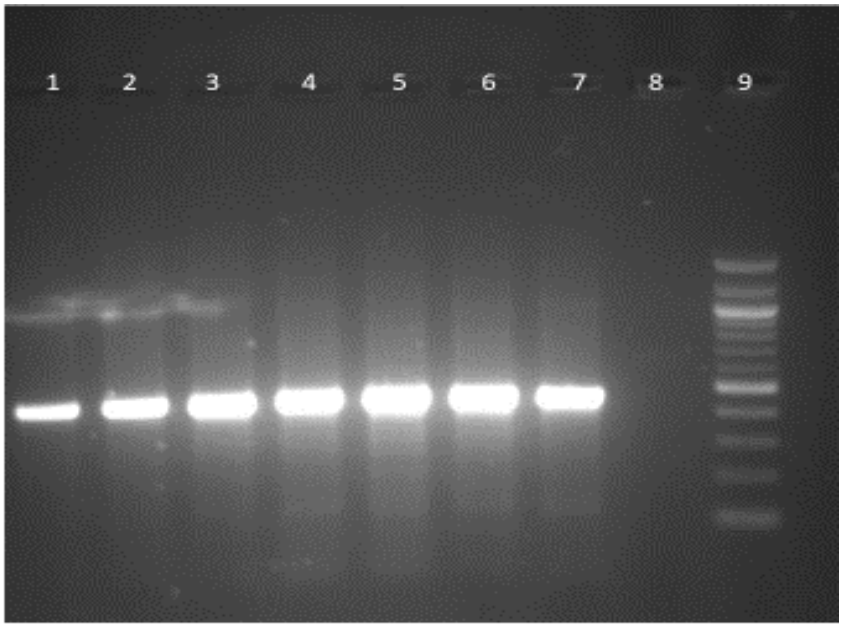

Figure S3: Virulence gene (*ail*) of *Yersinia enterocolitica* (singleplex PCR amplification).

Lane 9: 100 bp ladder; Lane 8: Negative control; Lane 1-6: Positive *ail* isolates (454bp); Lane 7: Positive control