

APPENDIX 1. *Questionnaire for owners of informal chicken Outlet used during visits to outlets*

**Serial #:** \_\_\_\_\_ **Area:** \_\_\_\_\_ **Date of Administration:**  
\_\_\_\_\_

**1. Name:** \_\_\_\_\_ (optional)

**2. Address:** \_\_\_\_\_ (optional)

**3. GPS coordinates of outlet:** \_\_\_\_\_

**4. Contact number(s):** \_\_\_\_\_

**5. Name of supplier:**  
i.  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

ii.

	Types of birds slaughtered			
	Spent hens	Culled Breeders	Broilers	Others/combinations (list)
Yes				
No				

iii. **Approximate number of each chicken type slaughtered daily:**

a. Spent hens: \_\_\_\_\_ b. Culled breeders: \_\_\_\_\_ Broilers: \_\_\_\_\_  
\_\_\_\_\_

**6. Number of processing days per week:**  
\_\_\_\_\_

**7. Key steps in the operation at the outlet (List in sequence):**

Key steps in the operations of the outlets (sequence)			
Serial no.		Yes	No
	Kept chicken in cages		
	Pick chickens for slaughter		
	Slaughter with knives		
	Scalding		
	De-feathering		
	Eviscerate chickens		
	Processing		
	Packaging and retailing		
	Refrigeration		

**8. Type of de-feathering method used**

	Types of de-feathering method			
	Drum type	Tube type	Hand picking	Others (list)
Yes				
No				

**9. Type of rinsing method**

	Types of rinsing method		
	Running water/pipeline	Stagnant water/Sink/Bucket	Briefly comment on the source of water(tap/river): _____
Yes			_____
No			_____

10. Number of years or months in operation:

\_\_\_\_\_

11. Opening hours

Types of operation			
	Weekly	Daily	Briefly comment on the source of water:
Yes			_____
No			_____

12. Average cost of a whole chicken (Rand): Spent hens: \_\_\_\_\_, Culled breeders: \_\_\_\_\_, Broilers: \_\_\_\_\_

13. Disposal of wastes from the outlet:

- a. Liquid waste: \_\_\_\_\_
- b. Solid waste: \_\_\_\_\_

14. Availability of refrigeration or freezing facilities at the outlet (tick):

Types of refrigeration method				
	Refrigerator	Freezer	Both	
Yes				
No				

15. Location of carcass for sale:

- a. Counter: \_\_\_\_\_ Refrigerator: \_\_\_\_\_ c. Freezer: \_\_\_\_\_ d. Bucket \_\_\_\_\_
- b. For outlets where carcasses are located on the counter for sale, how long do they remain there?
- i. Less than 30 min \_\_\_\_\_ ii. 30 min – 60 min \_\_\_\_\_ ii. Over 60 min: \_\_\_\_\_

16. Number and types of samples collected from the outlet?

- a. Chickens: Spent hens: \_\_\_\_\_ No.: \_\_\_\_\_ Culled breeders: \_\_\_\_\_ No.: \_\_\_\_\_ Broilers: \_\_\_\_\_ No.: \_\_\_\_\_
- b. Cloacal swabs: Spent hens: \_\_\_\_\_ No.: \_\_\_\_\_ Culled breeders: \_\_\_\_\_ No.: \_\_\_\_\_ Broilers: \_\_\_\_\_ No.: \_\_\_\_\_

- c. Post-evisceration carcass swab: \_\_\_\_\_
- d. *Rinse water*: \_\_\_\_\_
- e. *Drip water*: \_\_\_\_\_
- f. *Whole carcass* \_\_\_\_\_

**17. Any other comments/observations:**

- i. Do they wash the knives after processing each carcass? Yes. \_\_\_\_ No. \_\_\_\_
- ii. Clean the table with clean cloths? Yes \_\_\_\_ No. \_\_\_\_
- iii. Clean the table with dirty cloths? Yes. \_\_\_\_ No. \_\_\_\_
- iv. Wash hands with soap after processing each carcass? Yes. \_\_\_\_ No \_\_\_\_
- v. Wash hand without soap after processing each carcass? Yes. \_\_\_\_ No \_\_\_\_
- vi. Do not wash hand at all after processing each carcass? Yes. \_\_\_\_ No \_\_\_\_

**18. Type and identification of samples collected at outlet:**

**Codes**

- a. Chicken-Spent (CS): \_\_\_\_\_
- b. Chicken-Culled (CC): \_\_\_\_\_
- c. Chicken-Broiler (CB) \_\_\_\_\_
- d. Chicken-Spent Drip (CSD): \_\_\_\_\_
- e. Chicken-Culled Drip (CCD): \_\_\_\_\_
- f. Chicken-Broiler Drip (CB) \_\_\_\_\_
- g. Water rinse/drain liquid in bags with chicken carcass (WR):  
\_\_\_\_\_
- h. Chicken-Spent Cloaca (CSC): \_\_\_\_\_
- i. Chicken-Culled Cloaca (CCC): \_\_\_\_\_
- j. Chicken-Broiler Cloaca (CBC): \_\_\_\_\_

**19. Declaration and signature of owner or the person having charge of the animals/birds.**

I, (full name) \_\_\_\_\_,  
 hereby declare ***that I have consented*** to participate in the study and the samples  
 were collected by the authorized person mentioned above and that no relevant  
 information was withheld from the authorized person.

Date: \_\_\_\_\_

APPENDIX 2. Sanitation score sheet used in the study

Category	Score					
	1 (worst)	2	3	4	5 (best)	NA*
<b>1. Handlers of chickens at outlet</b>						
1.1 Wore clean clothes with sleeves						
Clean clothes with sleeves						
Clean clothes without sleeve						
Dirty clothes without						
Dirty clothes with sleeves						
Dirty clothes with dirty sleeves						
1.2 Wore aprons						
Wore very clean aprons						
Wore clean aprons						
Did not wear aprons						
Wore moderately dirty clothes						
Wore very dirty aprons						
1.3 Had hair covered						
Yes						
No						
<b>2 Cleanliness in cages or areas where live birds are kept</b>						
Relatively clean and not crowded						
Relatively clean and crowded						
Relatively dirty –faeces etc- and crowded						
Relatively filthy and crowded						
Very filthy and very crowded						
<b>3. Sanitation in slaughter area</b>						

Kept very clean—Little blood/feathers/faeces						
Kept clean---some blood/feathers/faeces						
Moderately kept clean—Blood/feathers/lot of flies						
Poorly kept—blood/feathers/lot of faeces/few flies						
Very poorly kept—blood/feathers/faeces/many flies						
<b>4. Sanitation in de-feathering or 'plucking' Area</b>						
Kept very clean—Little blood/feathers/faeces						
Kept clean---some blood/feathers/faeces						
Moderately kept clean—Blood/feathers/lot of faeces						
Poorly kept—blood/feathers/lot of faeces/few flies						
Very poorly kept—blood/feathers/faeces/many flies						
<b>5. Sanitation in evisceration area</b>						
Kept very clean—Little blood/feathers/faeces						
Kept clean---some blood/feathers/faeces						
Moderately kept clean—Blood/feathers/lot of faeces						
Poorly kept—blood/feathers/lot of faeces/few flies						
Very poorly kept—blood/feathers/faeces/ many flies						
<b>6. Sanitation in rinsing of carcasses</b>						
Use of 3 rinsing bucket in sequence/Clean water						
Use of 2 rinsing buckets in sequence/Clean water						
Use of 1 rinsing bucket/bloody water/feathers/1carcass						
Use of 1 rinsing bucket/bloody water/feathers/ 2 carcasses						
Use of 1 rinsing bucket/bloody water/feathers/3 or more Carcasses						
<b>7. Sanitation in packaging and sale areas</b>						

Kept very clean—No blood/No feathers/No faeces/No flies						
Kept clean---No blood/ No feathers/No faeces/Few flies						
Moderately kept clean—Some Blood/Few feathers/Some faeces/Some flies						
Poorly kept—blood/feathers/lots of faeces/ few flies						
Very poorly kept—Some blood/Lots of feathers/Lots of faeces/Many flies						

NA: Not applicable

APPENDIX 3. PCR conditions, primer sequences and reaction volumes for the detection of six virulence genes, three toxin genes and one antibiotic resistance gene in *C. coli*- and *C. jejuni*-positive broth cultures

Gene target cycling conditions	Primers Reaction volume	Nucleotide Sequence (5'-3')	Amplicon size (bp)	References	
<i>Dnaj/racR/cdtC</i>	dnajF	-AAG GCT TTG GCT CA-	720	(Datta et al., 2003)	
	dnajR	-CTT TTT GTT CAT CGT T-			
	<b>Assay 1</b> 94°C for 1 min 46°C for 1 min 72°C for 1 min 30 cycles	<b>0.5 µl</b>	racRF -GAT GAT CCT GAC TTT G-	584	(Datta et al., 2003)
			racRR -TCT CCT ATT TTT ACC C-		
	<b>1 µl</b>	cdtCF -TTG GCA TTA TAG AAA ATA CAG TT-	182	(Datta et al., 2003)	
	<b>0.25 µl</b>	cdtCR -CGA TGA GTT AAA ACA AAA AGA TA-			
<i>tetO/cdtA</i>	tetOF	-GGC GTT TTG TTT ATG TGC G-	559	(Kim et al., 2010)	
	tetOR	-ATG GAC AAC CCG ACA GAA GC-			
	<b>Assay 2</b> 94°C for 30 s 50°C for 30 s 72°C for 1 min 30 cycles	<b>0.25 µl</b>	cdtAF -CCT TGT GAT GCA AGC AAT	370	(Hickey et al., 2000)
			cdtAR C- -ACA CTC CAT TTG CTT TCT G-		
	<b>1 µl</b>				
<i>cdtB/ciaB/pldA</i>	cdtBF	-CAG AAA GCA AAT GGA GTG	620	(Datta et al., 2003)	
	cdtBR	TT-			
	<b>Assay 3</b> 94°C for 1 min 58°C for 1 min 72°C for 1 min 30 cycles	<b>1 µl</b>	-AGC TAA AAG CGG TGG AGT AT-	527	(Laprade et al., 2016)
			ciaBF -TGC GAG ATT TTT CGA GAA		
		<b>0.5 µl</b>	ciaBR TG - -TGC CCG CCT TAG AAC TTA CA-		
			pldAF -AAG AGT GAG GCG AAA TTC	385	(Laprade et al., 2016)
	<b>0.25 µl</b>	pldAR CA- -GCA AGA TGG CAG GAT TAT CA-			

<i>flaA/flaB</i>	flaAF	-GCA AGA TGG CAG GAT TAT	855	(Datta et al., 2003)
	flaAR	CA-		
<b>Assay 4</b>	<b>1 µl</b>	-AAT AAA AAT GCT GAT AAA		
94°C for 1 min		ACA G GT G-		
53°C for 1 min	flaBF	-TAC CGA ACC AAT GTC TGC	260	(Goon et al., 2003)
72°C for 1 min	flaBR	TCT GAT T-		
30 cycles		-AAG GAT TTA AAA TGG GTT		
	<b>0.25 µl</b>	TTA GAA TAA ACA CC-		

APPENDIX 4. Summary of processing methods used in the informal poultry outlets in six Gauteng townships

Parameter	No. (%) of outlets with practice in:											
	Alexandra*		Atteridgeville/ Phomolong*		Garankuwa*		Germiston*		Soweto**		Tembisa/Modise	
	No. (%) Positive	P- value	No. (%) Positive	P- value	No. (%) Positive	P- value	No. (%) Positive	P- value	No. (%) Positive	P- value	No. (%) Positive	P- value
<b>Types of birds slaughtered daily</b>												
Broilers	14 (70.0)	0.2419	0 (0.0)	0.4226	0 (0.0)	0.3555	0 (0.0)	0.1994	5 (8.3)	0.1334	7 (70.0)	0.2419
Culled breeders	0 (0.0)		0 (0.0)		16 (88.9)		12 (60.0)		22 (36.7)		0 (0.0)	
Spent hens	6 (30.0)		23 (100.0)		2 (11.1)		8 (40.0)		33 (55.0)		3 (30.0)	
<b>Chickens kept in cages</b>												
Yes	14 (70.0)	0.2422	16 (69.6)	0.2375	18 (100.0)	0.5000	20 (100.0)	0.5000	39 (65.0)	0.1855	5 (50.0)	a*
No	6 (30.0)		7 (30.4)		0 (0.0)		0 (0.0)		21 (35.0)		5 (50.0)	
<b>Chickens slaughtered with knives</b>												
Yes	20 (100.0)	0.5000	23 (100.0)	0.5000	18 (100.0)	0.5000	20 (100.0)	0.5000	60 (100.0)	0.5000	10 (100.0)	0.5000
No	0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
<b>Scalding</b>												
Yes	20 (100.0)	0.5000	23 (100.0)	0.5000	18 (100.0)	0.5000	20 (100.0)	0.5000	60 (100.0)	0.5000	10 (100.0)	0.5000
No	0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
<b>Eviscerated chickens</b>												
Yes	0 (0.0)	0.5000	13 (56.5)	0.0826	18 (100.0)	0.5000	20 (100.0)	0.5000	14 (23.3)	0.3119	10 (100.0)	0.5000
No	20 (100.0)		10 (43.5)		0 (0.0)		0 (0.0)		46 (76.7)		0 (0.0)	
<b>Type of defeathering method</b>												
Hand-picking	20 (100.0)	0.5000	16 (69.6)	0.2375	18 (100.0)	0.5000	20 (100.0)	0.5000	48 (80.0)	0.3440	3 (30.0)	0.2422
Knife shaving	0 (0.0)		7 (30.4)		0 (0.0)		0 (0.0)		12 (20.0)		7 (70.0)	
<b>Type of rinsing method</b>												
Bucket	20 (100.0)	0.5000	0 (0.0)	0.5000	0 (0.0)	0.5000	20 (100.0)	0.5000	31 (51.7)	0.0212	0 (0.0)	0.5000
Stagnant water in drums	0 (0.0)		23 (100.0)		18 (100.0)		0 (0.0)		29 (48.3)		10 (100.0)	

\*Based on the total number of samples collected from each township: Alexander (20 samples), Atteridgeville/Phomolong (23 samples), Garankuwa (18 samples), Germiston (20 samples), Soweto (60 samples), Tembisa/Modise (10 samples). [a\* = No statistics could be computed because the parameter is constant]