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Research Paper

Prevalence and Characterization of *Campylobacter* Species from Chickens Sold at Informal Chicken Markets in Gauteng, South Africa

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ABSTRACT

This study determined the prevalence, characteristics, and risk factors of *Campylobacter* species contamination of chicken carcasses sold at informal poultry outlets in Gauteng province, South Africa. Within six townships, 151 chicken carcasses were collected from 47 outlets. Carcass swab, cloacal swab, and carcass drip samples were collected from each chicken, along with a matched questionnaire on risk factors regarding *Campylobacter* contamination. Sample-inoculated Bolton broth (BB) was cultured to isolate *Campylobacter* species by bacteriological methods. Subsequent confirmation and characterization of *Campylobacter* were conducted using polymerase chain reaction (PCR). Isolated *Campylobacter* strains were evaluated for the presence of six virulence genes (*ciaB, dnaj, pldA, racR, flaA,* and *flaB*), three toxin genes (*cdtA, cdtB,* and *cdtC*), and one antimicrobial resistance gene (*tetO*). The overall prevalence of *Campylobacter* was 23.4% (106 of 453), with sample type–specific prevalence being 17.2% (26 of 151), 25.8% (39 of 151), and 27.2% (41 of 151) for the carcass swabs, cloacal swabs, and carcass drip, respectively, following bacteriological isolation and confirmation by PCR. The overall prevalence of *Campylobacter* species was 93.5% by PCR, which varied significantly ($P = 0.000$) by sample: 99.2, 98.4, and 82.8% for carcass swabs, cloacal swabs, and carcass drip, respectively, by using PCR to detect *Campylobacter* in BB. Important risk factors for carcass contamination by *Campylobacter* included the slaughter of culled breeders and spent chickens, the use of stagnant water, and poor sanitation. Virulence and toxin gene frequencies were higher in *C. jejuni*–positive (82.5%) than in *C. coli*–positive (71.4%) BB cultures, but tetracycline resistance gene (*tetO*) frequency was higher in *C. coli* (75.9%) than in *C. jejuni* (48.10%). The observed high frequencies in *C. jejuni* recovered from street-vended chickens may pose food safety and therapeutic concerns to consumers.

HIGHLIGHTS

- Prevalence of *Campylobacter* in chickens from informal markets was determined.
- Prevalence of *Campylobacter* was 23.4% (bacteriology) and 93.5% (PCR).
- Virulence and toxin genes were detected in *C. jejuni* (81.5%) and *C. coli* (74.1%).
- Tetracycline resistance gene was found in *C. coli* (75.9%) and *C. jejuni* (48.1%).
- Virulent strains of *C. jejuni* in chickens could pose a food safety risk.

Key words: *Campylobacter*; Chicken; Informal market; South Africa; Toxigenicity; Virulence

Campylobacteriosis, primarily caused by *Campylobacter coli* and *Campylobacter jejuni (19),* is a foodborne disease of major public health importance. It is a self-limiting disease because the infection only lasts for 3 to 5 days, and complications are rare *(55)*. Worldwide, *Campylobacter* spp. have been reported to be associated with foodborne campylobacteriosis in humans *(21, 50)* and have been reported to cause 500 million infections yearly *(54)*. Therefore, it is prudent to know the prevalence of *Campylobacter* spp. in their natural habitat and the prevalence of both *C. coli* and *C. jejuni,* because they are the most frequently isolated species in the avian gastrointestinal tract. It is also important to understand the pathogenesis and susceptibility of isolates to antimicrobials used in humans and animals.

Humans infected with *Campylobacter* spp. usually experience mild symptoms, the most common of which are

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watery or sticky diarrhea, abdominal cramps, fever, nausea, vomiting, muscle pain, and bloody feces *(17)*. Antimicrobials are rarely administered for campylobacteriosis in humans, but in severe gastroenteritis where the infection is prolonged, antibiotics are prescribed for treatment *(24, 45)*. The drugs of choice for severe gastroenteritis are macrolides, fluoroquinolones, and tetracyclines. These drugs are also added to poultry feed and used as prophylaxis and growth promoters *(33)*. The emergence of antimicrobial-resistant strains of *Campylobacter* is a public health concern because if an individual is infected with a drug-resistant strain, treatment drugs will be limited *(39)*.

In several countries, chickens processed and retailed at outlets of "wet markets," "informal markets," and "pluck markets" have been reported to be contaminated by many pathogens, including *Campylobacter* spp., *Salmonella,* and *Escherichia coli,* among others *(19, 37, 47)*. In South Africa, a few bacteriological and molecular studies have reported on the prevalence of *Campylobacter* spp. in chickens from retail outlets and commercial processing plants *(6, 18, 39, 40, 52)*. To date, there are no published reports on the occurrence and characteristics of *Campylobacter* spp. in chickens slaughtered, processed, and retailed at outlets of informal chicken markets in Gauteng province.

The informal poultry market of South Africa is a highly patronized market that is accessible to individuals from all economic strata. Even in its unorganized state, it sustains itself because the demand for chicken and chicken products in South Africa is extremely high, and most South Africans consume chicken because it has high protein content and is very affordable. The economic history of this country has allowed this type of market to thrive, whereby many South Africans and foreign residents can easily get into this business to support their families. The problem with an "industry" that is unregulated is that it is vulnerable to abuse from the lack of clear guidelines for operation, thereby allowing people to operate as they see fit. In the food industry, guidelines (farm-to-retail standards) do exist for food safety and security to protect both consumers and food handlers. Currently, there is no information on what happens during processing at these informal outlets from slaughter to processing to retailing, nor are there data on the potential contamination of carcasses with pathogens such as *Campylobacter, Salmonella,* and *E. coli,* among others, at these sale outlets, with a potential to negatively impact food safety.

The focus of this study is on *Campylobacter* spp. for which there is a dearth of information on the risk posed to consumers of chickens processed at unregulated informal chicken markets. The objectives of this study were to determine the prevalence of *Campylobacter* spp. in chickens sold at outlets in informal chicken markets in Gauteng province by using both standard bacteriological and polymerase chain reaction (PCR) assays. In addition, the study was designed to identify the risk factors for carcass contamination by *Campylobacter* spp. and to investigate the carriage of virulence, toxin, and resistance genes by *Campylobacter* strains.

MATERIALS AND METHODS

Data and sample collection. Over 8 months (from August 2017 to February 2018), we collected 151 chicken carcasses in total from 47 outlets located in six townships: Germiston, Atteridgeville–Phomolong, Garankuwa, Tembisa–Modise, Alexandra, and Soweto (Fig. 1). Prestudy assessment visits were made to obtain information on the global positioning system coordinates of locations and the throughput, processing practices, and number of workers at each facility. In addition, based on observations of the conditions and practices at each outlet (Supplemental Appendix S1), a sanitation score (Supplemental Appendix S2) was determined, without input from the owners or operators. The focus of the assessment was on the level of sanitation and cleanliness of handlers (e.g., wearing aprons), preslaughter chicken living areas, and areas where the following activities were conducted: slaughter, defeathering, evisceration, carcass rinsing, and packing in bags.

From each chicken carcass, three types of samples were obtained: cloacal swab, carcass swab, and carcass drip, resulting in a total of 453 samples being collected. At each outlet, a questionnaire also was administered to owners or primary operators. The sample size was calculated using the formula of Thrusfield *(51)*: $(n = 1.96^2 \times P_{exp}\,(1 - P_{exp})/d^2$, where $P_{exp} = 0.5$ (50%), with a margin of error (d^2) of 0.08. At an estimated prevalence of 50 and 95% confidence limits, a minimum of 150 samples was desirable. In this study, 151 samples in total were collected and transported in ice coolers to the laboratory for processing, within 4 h of collection, depending on the location of the outlet (Fig. 1).

Processing of samples. Two swab samples (cloacal and carcass) were each added to 10 mL of Bolton broth (BB) and incubated at 42°C for 48 h. A 3-mL aliquot of carcass drip was centrifuged (model 5702 centrifuge, Eppendorf India Private Limited, Chennai, India) for 10 min at 425 × *g*. The supernatant was discarded, and the pellet was resuspended with 3 mL of buffered peptone water.

Isolation and phenotypic identification of *Campylobacter* species. Isolation of *Campylobacter* species was conducted according to ISO 10272-1 and with PCR and methods as described in Lior *(26),* with a few modifications. One milliliter of the suspension in buffered peptone water was inoculated into 10 mL of BB and incubated at 42°C for 48 h in a microaerophilic atmosphere containing 6% O_2 (10% CO_2 and 84% N_2) created by a CampyGen sachet (Thermo Fisher Scientific [Johannesburg] Pty Ltd., South Africa) in an anaerobic jar (Thermo Fisher Scientific [Johannesburg] Pty Ltd.). Next, 100 μL was plated onto *Campylobacter* blood-free agar containing charcoal cefoperazone deoxycholate agar–selective supplement (Oxoid Ltd., Basingstoke, Hampshire, England). Inoculated BB was transferred into 1.5-mL Eppendorf tubes and stored at –80°C until characterization with PCR, to independently confirm the presence of *Campylobacter* spp. The inoculated plates were incubated under a microaerophilic atmosphere as described above. Representative colonies on the blood-free *Campylobacter* agar, which were grayish with a running and no-translucent appearance, were Gram stained. Representative isolates with the typical *Campylobacter* morphology on microscopic examination were subcultured onto blood agar and classified as presumptive *Campylobacter* spp. The isolates were subjected to further identification following ISO 10272-1 and using *C. jejuni* (ATCC 33560, Thermo Fisher Scientific [Johannesburg] Pty Ltd.) and *C. coli* (ATCC 43478, Thermo Fisher Scientific [Johannesburg] Pty Ltd.) type strains as positive controls for *Campylobacter* spp. The presumptive thermophilic *Campylobacter* spp. were stored in 50% brain heart infusion–50% glycerol broths

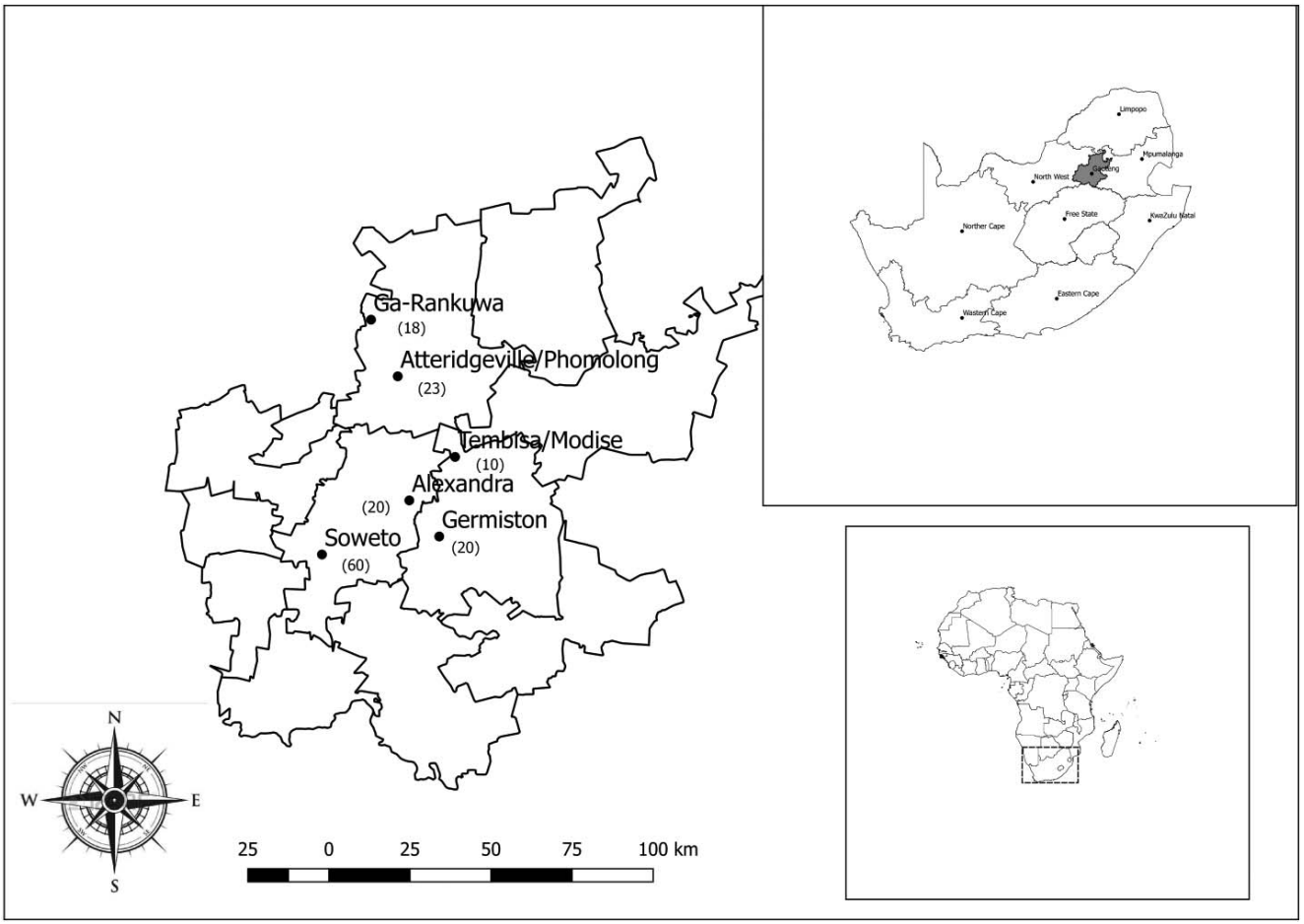

FIGURE 1. *Location of informal poultry outlets in Gauteng province inclusive of several samples collected from each township relative to the map of South Africa (33).*

at −80°C for further analysis with PCR to confirm and characterize the *Campylobacter* isolates.

DNA extraction and determination of DNA concentration. DNA was extracted from inoculated BB by boiling. BB (0.5 mL) was incubated using the heating block at 96°C for 10 min, and the tubes were then cooled down at room temperature. The mixture was separated by centrifugation at 20,817 × *g* for 5 min, and the supernatant was transferred into a sterile labeled tube and stored at −20°C for further analysis. DNA concentration of the total genomic DNA extracted by the heat method was determined by using the Qubit dsDNA BR assay kit (Thermo Fisher Scientific [Johannesburg] South Africa Pty Ltd.), and all steps were performed according to the manufacturer's instructions.

Confirmation of *Campylobacter* isolates and BB samples by PCR. Presumptive isolates of *Campylobacter* species were inoculated into BB that was screened using conventional multiplex PCR according to the method of Persson and Olsen *(38),* with a slight modification on the amount of primers added to the reaction mixture. One of the genes (16S) was for genus-specific identification, whereas the other two genes were for species differentiation (Whitehead Scientific, Cape Town, South Africa): *C. coli* (*Asp*) and *C. jejuni* (*hipO*). The sequences and amplicon sizes are listed in Table 1. The reaction mixture contained 12.5 μL of Qiagen multiplex PCR master mix kit (Whitehead Scientific), from 0.25 to 1 μL of 20 μM of each primer, and 2 μL of the template DNA, and the mixture was made up to 25 μL with nuclease-free water. A similar procedure used for the presumptive *Campylobacter* isolates was used to detect *Campylobacter* spp. in BB used as enrichment for all the samples (453) collected.

The genus *Campylobacter* was detected by a 1,062-bp band on 2% agarose gel. PCR was conducted in a 96-well thermal cycler (Veriti, Applied Biosystems, Waltham, MA) following the program and procedures provided by the manufacturer. In brief, the assay involved one cycle of denaturation for 10 s at 98°C, 30 cycles of annealing for 5 s at 57°C, and one cycle of extension for 20 s at 72°C. Amplicons (5 μL) were separated on a 2% agarose gel stained with ethidium bromide. GeneRuler 1-kb DNA ladder (Thermo Fisher Scientific [Johannesburg] South Africa Pty Ltd.) was used as a molecular marker. *C. jejuni* (ATCC 33560) and *C. coli* (ATCC 43478) type strains were used as positive controls. The separated bands were viewed with a UV transilluminator (ChemiDoc XRS+, BioRad, Johannesburg, South Africa). *C. jejuni* was detected by a 344-bp banding and *C. coli* by a 500-bp banding on 2% agarose gel.

Characterization of *Campylobacter* species. Confirmed *C. coli* and *C. jejuni* isolates from the BB cultures were evaluated for the presence of six virulence genes (*dnaj, racR, ciaB, pldA, flaA,* and *flaB*), three toxin-releasing genes (*cdtA, cdtB,* and *cdtC*), and one antibiotic resistance gene (*tetO*). The primers were obtained from Inqaba Biotechnical Industry (Pty) Ltd. (Pretoria, South Africa). The method used to screen for these 10 genes was adapted from Laprade et al. *(23),* with slight modifications in the amounts of primers added to the reaction mixture and the reduction in the number of cycles (Supplemental Appendix S3). The reaction mixture contained 12.5

TABLE 1. *Primers used for genus identification and speciation of* Campylobacter *into* C. coli *and* C. jejuni

Gene target	Primer	Nucleotide sequence (5′–3′)	Amplicon size (bp)	Reference
16S-rDNA	16S-F	GGG AGG CAG TAG GGA ATA	1,062	*5*
	16S-R	TGA CGG GCG GTG AGT ACA AG		
Hipurrate hydrolysis gene (*hipO*)	hipO-F	GAC TTC GTG CAG ATA TGG ATG CTT	344	*5*
	hipO-R	GCT ATA ACT ATC CGA AGA AGC CAT CA		
Aspartokinase gene (*asp*)	CC118-F	GGT ATG ATT TCT ACA AAG CGA G	500	*6*
	CC519-R	ATA AAA GAC TAT CGT CGC GTG		

μL of Qiagen multiplex PCR master mix kit (Whitehead Scientific), a primer concentration of 20 μM (from 0.25 to 1 μL; Supplemental Appendix S3), and 2 μL of the template DNA; the mixture was made up to 25 μL with nuclease-free water. PCR assay was conducted in a 96-well thermal cycler (Veriti, Applied Biosystems). The program set to run the four assays had unique denaturation, annealing, and initial elongation cycling conditions (Supplemental Appendix 3). However, all four assays had an initial denaturation cycle set at 95°C for 15 min and a final elongation cycle set at 72°C for 10 min. Amplicons (5 μL) were separated on a 2% agarose gel stained with ethidium bromide. The separated bands were viewed with a UV transilluminator (ChemiDoc XRS+, BioRad) and a 1-kb DNA ladder (Thermo Fisher Scientific [Johannesburg] South Africa Pty Ltd.). For all reactions, *C. jejuni* (ATCC 33560) and *C. coli* (ATCC 43478) type strains were used as positive controls. The virulence genes were detected at the expected bandwidth of 559 bp for *tetO* and 370 bp for *cdtA*.

Data analysis. Data obtained from the questionnaires were entered into Excel (Microsoft, Redmond, WA), matched with the laboratory data, filtered, and coded for analysis. All data were analyzed using IBM SPSS Statistics v25 (SPSS Inc., Chicago, IL). Specifically, the frequency of detection of *Campylobacte*r species was given a binary outcome (0 = absent, 1 = present) and used to determine the prevalence and to evaluate risk factors. The relationship between the nine virulence genes and one antibiotic resistance gene detected in *C. coli* and *C. jejuni* was analyzed using Pearson correction in STATA v15 (StataCorp LLC, College Station, TX), and the risk factors were analyzed using chi-square and logistic regression.

RESULTS

Demographic data and practices at informal chicken outlets in Gauteng province. The 47 outlets in six townships surveyed were situated near public transport stands (train station or taxi rank [garage]), in the household, or by the side of a busy road. The distribution of market locations varied across the townships. Alexandra township did not have street vendors, but individuals sold slaughtered chickens from homesteads with patronage from the local community.

The processing methods and practices, albeit similar, were customized per operator depending on the availability of resources, facilities, and infrastructures. Spent hens (old commercial egg-laying chickens) were the most slaughtered chickens in all outlets: Atteridgeville–Phomolong (23 of 23, 100%), Soweto (33 of 60, 55.0%), Germiston (8 of 20, 40.0%), Tembisa–Modise (3 of 10, 30.0%), Alexandra (6 of 20, 30.0%), and Garankuwa (2 of 18, 11.0%). Broiler chickens were the least slaughtered chickens in three areas: Alexandra (14 of 20, 70%), Soweto (5 of 60, 8.3%), and Tembisa–Modise (7 of 10, 70%). Culled breeders were slaughtered in Garankuwa (16 of 18, 88.9%) followed by Germiston (12 of 20, 60.0%), and the lowest frequency of slaughtered culled breeders was in Soweto (22 of 60, 36.7%; Supplemental Appendix S4).

Of the 151 chickens sampled from the 47 outlets, 112 (74.2%) originated from outlets where preslaughter chickens were kept in cages, whereas 39 (25.8%) were from outlets where the practice was to keep chickens tied together in groups of 5 to 10 on the ground before slaughter. In all the townships, the same knives were used for slaughtering and scalding the carcasses. However, although evisceration of carcasses was a routine procedure (100.0%) in Garankuwa, Germiston, and Tembisa–Modise, in Atteridgeville and Soweto, only 56.5% (13 of 23) and 23.3% (14 of 60), respectively, of the interviewed operators eviscerated the carcasses, and in Alexandra, no evisceration was carried out. All the outlets defeathered the chickens before presentation for sale, but the defeathering method varied. Operators in outlets in Alexandra, Garankuwa, and Germiston only used the hand-picking method (100.0%); the others used a combination of hand-picking and knife-shaving methods of defeathering (Supplemental Appendix S4).

All the outlets in Atteridgeville–Phomolong, Garankuwa, and Tembisa–Modise used stagnant water in drums for rinsing chicken carcasses postprocessing. Those in Alexandra and Germiston only used water in the bucket to rinse chicken carcasses, but outlets in Soweto used a combination of the two methods, with 31 (51.7%) of 60 using a bucket and the remaining 29 (48.3%) using stagnant water in drums (Supplemental Appendix S4).

From all the selected outlets, a cumulative proportion of 49.0% of the chickens slaughtered daily was spent hens. All the operators slaughtered chickens by using knives, and 82.0% defeathered chickens by using the hand-picking method. From all the outlets, the water source was either stagnant water (52.0%) or a bucket (47.0%), and rarely other sources (1.0%). The processing methods investigated were not statistically significant ($P > 0.05$; Supplemental Appendix S4); however, the data collected provide insight into the processing methods used in Gauteng informal poultry markets.

Risk factors for contamination of carcasses by *Campylobacter* spp. Overall, most of the outlets in Gauteng placed their slaughtered chickens in the rinse bucket (64 of 1,551, 42.4%), and some displayed them on the counter (53 of 151, 35.1%). Only a few outlets placed their slaughtered chickens in the freezer (28 of 151, 18.5%), and it was rare to find outlets that placed them in drums (6 of 151; 4.0%; Table 2). Of the 47 outlets sampled, only 81 (53.6%) of 151 washed

TABLE 2. *Summary of risk factors for carcass contamination with* Campylobacter *species in six Gauteng townships*

Risk factor	No. (%) of outlets with practice in the township of[a]:											
	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
Location of the carcass for sale		0.01		0.0005		0.01		0.01		0.0005		<0.005
Bucket	6 (30.0)		10 (43.5)		0 (0.0)		0 (0.0)		43 (71.7)		5 (50.0)	
Counter	14 (70.0)		7 (30.4		18 (100.0)		0 (0.0)		9 (15.0)		5 (50.0)	
Drum	0 (0.0)		6 (26.1)		0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
Freezer	0 (0.0)		0 (0.0)		0 (0.0)		20 (100.0)		8 (13.3)		0 (0.0)	
Length (min) of the carcass on the counter		0.001		—[b]		0.01		0.01		0.0005		<0.005
<30	8 (40.0)		NA[c]		18 (100.0)		0 (0.0)		21 (35.0)		0 (0.0)	
30–60	6 (30.0)		NA		0 (0.0)		0 (0.0)		15 (25.0)		0(0.0)	
>60	0 (0.0)		NA		0 (0.0)		0 (0.0)		24 (40.0)		5 (50.0)	
NA	6 (30.0)		NA		0 (0.0)		20 (100.0)		0 (0.0)		5 (50.0)	
Owner washes knives after slaughtering		0.01		<0.01		<0.0001		0.71		0.47		<0.0001
Yes	6 (30.0)		17 (73.9)		18 (100.0)		8 (40.0)		32 (53.3)		0 (0.0)	
No	14 (70.0)		6 (26.1)		0 (0.0)		12 (60.0)		28 (46.7)		10 (100.0)	
Overall level of sanitation		0.002		0.002		<0.01		<0.01		0.002		<0.01
Fair	6 (30.0)		2 (8.7)		0 (0.0)		20 (100.0)		3 (5.0)		0 (0.0)	
Good	13 (65.0)		0 (0.0)		0 (0.0)		0 (0.0)		5 (8.3)		10 (100.0)	
Poor	1 (5.0)		21 (91.3)		18 (100.0)		0 (0.0)		52 (86.7)		0 (0.0)	

[a] 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), and Tembisa–Modise (10 samples).

[b] —, *P* value could not be generated because there were no data to compare.

[c] NA, not available. At Atteridgeville–Phomolong township, the risk factor could not be assessed because all of the outlets do not display carcasses on counters.

TABLE 3. *Odds ratio for risk factors associated with* Campylobacter *species contamination of carcasses at outlets in six Gauteng townships*

Risk factor	Odds ratio[a]	*P* value
Culled breeders	9.7	0.000
Spent hens	7.2	0.000
Defeather	1.8	0.041
Stagnant water	2.0	0.006
Poor sanitation	2.6	0.002

[a] Based on the frequency of isolation of *Campylobacter* spp. from chicken carcasses.

their knives after slaughtering the chicken. Most of the outlets had poor sanitation (92 of 151, 60.9%). The differences in the risk factors for carcass contamination were statistically significant ($P < 0.05$) for one parameter in Soweto, whereby owners not washing their knives after slaughtering each chicken increased the risk of *Campylobacter* contamination; the other five areas (Atteridgeville–Phomolong, Tembisa–Modise, Germiston, Garankuwa, and Alexandra) had no significant findings in this parameter ($P > 0.05$).

Risk factors for contamination of chicken carcasses across 47 informal outlets suggested an increased risk of *Campylobacter* contamination associated with the slaughtering of culled breeders (odds ratio [OR] = 9.7), poor sanitation in the operating environment (OR = 2.6), slaughtering of spent hens (OR = 7.2), use of stagnant water to wash carcasses (OR = 2.0), and defeathering of chickens (OR = 1.8; Table 3).

Frequency of isolation of presumptive *Campylobacter* spp. in chicken samples. Overall, the frequency of isolation of *Campylobacter* species from all types of samples from the six townships was 34.2% (155 of 453; Table 4). The sample type–specific frequency of isolation of *Campylobacter* spp. was 24.5% (37 of 151), 40.4% (61 of 151), and 37.7% (57 of 151) for carcass swabs, carcass drip, and cloacal swabs, respectively. The differences were statistically significant ($P < 0.05$).

For carcass swab samples collected from outlets in the six townships, the frequency of isolation of *Campylobacter* spp. ranged from 0.0% (Tembisa–Modise) to 50.0% (Garankuwa; $P = 0.0001$); for cloacal swabs, the range was from 0.0% (Tembisa–Modise) to 65.0% (Germiston; $P = 0.0001$); and for carcass drip, the range was from 0.0% (Tembisa–Modise) to 100.0% (Germiston; $P = 0.0001$). Within each of the six townships, there were statistically significant differences in the frequency of isolation of *Campylobacter* spp. for the three types of samples (carcass swab, cloacal swab, and carcass drip), except for Tembisa–Modise township (Table 4).

Confirmation of *Campylobacter* spp. by conventional PCR. Of a total of 155 isolates of *Campylobacter* spp. recovered by bacteriological methods (Table 4), 106 (68.4%) were confirmed by conventional PCR. The frequency of confirmation of isolates of *Campylobacter* spp. by sample type was 70.3% (26 of 37), 68.4% (39 of 57), and 67.2% (41 of 61) from carcass swabs, cloacal swabs, and carcass drip, respectively (Table 4). The

TABLE 4. *Frequency of isolation of* Campylobacter *species from carcass swabs, cloacal swabs, and carcass drip by township by using standard bacteriology methods and from sample-inoculated BB by using conventional PCR*

		No. (%) positive for *Campylobacter* spp.				Total for all types of samples	
Township	No. tested	Carcass swabs	Cloacal swabs	Carcass drip	*P* value	No. tested	No. (%) positive
Isolation from carcass swabs, cloacal swabs, and carcass drip by using standard bacteriology methods							
Atteridgeville–Phomolong	23	7 (30.4)	13 (56.5)	11 (47.8)	0.002	69	31 (44.9)
Garankuwa	18	9 (50.0)	7 (38.9)	1 (5.5)	0.002	54	17 (31.5)
Tembisa–Modise	10	0 (0.0)	0 (0.0)	0 (0.0)	NA[a]	30	0 (0.0)
Alexandra	20	4 (20.0)	4 (20.0)	7 (35.0)	<0.01	60	15 (25.0)
Germiston	20	7 (35.0)	13 (65.0)	20 (100.0)	0.002	60	40 (66.7)
Soweto	60	10 (16.7)	20 (33.3)	22 (36.6)	0.002	180	52 (28.9)
P value		0.0001	0.0001	0.0001			0.0001
Total	151	37 (24.5)	57 (37.7)	61 (40.4)		453	155 (34.2)
Detection from sample-inoculated BB by using conventional PCR[b]							
Garankuwa	18	18 (100.0)	18 (100.0)	17 (94.4)	<0.01	54	53 (98.1)
Tembisa–Modise	10	10 (100.0)	10 (100.0)	9 (90.0)	<0.01	30	29 (96.7)
Alexandra	20	19 (95.0)	18 (90.0)	20 (100.0)	0.002	60	57 (95.0)
Germiston	20	20 (100.0)	20 (100.0)	0 (0.0)	<0.01	60	40 (66.7)
Soweto	60	60 (100.0)	60 (100.0)	60 (100.0)	1.00	180	180 (100.0)
P value		0.02	0.02	0.0002			0.0001
Total	128	127 (99.2)	126 (98.4)	106 (82.8)		384	359 (93.5)

[a] No statistics could be computed because the parameter is constant. Of the 155 *Campylobacter* spp.–positive isolates, only 106 (68.4%) were typed and confirmed by PCR.

[b] Atteridgeville–Phomolong was not included in the BB culture analysis because samples from this township were lost during storage in a freezer used by multiple researchers.

TABLE 5. *Frequency of detection of species of* Campylobacter *in sample-inoculated BB by using conventional PCR*

Township	No. tested	No. (%) positive for: *C. coli*	*C. jejuni*	*P* value
Garankuwa	53	11 (20.8)	8 (15.1)	0.47
Tembisa–Modise	29	7 (24.1)	6 (20.7)	0.76
Alexandra	57	4 (7.0)	13 (22.8)	0.02
Germiston	40	16 (40.0)	0 (0.0)	<0.0001
Soweto	180	16 (8.9)	0 (0.0)	<0.0001
P value		0.0001	0.0002	
Total	359	54 (15.0)	27 (7.5)	

frequency of PCR-confirmed isolates of *Campylobacter* spp. varied across the five townships for all sample types.

Based on the PCR-confirmed isolates of *Campylobacter* spp., the overall prevalence of *Campylobacter* spp. was 23.4% (106 of 453), with sample type–specific prevalence being 17.2% (26 of 151), 25.8% (39 of 151), and 27.2% (41 of 151) for carcass swabs, cloacal swabs, and carcass drip, respectively. The differences were not statistically significant ($P = 0.09$).

Frequency of detection of *Campylobacter* spp. in sample-inoculated BB by PCR. For the samples enriched and inoculated in BB for enhanced PCR detection, *Campylobacter* spp. were detected in carcass swabs (99.2%), cloacal swabs (98.4%), and the carcass drip (82.8%; Table 4). The differences in prevalence among the three types of samples were statistically significant for carcass swabs ($P = 0.02$), cloacal swabs ($P = 0.02$), and carcass drip ($P = 0.0002$; Table 4). The overall frequency of detection of *Campylobacter* species from these five areas was 93.5% (359 of 384) by using BB grown followed by confirmation by PCR. The differences in the detection rate for *Campylobacter* species in the three types of samples within each of the areas were statistically significant in Garankuwa ($P < 0.01$), Tembisa–Modise ($P < 0.01$), Alexandra ($P = 0.002$), and Germiston ($P < 0.01$). Soweto had the highest frequency of detection (100%) followed by Garankuwa (98.1%), Tembisa–Modise (96.7%), Alexandra (95.0%), and Germiston (66.7%). The difference in prevalence in the five areas was statistically significant ($P = 0.0001$; Table 4).

Frequency of confirmed *C. coli* and *C. jejuni* in sample-inoculated BB. From the total samples evaluated ($n = 384$ BB), 340 (88.5%) were confirmed by PCR to be *Campylobacte*r species and of these, 54 (15.0%, $P = 0.01$) and 27 (7.5%, $P = 0.10$) were identified as *C. coli* and *C. jejuni,* respectively (Table 5). The difference in the frequency of detection of *C. jejuni* and *C. coli* was statistically significant in isolates from Alexandra ($P = 0.02$), Germiston ($P < 0.0001$), and Soweto ($P < 0.0001$).

Frequency of detection of virulence, toxin, and antimicrobial resistance genes in *C. jejuni* and *C. coli* isolates. The frequencies of detection of six virulence genes, three toxin genes, and one resistance gene by township are summarized for *C. jejuni*– and *C. coli*–positive broth cultures (Table 6). Overall, virulence and toxin genes were detected at a higher frequency in *C. jejuni* isolates, 81.5% (22 of 27), than in *C. coli* isolates, 71.4% (40 of 54). For the six virulence and three toxin genes assayed, the frequencies of both types of genes were detected at higher frequencies in *C. jejuni*–positive than in *C. coli*–positive broth cultures, except for *flab,* wherein a slightly higher percentage was detected in *C. coli*–positive broth cultures (74.1%) than in *C. jejuni*–positive broth cultures (70.4%).

Overall, the antibiotic resistance gene (*tetO*) was detected in the broth cultures at a significantly ($P = 0.012$) higher frequency in *C. coli* isolates, 75.9% (41 of 54), than in *C. jejuni* isolates, 48.1% (13 of 27). In the *C. jejuni*–positive broth cultures, the *tetO* gene was detected at the highest frequency in samples from Tembisa–Modise (83.3%); the lowest level of detection was in Alexandra (30.8%). The differences across the townships were significant ($P = 0.0329$). For *C. coli*–positive broth cultures, the highest frequency of detection of the *tetO* gene was in Germiston (100.0%) and the lowest frequency was in Garankuwa (27.3%; Table 6).

DISCUSSION

Using phenotypic and molecular methods, we determined the prevalence of *Campylobacter* spp. from informal outlets selling chickens and explored the associated risk factors for carcass contamination by *Campylobacter* spp. as well as the influence of informal sale outlets' practices on microbial contaminations by *Campylobacter* species and other pathogens. Overall, the prevalence of presumptive *Campylobacter* spp. was 34.2% based on isolation and 23.4% for PCR-confirmed isolates of *Campylobacter* spp. in chicken samples (carcass swabs, cloacal swabs, and carcass drip). These values, although low, agree with those of other studies similarly conducted on chickens sampled from wet market outlets in Tunisia (22.4% *(13)*) and Malaysia (26.6% *(47)*), but are higher than those from Brazil (7.7% *(27)*) and China (19.3% *(29)*). Similarly, a higher prevalence of *Campylobacter* spp. in chickens at outlets of wet markets has been reported in Sri Lanka (48% *(22)*), Malaysia (75.6% *(32)*), and Trinidad (89.6% *(43)*). These variable isolation rates may reflect differences in the influence of the type of transport and in the sensitivity of the isolation methods used; for example, delays in postsampling transport of chicken carcasses (ice cooled and not in transport media) to the laboratory *(41)* may reduce the chances of isolation. They may also reflect the prevalence of *Campylobacter* spp. in slaughtered chickens, types of chickens slaughtered, and sanitary practices during processing and retailing *(22)*.

In our study, the frequency of isolation of *Campylobacter* spp. by using standard bacteriological methods confirmed by conventional PCR was not significantly different among carcass swabs (17.2%), cloacal swabs (25.8%), and carcass drip (27.2%). Other studies have reported relatively similar or differing prevalence of *Campylobacter* spp. of 80.2 and 83.9% in cloacal and carcass swabs, respectively *(42),* 58.9 and 37.8% in cecal

TABLE 6. *Frequency of six virulence genes, three toxin genes, and one resistance gene in* C. jejuni– *and* C. coli–*positive broth cultures by location of retail outlets*

Township[a]	No. of positive *Campylobacter* spp. broth cultures	Virulence, *n* (%)						*P* value	Toxin, *n* (%)			*P* value	Antibiotic resistance, *n* (%)
		dnaj	*racR*	*flaA*	*flaB*	*ciaB*	*pldA*		*cdtA*	*cdtB*	*cdtC*		*tetO*
Frequency of six virulence genes, three toxin genes, and one antibiotic resistance gene in *C. jejuni*–positive broth cultures by location of the market													
Garankuwa	8	2 (25.0)	4 (50.0)	6 (75.0)	5 (62.5)	4 (50.0)	4 (50.0)	0.0006	4 (50.0)	5 (62.5)	4 (50.0)	0.0059	4 (50.0)
Tembisa–Modise	6	4 (66.7)	5 (83.3)	2 (33.3)	3 (50.0)	6 (100.0)	6 (100.0)	0.0013	6 (100.0)	6 (100.0)	5 (83.3)	0.0034	5 (83.3)
Alexandra	13	9 (69.2)	10 (76.9)	11 (84.6)	11 (84.6)	11 (84.6)	11 (84.6)	0.0001	9 (69.2)	11 (84.6)	10 (76.9)	0.0033	4 (30.8)
P value		0.1383	0.0762	0.1355	0.1189	0.0782	0.0782		0.0488	0.0585	0.0762		0.0059
Total	27	15 (55.5)	19 (70.4)	19 (70.4)	19 (70.4)	21 (77.8)	21 (77.8)		19 (70.4)	22 (81.5)	19 (70.4)		13 (48.1)
Frequency of six virulence genes, three toxin genes, and one antibiotic resistance gene in *C. coli*–positive broth cultures by location of the market													
Garankuwa	11	2 (18.2)	3 (27.3)	7 (63.6)	8 (72.7)	2 (18.2)	2 (18.2)	0.0163	1 (9.1)	3 (27.3)	3 (27.3)	0.0728	3 (27.3)
Tembisa–Modise	7	3 (42.9)	2 (28.6)	5 (71.4)	7 (100.0)	3 (42.9)	3 (42.9)	0.0037	4 (57.1)	3 (42.9)	3 (42.9)	0.0099	4 (57.1)
Alexandra	4	2 (50.0)	4 (50.0)	3 (75.0)	3 (75.0)	2 (50.0)	0 (0.0)	0.0086	2 (50.0)	2 (50.0)	2 (50.0)	NA[b]	3 (75.0)
Germiston	16	10 (62.5)	3 (18.7)	15 (93.7)	15 (93.7)	12 (75.0)	12 (75.0)	0.0016	7 (43.7)	12 (75.0)	10 (62.5)	0.0219	16 (100.0)
Soweto	16	5 (31.2)	0 (0.0)	5 (31.2)	7 (43.7)	1 (6.2)	1 (6.2)	0.0421	1 (6.2)	2 (12.5)	4 (25.0)	0.1181	15 (93.8)
P value		0.0429	0.024	0.0289	0.0148	0.1194	0.1707		0.0581	0.0829	0.0375		0.0518
Total	54	22 (40.7)	10 (18.5)	35 (64.8)	40 (74.1)	20 (37.0)	18 (33.3)		15 (27.8)	22 (40.7)	22 (40.7)		41 (75.9)

[a] Townships where samples from outlets were positive for *C. coli* or *C. jejuni*.
[b] NA, no statistics could be computed because the parameter is constant.

and cloacal swabs *(32)*, 67.0 and 17.2% in ceca and carcasses *(15)*, and 77.2 and 87.5% in ceca and carcasses *(16)*. Differences in the sample type–specific frequencies of isolation of *Campylobacter* spp. within outlets of wet markets may reflect the preslaughter prevalence of the organism in the gastrointestinal tracts of the chickens and/or postslaughter practices. It was not surprising that the overall (23.4%) and sample type–specific (carcass swab, 17.2%; cloacal swab, 25.8%; carcass drip, 27.2%) isolation rates for which the *Campylobacter* isolates were confirmed by PCR were low; however, a high frequency of the sample-inoculated BB (93.5%) from the outlets had significantly high levels of *Campylobacter* spp. contamination (carcass swab, 99.2%; cloacal swab, 98.4%; carcass drip, 82.8%). Furthermore, PCR is more sensitive and specific than bacteriological methods for the detection of *Campylobacter* spp. *(31, 34, 44, 46)*.

Campylobacter spp. detection in chicken carcasses from wet market outlets, determined using conventional bacteriological or PCR assays, has produced similar outcomes *(10, 44)*. The major disadvantage of the current "gold standard" for the detection of *Campylobacter*, i.e., culturing, is the length of the procedure *(10)*. It cannot be overemphasized that most PCR protocols detect DNA from both live and dead microorganisms, whereas isolation procedures can only detect live microorganisms, a factor often associated with higher detection rates associated with PCR *(9)*. However, some multiplex real-time PCR protocols capable to determine DNA from live cells have been reported previously *(3, 49)*. It is also pertinent to mention that the overall isolation rate for presumptive *Campylobacter* spp. (34.2%) in our study, which was reduced to 23.4% through PCR confirmation, suggests that the isolation method used had comparatively lower sensitivity and specificity than the frequency of detection through BB.

In this study, a total of 93.5% of all sample-inoculated BB were positive for *Campylobacter* DNA, but only 22.6% (81 of 359) were speciated into pathogenic *C. coli* (15.0%) and *C. jejuni* (7.5%). Jonker and Picard *(18)* reported a much higher prevalence of *C. jejuni* (31.9%) and *C. coli* (14.2%) in poultry from South Africa. Similarly, higher frequencies of isolation of *C. jejuni* than *C. coli*, respectively, in chickens from wet markets have been documented in the Philippines (64.2 and 12.1% *(25)*), Tunisia (64.7 and 14.0% *(13)*), Trinidad (54.7 and 45.3% *(43)*), Malaysia (69.5 and 16.2% *(47)*), and Vietnam (45.2 and 25.8% *(28)*).

A dominance of *C. coli* (15.0%) over *C. jejuni* (7.5%) was observed, as was previously confirmed by Van Nierop et al. *(52)*, from fresh chickens sold by street vendors (2.0 versus 1.0%), and by Mabote et al. *(30)* in fresh chickens sold in supermarkets in North West province, South Africa (48.1 versus 3.9%). The reason behind the unconventional change in prevalence between these two species is not understood and may warrant more research into the microbial ecology of the poultry gut and *Campylobacter* spp. It was suggested that the high prevalence of *C. jejuni* in poultry meat is due to high genetic diversity, which

allows it to have a more stable population than that of the *C. coli (48, 53)*.

The lack of basic infrastructures, such as a pipe-borne municipal water supply, at these "illegal" informal outlets, where chicken carcasses are processed as food for humans, was obvious. This lack is directly linked with observed risk factors such as poor sanitation in the operating environment and the use of stagnant water to wash carcasses. The negative impact of the absence of such infrastructure at food processing outlets on contamination and cross-contamination of foods by pathogens, such as *Campylobacter* spp. and *Salmonella,* has been emphasized by previous work *(7, 35)*. Studies conducted at the outlets of wet market pluck shops in Trinidad showed that the rinsing of carcasses in a stagnant water system was a significant ($P < 0.05$) risk factor for *Campylobacter* contamination of chickens compared with those rinsed under constantly running water *(42, 43)*; furthermore, the studies revealed that the length of time a bird is kept in the shop and the location of the carcass for sale, as well as the level of activities in the shops (medium-activity sale shops versus low-activity sale shops), influences the prevalence of *Campylobacter* spp.

This observation contrasts with what was obtained at legal or formal chicken retail outlets, where pipe-borne water is available unrestrictedly, compliance with ISO standards and hazard analysis critical control point guidelines were conducted, and intense monitoring and evaluation of good manufacturing practice are ensured *(7, 14)*. In our study, 47 outlets in six broad locations and townships across Gauteng province, South Africa, were investigated, and evidence abounds that informal market outlets remain popular within the townships *(1, 2, 4)*. The locations studied have a combined human population of more than 2.7 million, and many of the outlets are located near tarred and untarred roads, with evidence of dust contamination and unhygienic environments. Furthermore, slaughter, processing, and display procedures facilitate cross-contamination. Such activities, including defeathering, evisceration, use of stagnant water in buckets for cleansing, use of unwashed knives, and use of dirty linen to wipe display table surfaces, further encourage microbial contamination of carcasses *(7, 35, 36)*. In addition, we confirmed that the slaughter of culled breeders and spent hens was significantly associated with the risk of contamination of chicken carcasses by *Campylobacter* species.

Of clinical significance is the detection of six virulence genes and three toxin genes in chickens in this study; the frequency of detection was considerably higher in the 27 broth cultures positive for *C. jejuni* than in the 54 broth cultures positive for *C. coli,* except for the *flaB* virulence gene. The frequency of virulence-associated genes detected was 76.5% from 81 speciated broth cultures, with 81.5% detected from 27 *C. jejuni*–positive broth cultures and 74.1% detected from 54 *C. coli*–positive broth cultures. This observation may partially explain the reason why more clinical infection is associated with *C. jejuni* than with *C. coli*. However, the high frequency of virulence and toxin genes indicated that both *C. coli* and *C. jejuni* have the potential to cause foodborne campylobacteriosis in humans. Reddy and Zishiri *(40)* studied 100 fecal isolates of *Campylobacter* spp. (*C. jejuni* and *C. coli*) obtained from poultry farms in Durban metropolis, South Africa, and screened 78 isolates for nine virulence and toxin genes (*cadF, hipO, asp, ciaB, dnaJ, pldA, cdtA, cdtB,* and *cdtC*). Although the current study considered six genes (*dnaj, ciaB, pldA, cdtA, cdtB,* and *cdtC),* Reddy and Zishiri *(40)* and the current study both confirmed that virulence and toxin genes are more prevalent in *C. jejuni* than in *C. coli,* as reported by other work *(12, 20)*. Zheng et al. *(56)* had similarly detected virulence and toxin genes (*flaA, cadF, pldA, cdtA, cdtB,* and *cdtC*) in all *Campylobacter* spp. tested, whereas 91% also contained *ciaB*. Despite the presence of the putative virulence genes, some, but not all, *Campylobacter* strains isolated from retail meat can effectively invade human intestinal epithelial cells in vitro. The detection of these genes does not directly confer pathogenicity on the microorganisms possessing them because these genes have to be activated for expression *(48)*. Overall, the general higher frequency of detection of virulence and toxin genes in *C. jejuni* than in *C. coli* in chicken may be responsible for the reported involvement of *C. jejuni* in human and animal campylobacteriosis *(40, 50)*.

The possession of resistance genes to tetracycline among *Campylobacter* species was also investigated in our study. A higher frequency of potential tetracycline resistance in positive *C. coli* broth cultures (75.9%) than in positive *C. jejuni* broth cultures (48.1%) was obtained, indicating significant findings for resistance to antimicrobials in humans and animals. In South Africa, tetracycline was reported as one of the main growth-promoting antimicrobials in food animals *(8)* and is a frequently used antibiotic in South Africa *(11)*. In other studies, the assessments of strains of *Campylobacter* isolated from chickens sampled from slaughtered broilers and layers at commercial processing plants have also detected high resistance to tetracycline *(5, 6)*. That *Campylobacter* species are zoonotic agents indicates that tetracycline-resistant strains of *Campylobacter* species may be transmitted to consumers of chickens from these sources, which could have therapeutic implications.

In conclusion, findings from this study should (i) inform policy change and innovative measures aimed at improving service delivery to the informal sector, (ii) assist in developing a monitoring and evaluation framework to closely guide outlets' operations, and (iii) provide risk communication and community engagement messaging on health- and hygiene-related education that promotes good manufacturing practices among operators.

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SUPPLEMENTAL MATERIAL

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