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Microbiological Status and Food Safety Compliance of Commercial Basil Production Systems

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ABSTRACT

Basil has been implicated in a number of microbe-associated foodborne illnesses across the world, and the source of contamination has often been traced back to the production and/or processing stages of the supply chain. The aim of this study was to evaluate the microbiological quality of fresh basil from the point of production to the retail outlet in the Gauteng and Northwest Provinces of South Africa. A total of 463 samples were collected over a 3-month period from two large-scale commercial herb producing and processing companies and three retail outlets. The microbiological quality of the samples was assessed based on the presence or absence of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium and the levels of the indicator bacteria *E. coli* and total coliforms. *Salmonella* Typhimurium was detected on four basil samples (0.9%) arriving at the processing facility and at dispatch, but no *E. coli* O157:H7 was detected throughout the study. Total coliform counts were 0.4 to 4.1 CFU/g for basil, 1.9 to 3.4 log CFU/ml for water, and 0.2 to 1.7 log CFU/cm^2 for contact surfaces, whereas *E. coli* was detected in the water samples and only once on basil. The Colilert-18 and membrane filter methods were used to analyze water samples, and a comparison of results revealed that the Colilert-18 method was more sensitive. Strong evidence suggests that high numbers of coliforms do not necessarily indicate the presence of *Salmonella* Typhimurium. The study results highlight the importance of effective implementation of food safety management systems in the fresh produce industry.

Globally there has been an increase in the consumption of leafy vegetables as consumers are encouraged to increase their daily intake of fresh fruit and vegetables *(22)*. For a variety of reasons, the increased consumption of fresh produce has been linked to an increase in the number of foodborne disease outbreaks *(28)*. Recently the Food and Agricultural Organization of the United Nations *(17)* evaluated the microbial hazards in fresh fruit and vegetables and assigned the highest microbiological risk ranking to leafy greens. This risk is high partly because leafy greens grow close to the soil where they are subject to contamination from splashing (soil to leaves) and because the product is extensively traded across the world and has been the source of multiple foodborne disease outbreaks that have occurred in various countries.

The most common bacterial foodborne pathogens on leafy greens are *Escherichia coli* O157:H7 and *Salmonella (12)*. These foodborne pathogens can contaminate fresh produce at any point during the production or processing stages and can cause illness after ingestion *(3, 4)*. Possible sources of contamination include noncomposted or incompletely processed manure used as fertilizer *(36, 47)*, irrigation water *(26, 45)*, and contact surfaces such as hands, crates, and processing equipment *(4)*.

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Total coliforms and fecal coliforms are commonly used as indicators of potential microbial contamination. The presence of coliforms can indicate unhygienic working conditions, fecal contamination, or the presence of ecologically similar enteric pathogens. Indicator bacteria are not necessarily pathogenic but are often found where intestinal pathogens are likely to exist *(32)*.

Basil is a popular culinary herb used in a variety of dishes all over the world. Often it is used as a minimally cooked or raw ingredient in foods such as pesto. The consumption of contaminated basil has been linked to foodborne illnesses. The most recent outbreak caused by the consumption of fresh basil occurred in Norway in 2011 and resulted in 46 human illnesses *(21)*. In 2007, *Salmonella* Senftenberg was responsible for two international outbreaks associated with fresh basil imported from Israel, which resulted in 32 and 19 cases of *Salmonella* infection, respectively *(15, 37)*.

The objectives of this study were to (i) evaluate the microbial quality of basil from the seedling stage to the final retail product and (ii) assess the reliability of indicator bacteria to indicate the presence of the common foodborne pathogens *E. coli* O157:H7 and *Salmonella* Typhimurium.

MATERIALS AND METHODS

A total of 143 basil, 65 water, 162 contact surface swab, and 54 hand swab samples were collected from two South African basil production chains (production chains 1 and 2). The two chains

TABLE 1. *Comparison of the cultivation and processing practices of the two basil production chains assessed in this study*

Practice	Production chain 1	Production chain 2
Certification status	Global-GAP	Global-GAP and "Farming for the Future"[a]
Cultivation method	Open field cultivation	Tunnels with plastic cover
Growing medium	Soil	Soil covered with plastic mulch
Irrigation source	Surface dam water	Borehole, groundwater
Irrigation water storage	Surface dam water	Dam covered with netting
Irrigation application method	Overhead sprinkler	Drip irrigation

[a] Commercial standard required by one of the retailers.

consisted of two commercial large-scale primary producers (each with their own processing company) and three major retail vendors that these companies supply. The two companies and their associated retail vendors were situated in the Gauteng and Northwest Provinces, respectively. For production chain 1, basil samples were collected at two major retail vendors in Gauteng Province; for production chain 2, one major retail vendor was visited in Northwest Province. A comparison of the cultivation and processing practices used by the two chains is given in Table 1.

The two production chains were evaluated from February to April 2013 (summer and autumn) at four stages during the basil growing cycle: the seedling stage, 2 weeks before harvest, 1 week before harvest, and at harvest. The processing facility was not visited at the seedling stage. Basil (cultivar Genovese) samples, water samples, and swab samples from contact surfaces and hands were obtained from the field, processing facility, and retail vendors. All samples were evaluated for coliform and *E. coli* counts as an indication of basic sanitary conditions and potential fecal contamination *(20, 39),* respectively. The presence of *E. coli* O157:H7 and *Salmonella* Typhimurium also was determined throughout the chains. The samples were transported in cooler boxes to the Plant Pathology laboratories of the University of Pretoria (Pretoria, South Africa), where sample processing commenced within 24 h of sample collection.

Farm sampling (primary sampling). At each sampling visit, three basil samples were chosen at random diagonally throughout the same field (approximately 100 m^2; production chain 1) or tunnel (approximately 50 m long, containing nine rows grouped together in rows of three; production chain 2). The basil samples were collected aseptically and placed in brown paper bags. One sample consisted of the leaves of three basil plants that were pooled for a total weight of 40 g. Three water sites were sampled on each farm: the water source used, the on-farm storage dam, and the outlet of the irrigation pipes. Three 1-liter and three 100-ml water samples were collected *(24)* from each water sampling site during each visit. Contact surfaces were sampled using transport swabs (Copan, Cape Town, South Africa) from the hands of three basil pickers and from three harvesting crates. Sampling areas of 25 and 50 cm^2 were swabbed on the pickers' hands and the harvesting crates, respectively.

Processing facility sampling (processing sampling). Three samples of basil entering the processing line and three samples of the basil end product leaving the processing facility were sampled at both processing facilities during each visit. Each basil sample consisted of 40 g of basil. Swab samples were taken from each of the following contact surfaces: three incoming crates, three different areas on the conveyer belts, and three plastic clamshell containers. The hands of three packers were also swabbed. Swabs were taken as described for farm sampling (25 cm^2 for the hands and 50 cm^2 for all other surfaces). All samples were collected at three times during the day (beginning, middle. and end). The hands of the same three packers and the same areas of the conveyer belt were sampled at all three times.

Retail vendors sampling (final sampling). During each site visit, nine basil packages were purchased from all three local retail outlets that the processing companies supplied. Three packages were pooled to represent one retail basil sample.

Climatic parameters. Throughout the study, ambient temperatures were recorded in the field, processing facilities, and retail outlets with an ExtechEasyView hygro-thermometer EA25 (SKC, Johannesburg, South Africa). Temperatures of the water samples were documented with an infrared Extech thermometer (SKC).

Sample processing. Basil samples were aseptically cut into smaller pieces and weighed to make units of 10 and 25 g. The units were then placed in stomacher bags, and 90 ml of peptone buffered water (PBW; Merck, Johannesburg, South Africa) was added to each 10-g unit and 225 ml of PBW was added to each 25-g unit. The two bags of each sample were homogenized for 30 s at 230 rpm (Stomacher 400 Circulator, Lasec, Cape Town, South Africa). The swabs were removed from their holding tubes, placed in 9 ml of sterile PBW, and vortexed for 10 s. The 1-liter water samples were filtered through a Millipore filter (0.45-μm pore size; National Separation, Johannesburg, South Africa) using a vacuum pump (Balzers, Bingen, Germany), after which the membrane was transferred to 90 ml of PBW. The remaining three 100-ml water samples were used in three Colilert-18 tests (IDEXX, Westbrook, Maine) as specified by the manufacturer to indicate the presence of *E. coli* and coliforms. Samples in PBW were either serially diluted for quantitative purposes or incubated for 24 h at 37°C for detection of pathogens. For enumeration, 1 ml of the PBW culture was plated onto Petrifilm (*E. coli*/coliform count plate, 3M, Johannesburg, South Africa), and all plates were incubated for 24 h at 37°C. The number of colonies was used to determine the level of indicator bacteria in each sample. For the detection of pathogens, PBW samples were shake incubated at 37°C and 150 rpm for 18 to 24 h. Enriched samples were held at −80°C in 25% glycerol until needed.

MALDI-TOF-MS identification of coliforms. Dominant coliform colonies were isolated from Petrifilms, purified. and identified using matrix-assisted laser desorption ionization–time of flight–mass spectrometry (MALDI-TOF-MS; Bruker Daltonics, Bremen, Germany) as described below. A single colony was transferred with a toothpick onto MALDI plates in duplicate (Sigma-Aldrich, St. Louis, MO). The preparation was covered with 1 μl of cyano-4-hydroxycinnamic acid in an organic solution (50%

acetonitrile, 2.5% trifluoroacetic acid), crystallized by air drying at room temperature, and then screened. Spectra were recorded by Flex Control software (Bruker Daltonics) using the recommended instrument setting for bacterial identification. The peak lists generated were used for matches against the reference library. MALDI Biolayer 3.0 software (Bruker Daltonics) was used to analyze the raw spectra of the bacterial isolates with default settings and expressed the degree of spectral pattern matching as a logarithmic identification score.

PCR and sequencing. After enrichment, DNA extraction was conducted with all samples ($n = 463$) using a Quick-g DNA MiniPrep kit (Zymo Research Corp., Pretoria, South Africa), and DNA concentrations were measured using a Qubit 2.0 Fluorometer (Life Technologies, Johannesburg, South Africa). A duplex PCR was used for the detection of *E. coli* O157:H7 and *Salmonella* Typhimurium. Primers specific to each pathogen were selected. Universal primers used to amplify a 1,500-bp DNA fragment of the 16S rDNA gene served as an internal positive control for the PCR. The 25-μl reaction mixture included the following: 5.0 μl of My BioTaq reaction buffer (Bioline, London, UK), 0.3 μl of My BioTaq polymerase (5 U/μl; Bioline), sample DNA, and 0.3 μl of the primers at their optimal concentrations: 30 pmol of UidAaF (5′-GCG AAA ACT GTG GAA TTG GG-3′) and UidAbR (5′-TGA TGC TCC ATA ACT TCC TG-3′), 50 pmol of SLDF (5′-CCT GTG AAT GCC CTG ATG AT-3′) and SLDR (5′-TTG CCG GTG GTA CTG ATA GG-3′), and 10 pmol of 27F (5′-GAG TTT GAT CCT GGC TCA G-3′) and 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) (Intergrated DNA Technologies, Coralville, IA). A Mastercycler Pro thermocycler S (Eppendorf, Hamburg, Germany) was used with the following cycling conditions: initial denaturing at 95°C for 2 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 45 s, and extension at 72°C for 90 s, and a final extension at 72°C for 7 min. The PCR amplicon was visualized on a 2% (wt/vol) agarose gel containing 0.1% (vol/vol) ethidium bromide with a VilberLourmat Gel Documentation System (VilberLourmat, Marne La Valle, France), and digital images were captured with E-capt software (VilberLourmat).

For the samples that gave positive PCR results, 100 μl of the original enriched broth was placed in 9 ml of tryptone soy broth (TSB; Merck) and shake incubated (150 rpm) at 37°C. After 24 h, 100 μl of this culture was placed in 9 ml of Rappaport-Vassiliadis *Salmonella* enrichment broth (Merck) and shake incubated under the same conditions as for the TSB. After another 24 h, a single loop of culture was plated onto Brilliance *Salmonella* agar base (containing *Salmonella* selective supplement; Oxoid, Thermo Scientific, Johannesburg, South Africa) and incubated at 37°C for 24 h. Five single presumptive-positive colonies per plate were then placed in 9 ml of TSB and shake incubated at 37°C for 24 h. DNA was then extracted, and DNA concentrations were measured as before. A PCR assay was used for the detection of *Salmonella* Typhimurium, followed by gel electrophoresis. The amplified PCR products were purified from the gel using a Geneclean kit (Zymo Research), and the PCR products were sent for sequencing to an external laboratory (Inqaba Biotechnical Industries, Pretoria, South Africa). Homology studies were performed by comparison with the NCBI (Bethesda, MD) database for sequence similarity using the BLAST (Basic Local Alignment Search Tool) program.

Food safety risk assessment. A diagnostic tool, developed in the form of a questionnaire by Kirezieva et al. *(27),* was used to indicate the application of good agricultural practices in the separate production chains. The tool can be used to analyze factors such as system design, equipment, procedures, and the specific context of the production chain to indicate possible food safety risk areas. The questionnaire specifically deals with issues relating to (i) control (aimed at keeping product and process conditions within certain limits) and assurance (setting, evaluating, and modifying the system) activities and (ii) system output activities (measured by the performance of activities aimed at maximizing product safety). The control and assurance activities (indicated by a darker area on a graph) are assigned different levels to indicate the situation, where 1 is low, 2 is basic, 3 is average, and 4 is advanced. Level 1 indicates that the activity is not possible or not acceptable, whereas level 4 indicates that the activity is highly acceptable. The system output (indicated by lighter areas on a graph) is also assigned different levels, where 1 represents no output activities, 2 is poor, 3 is moderate, and 4 is good. Level 4 indicates that comprehensive external systems and structured and comprehensive sampling are used and that no safety concerns exist within the food safety management system (FSMS). For each of these aspects, feedback findings were formulated, and data were expressed in spider diagrams to reflect on effectiveness of the FSMS (questionnaire available as supplemental material, https://drive.google.com/file/d/0B4Rh9ySt3ik-ZzQycURwQ2g5Tkk/view?usp=sharing).

Statistical analysis. An investigation was conducted on the colony count in the production process of basil until the marketing stage. The input was two production companies (A and B) at different stages in the production process (including the time of day). The whole process was repeated four times. The colony counts were log transformed to $\log(x+1)$ before being subjected to an appropriate analysis of variance (ANOVA) of the company data combined. Replications over time were used as block replicates *(43)*. Two ANOVAs were performed, one with company and sample as variables and one with company and stage as variables. A Shapiro-Wilk test was performed with the standardized residuals to test for deviations from normality *(40)*. Student's *t* LSD (least significant difference) was calculated at a 5% significance level to compare means of significant source effects. All analyses were performed with SAS version 9.3 statistical software *(38)*. To test for a significant difference between the coliform colony counts and *Salmonella* presence or absence, a one-way ANOVA was performed on the transformed data using XLSTAT, an add-in Statistical package to Excel 2013 (Microsoft, Redmond, WA).

RESULTS

Indicator organisms (basil). Figure 1 shows the total number of coliforms recovered from basil samples at different stages in the production, processing, and retail line. The coliform counts on basil leaves for production chain 1 (2.6 to 4.0 log CFU/g) were significantly higher than those for production chain 2 (0.4 to 1.3 log CFU/g) on three occasions in samples collected from the field and the processing facility (basil arrival at the end of the day and basil ready for dispatch in the middle of the day). Total coliform counts were the highest at the production and dispatch stages (beginning of the day) for production chains 1 and 2, respectively. At the production stage, both companies had a higher ambient temperature (23.5 and 19.4°C) than at the point of entry (18.3 and 19.8°C) or dispatch (18.1 and 19.8°C) of the processing facility and at retail (17.9 and 19.8°C). An *E. coli* count of 4.1 log CFU/g was detected for only one basil sample obtained during the second visit at the processing facility of production chain 2. This sample was isolated from basil plants entering the

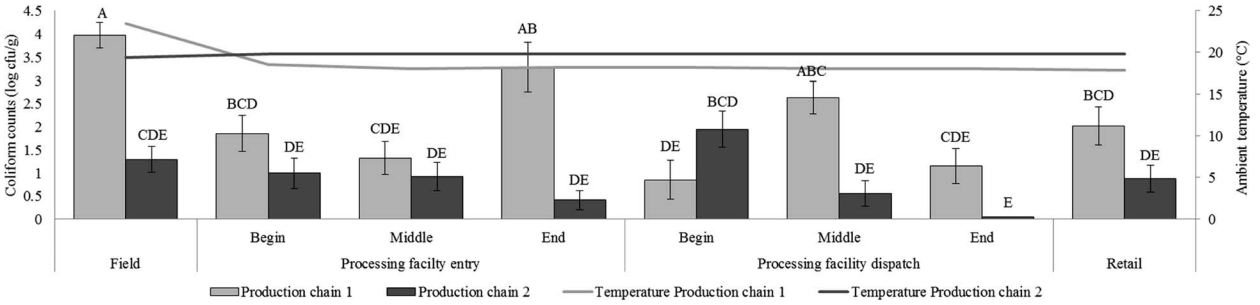

FIGURE 1. *Mean coliforms counts from 143 basil samples collected at different stages in the production chain of two companies. Bars with different capital letters indicate mean counts that are significantly different at the 5% level, whereas bars with the same capital letters indicate mean counts that are not significantly different.*

processing facility at the beginning of the day. No further *E. coli* was detected on basil throughout the study.

Dominant isolates (basil). Of the 73 dominant coliform isolates identified, 58.3% were *Acinetobacter calcoaceticus* and 41.7% were *Pantoea agglomerans*.

Indicator organisms (water). A comparison of the water samples analyzed with the Colilert-18 and membrane filter methods reveals a marked difference in *E. coli* and coliform levels. With the membrane filter method, no *E. coli* was detected in any water samples throughout the study. Overall, the total coliform counts were lower with the membrane filter method than with the Colilert-18 method. The average total coliform count (2.4 log CFU/ml) for production chain 1 was higher than that for production chain 2 (1.6 log CFU/ml). The counts in source and storage dam water in production chain 1 differed significantly ($P = 0.02$ and $P = 0.04$) from those in the source and storage water in production chain 2. For production chain 2, no coliforms were found in the source water throughout the study. The coliform count in irrigation water of production chain 1 was the same as that of production chain 2 (2.5 log CFU/ml). For the Colilert-18 method, *E. coli* counts remained constant for the source, storage dam, and irrigation system. Higher levels of *E. coli* were observed for production chain 1 than for production chain 2. however, total coliform counts for production chains 1 and 2 were within the same order of magnitude, except for the counts in the irrigation system of production chain 2, which were significantly higher. Water temperatures for production chain 1 were higher for all three water sources compared with production chain 2. The higher coliform counts were consistent with higher water temperatures throughout the study (Fig. 2).

Dominant isolates (water). Twelve dominant coliforms isolates were identified: 75% were *Klebsiella pneumoniae* subsp. *pneumoniae* and 25% were *Enterobacter aerogenes*.

Indicator organisms (contact surfaces). No *E. coli* was detected on any of the contact surfaces throughout the

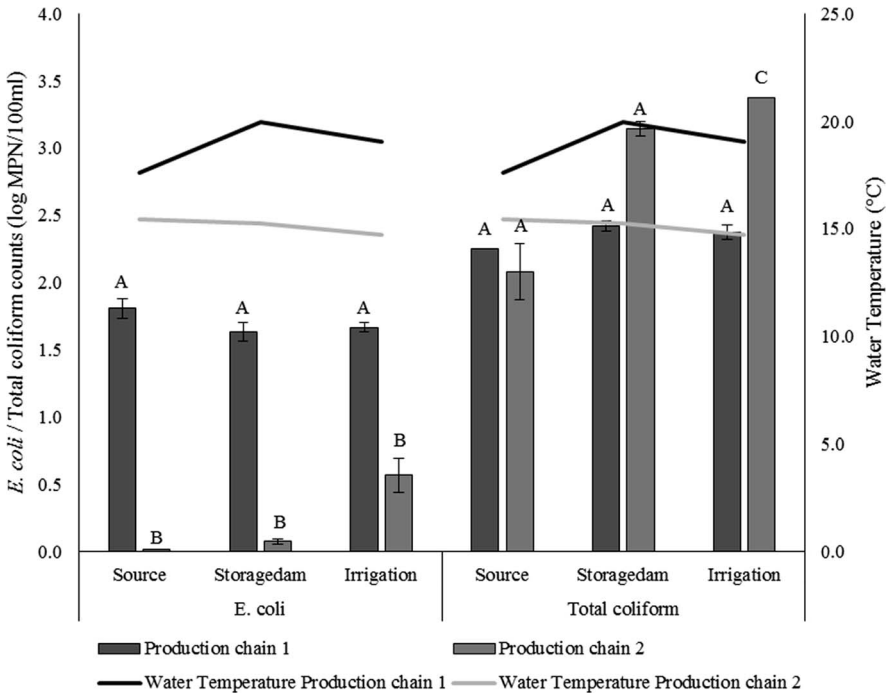

FIGURE 2. *Mean coliform counts from 65 water samples obtained during four visits to two basil producing companies (Colilert-18 method). Bars with different capital letters indicate mean counts that are significantly different at the 5% level, whereas bars with the same capital letters indicate mean counts that are not significantly different.*

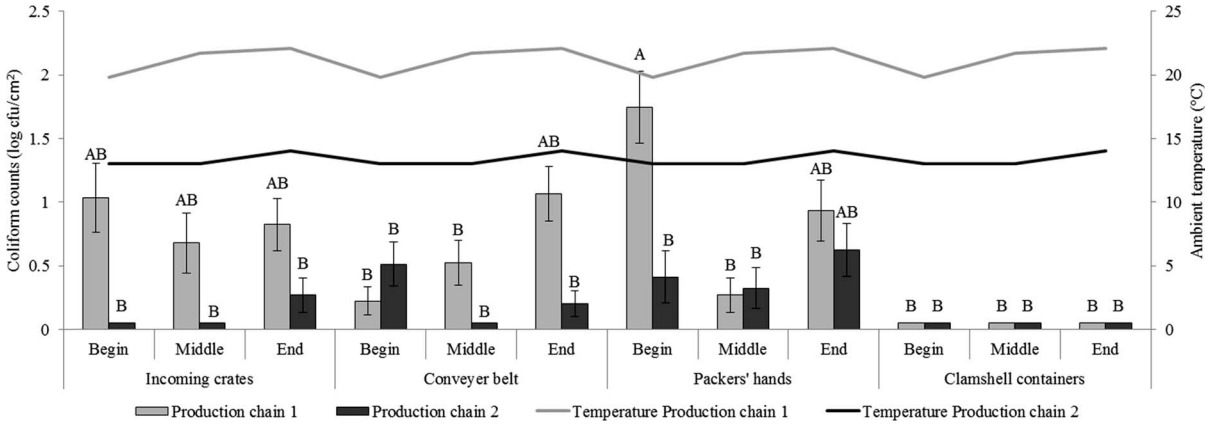

FIGURE 3. *Mean coliform counts from 162 contact surfaces samples (clamshell containers, incoming crates, and conveyer belts) and 54 samples from packers' hands at three different times (beginning, middle, and end of the day) at both basil producing companies. Bars with different capital letters indicate mean counts that are significantly different at the 5% level, whereas bars with the same capital letters indicate mean counts that are not significantly different.*

study. The highest coliform counts were found on the hands of basil pickers (1.7 log CFU/cm^2) (data not shown) and packers (1.7 log CFU/cm^2) and on the conveyer belts (1.3 log CFU/cm^2) of both production chains. No coliform bacteria were detected on the clamshell containers throughout the study. For production chain 1, coliform counts on the conveyer belts increased as the processing day progressed. Counts on the packers' hands decreased from the beginning of the day (1.7 log CFU/cm^2) to the middle of the day (0.3 log CFU/cm^2) but then increased toward the end of the day (0.9 log CFU/cm^2). For production chain 2, at the beginning of the day coliforms were found on only the conveyer belt and packers' hands (0.5 and 0.4 log CFU/cm^2, respectively). In the middle of the day, coliform counts on the packers' hands were 0.3 log CFU/ cm^2. At the end of the day, coliform counts were 0.3 log CFU/cm^2 on the incoming crates, 0.2 log CFU/cm^2 on the conveyer belts, and 0.6 log CFU/cm^2 on packers' hands. For production chain 1, the ambient temperatures (19.8 to 22.6°C) and coliform counts (0.2 to 1.7 log CFU/cm^2) were higher than those for production chain 2 (13.0 to 15.0°C and 0.2 to 0.6 log CFU/ cm^2, respectively) (Fig. 3).

Dominant isolates (contact surfaces). Thirty-one dominant coliform isolates were identified: 71.4% were *P. agglomerans* and 28.6% were *Lysinibacillus fusiformis*.

Prevalence of *Salmonella* Typhimurium on fresh basil. *Salmonella* Typhimurium was detected in 4 (0.9%) of the 463 samples collected throughout the production chains (farm to retail). The four contaminated samples were all from basil that originated from production chain 1. Two of the samples were isolated from basil collected at the end of the day in the processing facility (third visit). One of these two samples was obtained from the basil entering the packing line, and the other was obtained from basil at dispatch. The other two basil samples contaminated with *Salmonella* Typhimurium came from basil entering the packing line at the beginning of the day on the fourth visit. A BLAST search for the sequences of the PCR products obtained from these four positive isolates confirmed homology to *Salmonella enterica* subsp. *enterica* serovar Typhimurium.

***Salmonella* Typhimurium prevalence and coliform levels.** The 143 basil samples were analyzed to determine any significant association between total coliform counts and the presence or absence of *Salmonella* Typhimurium. Results revealed that total coliform level was not an indication of the presence of *Salmonella* Typhimurium; *Salmonella* Typhimurium was absent when the average total coliform count was 1.6 log CFU/g and present when no coliforms were detected ($P < 0.001$) (Fig. 4).

Food safety risk assessment. The diagnostic tool assessment was completed for both companies, and the possible risk areas were identified. Both companies were assigned level 3 for the microbial risk of initial material, microbial risk of final products, FSMS evaluation, and microbiological complaints. Production chain 1 had a score

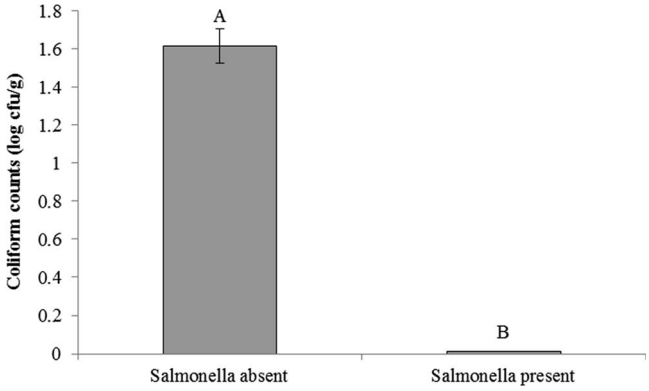

FIGURE 4. *Mean coliform counts from 143 basil samples compared with presence or absence of* Salmonella *Typhimurium in the basil supply chain. Bars with different capital letters indicate mean counts that are significantly different at the 5% level, whereas bars with the same capital letters indicate mean counts that are not significantly different.*

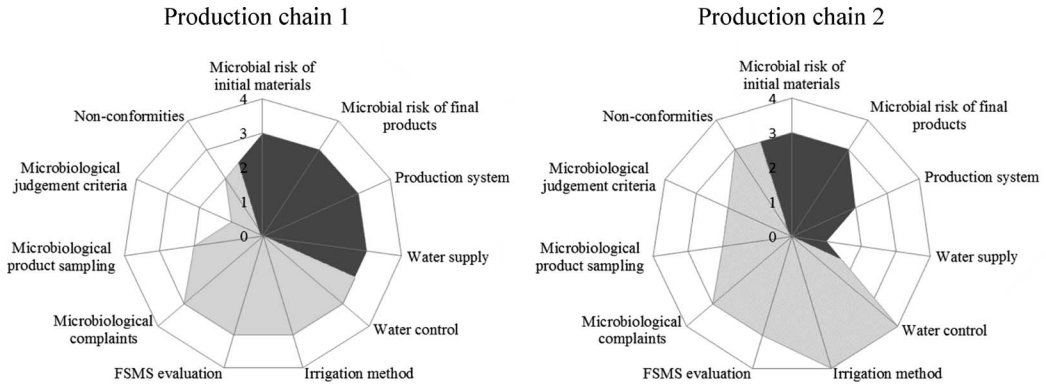

FIGURE 5. *Diagnostic tool for food safety compliance in two basil producing companies, with scores from 0 to 4. Dark shading indicates more risky food safety environment, and light shading indicates a better systems output for the current food safety management system.*

of 3 for the water supply, water control, and irrigation method, whereas production chain 2 had a score of 1 for the water supply and a score of 4 for water control and irrigation method. Production chain 2 had a score of 2 for the production system, microbiological product sampling, and microbiological judgment criteria. However, production chain 1 had a score of 3 for the production system, a score of 2 for the microbiological product sampling, and a score of 1 for the microbiological judgment criteria. For the nonconformities, production chain 1 had a score of 2 and production chain 2 had a score of level 3 (Fig. 5).

DISCUSSION

To the best of our knowledge, this is the first study in which the microbiological quality of basil from production, through processing, and up to the retail stage was assessed. No *E. coli* O157:H7 was detected in any of the 463 samples analyzed in this study, but 4 (0.9%) of the samples contained *Salmonella* Typhimurium. *Salmonella* is commonly associated with the intestines of humans and animals, and its presence on basil may indicate fecal contamination from an external source. *Salmonella* has been the cause of foodborne disease outbreaks associated with lettuce, cilantro, basil, and parsley *(8, 13, 23, 29, 46)*; however, the only foodborne outbreak of *Salmonella* infection reported for basil was caused by *Salmonella* Senftenberg *(15, 37)*. In South Africa, outbreaks of foodborne disease in humans are common but markedly underreported *(42)*. Eleven percent of mortality in children below age 5 years are caused by diarrheal diseases *(5),* and *Salmonella* Typhimurium is the second most common *Salmonella* serotype isolated in South Africa *(19)*.

The basil samples that contained *Salmonella* Typhimurium all originated from the processing facility of production chain 1. Limitations in the sampling size of this study did not allow establishment of a direct link between the contaminated samples and the source of contamination. However, one of the main differences between production chains 1 and 2 was the irrigation water type, application, and storage method. The uncovered water source and storage facility of production chain 1 could have been sources of *Salmonella* contamination, as suggested by the higher *E. coli* counts in water samples from these sources. The open water storage facility of production chain 1 was in close proximity to a sheep paddock. Previous studies have revealed the association between livestock, surface runoff, and fecal contamination of water sources *(2, 30)*. The presence of farm animals has been linked to the deterioration of the microbial quality of surface waters in general *(41)*. Production chain 1 also made use of overhead irrigation, which can contribute to contamination due to splash dispersal. Markland et al. *(31)* compared drip and overhead irrigation and found that the drip method reduces the risk of contamination because less water comes into direct contact with the edible parts of the plant. Fresh water in South Africa is limited and mainly originates from rivers and impoundments, which can be contaminated with waterborne pathogens *(1, 10, 48)*. This information is correlated with the water supply and water control scores assigned to each company in the diagnostic tool assessment.

In the present study, *E. coli* was detected only in water samples analyzed with the Colilert-18 method; no *E. coli* was isolated with the membrane filter method. Higher levels of coliforms were also observed with the Colilert-18 method than with the membrane filter method. For testing fresh water, results of several studies support the idea that the Colilert method is a more accurate detection system for coliforms *(9)* because it can be used to precisely quantify low and high bacterial levels *(6)*. Solo-Gabriele et al. *(44)* found that coliform and *E. coli* levels were lower as determined with the membrane filter method during dry times when the microorganisms are stressed and presumably nonculturable. Our results, coupled with the findings of these authors, suggest that the Colilert-18 method should be favored over the membrane filter method when testing agricultural waters.

The coliform levels in production chain 1 were generally higher than those in production chain 2, a difference that may be attributed to higher ambient and water temperatures. Isobe et al. *(25)* found a positive correlation between the water temperature and the level of indicator bacteria present in a sample. Several other researchers have similarly reported that the growth and survival of indicator bacteria are sensitive to environmental factors, such as temperature and sunlight *(44)*.

Analysis of the nonconformities for each production chain with the diagnostic tool revealed that production chain 1 had more nonconformities than production chain 2.

Nonconformities exist when initial materials, intermediate products, or the final product do not comply with standards set for microbial hygiene indicators or when foodborne pathogens are detected. The presence of *Salmonella* Typhimurium on basil and higher total coliform counts throughout production chain 1 reflect the finding that this production chain had less control over the microbiological status of the processing facility. Contact surfaces and water may be sources of bacterial pathogen contamination *(4)*. Some of the coliforms identified are part of the normal flora of the human body and soil, such as *K. pneumoniae* subsp *pneumoniae, A. calcoaceticus,* and *E. aerogenes,* but these coliforms can also cause opportunistic infections. *E. aerogenes* lives in the intestines of animals, and its presence on basil leaves and contact surfaces likely indicates that fecal contamination had occurred during the handling of the product *(16)*.

Our study conclusively showed that the association between total coliform counts and the presence of *Salmonella* Typhimurium is weak. Our observation supports findings of others, who have reported detection of *Salmonella* in the absence of indicator bacteria *(18, 33)*. Efstratiou et al. *(14)* found that this relationship depends on the level of pollution. In areas of moderate pollution, a stronger association exists between *Salmonella* and total coliforms, but this association is weaker in highly polluted areas. The limitations of using coliforms as contamination indicators *(14)* has been widely debated *(34, 35)*. Although total coliform counts are commonly used as an indicator of the sanitary quality of water and food, the persistence of these indicator bacteria and pathogens in naturally variable aquatic environments can differ greatly *(32)*. An effective indicator system must be ubiquitous in nature, and the pathogenic organisms should be easily cultured, identified, and quantified *(7, 44)*.

Microbiological contamination of basil can be prevented from the start of the production line to the retail stage by implementing good agricultural practices *(49),* and the effectiveness of these practices can be determined by analyzing critical sampling locations. These sampling locations are selected based on potential risk factors that contribute to the microbiological contamination of fresh produce and that have been identified through literature searches *(11)*. Participants in production chains can use the information gained from sampling to assess their systems and position themselves in the market. This approach also highlights the importance of having a proper audit conducted by an external entity. There are inherent limitations in sampling schemes due to financial and time constraints, microbiological variability found in natural systems, and the low prevalence of pathogenic organisms. In this study we demonstrated the value of using several support systems to manage product safety. For optimum functionality, analysis of critical sampling locations should be complemented with use of the diagnostic tool and regular water testing. Growers should give careful consideration to their choice of water source *(11),* especially in South Africa where water is scarce and often below acceptable quality due to inadequate sanitation and waste-removal practices, sewage spills, storm water runoff, and the activities of human settlements *(10)*.

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