Evaluation of Detection Tool for Some Bacterial Pathogens from Chicken Meat Production Line

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ABSTRACT

Foodborne microorganisms can trigger severe outbreaks. Rapid detection is essential for preventing the spread of disease before it causes an outbreak. This study investigates various laboratory techniques, including conventional detection, immunoassay using VIDAS, and automated identification systems using BD Phoenix M50. It targets the four most prevalent foodborne pathogens: Staphylococcus aureus, Shigella spp., and Salmonella spp., along with Campylobacter spp., by analyzing 174 samples of poultry. The SPSS statistical analysis system was used for every study. Staphylococcus aureus was found in 20.7% of samples using conventional detection and automated system, whereas it was presented in 25.3% of samples using the immunoassay technique. For Shigella spp. Shigella flexneri was identified by Phoenix M50 in 4% of samples similarly it was detected using conventional methods. In contrast, 33.3% of Salmonella spp. were detected using VIDAS, 28.7% were detected using the conventional method, 70% were confirmed to be Salmonella enterica, and 30% were confirmed to be other Salmonella spp. For Campylobacter spp. Campylobacter jejuni was identified in 9.8% of samples by conventional detection and 29.3% by immunoassay. The turn-around time for Shigella and Salmonella spp. was decreased by BD Phoenix M50 in comparison to conventional methods, whereas it was shorter for Staphylococcus aureus detection using a conventional method.

Keywords: *Conventional detection, Poultry slaughterhouse, Staphylococcus, Salmonella, Shigella, Campylobacter*

INTRODUCTION

The global production and consumption of poultry meat have increased significantly and are projected to continue to rise in the future (**Cavani et al., 2009**). Microorganisms, primarily bacteria, are inhabitants in the intestines and skin of the human body and in plants. Most of these microorganisms are nonpathogenic and some could be beneficial to the body in many ways. However, there are numerous pathogenic microorganisms, including bacteria, fungi, and viruses. The digestive system is a way that pathogens enter the body and cause various foodborne diseases (**Rejab et al., 2011**).

Foodborne pathogens can enter the body through contaminated and raw foods. Therefore, it is important to detect pathogens in food and water before they enter the body and cause an outbreak of these diseases. The majority of such pathogens include *Listeria monocytogenes*, Shiga toxin-producing *Escherichia coli*, *Salmonella* spp., *Campylobacter jejuni*, *Cronobacter sakazakii*, *Vibrio parahaemolyticus*, *Bacillus cereus*, and *Clostridium perfringens* **(Marshall and Levy 2011).**

It has been observed that foodborne pathogens can cause severe disease outbreaks regardless of region or age, properly infants and elderly people being the most susceptible ones. Therefore, rapid detection is necessary to prevent the disease from spreading and causing a serious outbreak. However, there is a discrepancy between the reference methods used to detect microbes in various foods, as well as a significant development in various rapid techniques in foodborne pathogen detection.

Recently, the poultry market has undergone a dramatic transformation, shifting from a nearly whole-bird product to modern, highly diversified manufacturing centered on cold cuts, deboned meat, and ready-to-eat processed products. This caused a substantial change in quality expectations. Between 1961 and 2020, global poultry meat production increased from 9 to 133 million tons to meet rising demand, while egg production increased from 15 to 93 million tons. In 2020, nearly 40% of global meat production was poultry meat. The global egg supply has increased by

150% over the past three decades. Most of these increases occurred in Asia, where production nearly quadrupled **(USDA 2022).**

A clear distinction is emerging between large and medium-sized industrialized production systems that feed into integrated value chains and extensive production systems that support livelihoods and supply local or niche markets. The primary function of the former is to provide inexpensive and safe food to populations located far from the source of supply, while the latter serves as a safety net for livelihoods, often as part of a diverse portfolio of income sources (**Vesper et al., 2016**).

Traditional small-scale, rural, family-based poultry systems continue to play a vital role in sustaining livelihoods in developing nations, supplying poultry products to rural areas, and providing crucial support to women farmers. Thus, as long as rural poverty exists, small-scale poultry production will continue to provide opportunities for income generation and food quality (**USDA 2022**).

The global egg supply has increased by 150% over the past three decades. Most of this growth has occurred in Asia, where production has nearly quadrupled. Approximately 80% of rural households in developing countries raise poultry (**Akinola and Essien 2011**).

Meanwhile, there are various available laboratory methods for measuring the quality of poultry products, each of which is based on a distinct test principle or instrument. Given the complexity of poultry processing after slaughter and quality trait determination, it is not surprising that laboratory results do not always coincide. For the evaluation of results, it is necessary to adhere to measurable specifications; hence, standardization of testing is essential. Thus, laboratory result comparability can be achieved by establishing metrological traceability. This ensures that measurement procedures that measure the same quantity and the calibration of measurement procedures are traceable to a common reference method consisting of reference materials and methods. The application of non-commutable reference materials or procedures will yield inaccurate results. Although standardization verifies traceability to the international system of units,

harmonization verifies traceability to an agreed-upon reference method (**Vesper et al., 2016**).

Therefore, efforts to enhance, develop, and evaluate detection methods are ongoing. This study aims to provide a comprehensive understanding of the available techniques for the detection of foodborne pathogens and to select the most suitable ones for implementing the traceability process in the food production chain.

MATERIALS AND METHODS

Sample Collection and Preparation

From March to June 2022, 174 samples were collected from two semiautomated poultry slaughterhouses in Egypt. Each set is associated with a particular poultry farm. Six critical control points (CCPs) have been defined **(Table 1)** and several types of microbial samples, including chicken feces, cages and table swabs, chicken parts, and carcasses from these points were collected. Each sample weighed approximately 100 g and was collected from the entire supply chain, from the receiving area to the final product; all samples were transported to the processing laboratory at 4°C.

Samples Pre-enrichment, Inoculation, and Incubation

For *Salmonella* spp. pre-enrichment, 225 mL of buffered peptone water (BPW) was prepared according to ISO 6579-2017 **Adeyanju and Ishola (2014**), inoculated 25 g of the tested sample, and incubated at 37°C for 24h. In terms of *Shigella* spp. (**Shad and Shad 2021**) the Shigella broth used for pre-enrichment was inoculated with 1 g of the tested sample portion at a dilution of 1:10, then incubated for 24h in anaerobic conditions at 41.5°C. In accordance with ISO 6888-2 (2021), ten grams of sample was diluted in 90 mL of diluent water containing BPW supplemented with sodium chloride (Diluent media). From this mixture, 0.1 mL was transferred to the supplemented Baird Parker enrichment (HiMedia, USA) medium (1:10), which was then incubated at 37°C for 24 and 48h. Concerning *Campylobacter* spp. the supplemented Bolton broth (1:10) was used for pre-enrichment in accordance with **ISO 10272** (2017) and incubated at 37°C for 4h and

then at 41.5°C for 48h. This culture was streaked onto Skirrow agar (Sigma Aldrich, USA) and incubated at 42°C for 48h.

Laboratory Detection Methods

Conventional Identification

In this method, the detection of pathogens frequently depends on the precise identification using microbiological and biochemical elements (**Velusamy et al., 2010**). Several types of selective and differential media were used based on the targeted bacteria including MacConkey agar, and Hektoen agar, followed by Gram stain, spot testing, and biochemical analysis.

Aerobic Plate Count (APC)

Plate count agar is a nonselective medium used in accordance with **ISO 4833** (2013) in this section. In order to obtain a homogenous suspension, each sample was added to Diluent media (1:10 m/v) in a Stomacher-type bag and blended at 260 rpm for 3 min. Then, until the fifth dilution, each diluted sample was serially diluted 10-folds into 9 mL of Diluent media. Subsequently, 1 mL of the test sample dilutions (3, 4, and 5) were transferred into a sterile petri dish, followed by the addition of 30 mL of APC media and incubated at 37°C for 24h (NFSA 2021).

Automated Identification System

(BD Phoenix M50, USA) BD Gram-negative (NID-448007) and positive panels (PID-448008) were used. The system identified the bacteria in less than 4h.

Immunoassay Detection

A Biomerieux automated Vitek Immuno-Diagnostic Assay System (VIDAS system, USA) portion of the enrichment broth was dispensed into the reagent strip, then the identification process completely runs automatically. Multiple times, the enrichment broth is cycled in and out of the solid phase receptacle device.

Statistical Analysis

All turn – around - time (TAT) calculations were subjected to statistical analysis to determine the variation for each detection method within the targeted microorganism. In order to compare the various detection methods, the analysis of variance compares group mean values. It was deemed statistically significant if $P < 0.05$. All analyses were conducted utilizing the SPSS

statistical analysis system (**Abo-Allam 2003**).

RESULTS AND DISCUSSION

The samples were regrouped into four groups as follows:

Group 1: Chicken thigh meat, Group 2: Chicken breast meat, Group 3: Chicken feces, and group 4: Other poultry parts, such as chicken liver and gizzard, as well as water for rinsing and table swabs.

Bacterial Count Analysis

Notably, in the first slaughterhouse, the fourth group contained the most Colony forming units (CFUs), while the second group contained the fewest CFUs. In general, the average number of CFU was nearly identical across all groups, and the mean was 23, 11, 19, and 29 For Group 1, Group 2, Group 3, and Group 4, respectively.

In present observations, in the second slaughterhouse, the APC results were nearly identical and followed the same pattern, albeit with a smaller proportion of CFUs. Group 4 contained the highest number of CFUs, while Group 2 contained the least number of CFUs. In general, the average number of CFU was almost the same in all groups, and the mean was 17, 3, 14, and 23 For Group 1, Group 2, Group 3, and Group 4, respectively.

Microorganisms Detected by Conventional Methods and Confirmed by Automated System

The total number of isolates detected by conventional methods for *S. aureus*, *Shigella* spp., *Salmonella* spp., and *Campylobacter* spp. 36 (20.7%), 7 (4%), 50 (28.7%), and 17 (9.8%) detection from both sites, respectively **(Fig 1),** and the same number was confirmed by the Phoenix M50. The automated system does not have the capability to identify *Campylobacter* spp.

Microorganisms Detected by Immunoassay Method

The total isolates for all organisms studied (*S. aureus*, *Salmonella* spp., and *Campylobacter* spp.) were as follows: 44 (25.3%), 58 (33.3%), and 51 (29.3%), respectively **(Fig 1)**. The VIDAS system is not competent to detect *Shigella* spp.

Bacterial Detection Across the Critical Control Points (CCPs) with Method Comparisons

S. aureus was detected by all laboratory methods at the majority of CCPs for both sites **(Fig 2)**. Using a conventional identification method, *Shigella* spp. was detected predominantly in CCP 1, 3, and 4 for the first site but only in CCP 3 and 6 for the second site. The automated system verified that all seven isolates were *S. flexneri* **(Fig 3).** All laboratory methods detected *Salmonella* spp. at the majority of CCPs at both locations. The automated system confirmed that 70% of the isolated *Salmonella* spp. was *S. enterica* and was resistant to antibiotics, and the rest required an additional serotyping test to identify their serotypes **(Fig 4)**. *Campylobacter* spp. was detected by all laboratory methods at the majority of CCPs for both sites **(Fig 5)**. Among the CCPs, the first site has the highest number of detections for all bacterial pathogens studied. The study's findings were consistent with many previous studies, including those by **Ahmed et al., (2014); Jackson et al., (2013); Monaco et al., (2013).**

Other Important Studied Factors

Turnaround time is an important factor to consider in this study, as it is one of the most obvious indicators of laboratory performance and one of the most visible indicators of laboratory services **(Table 2)**. The average plate counting test time per sample, including incubation and plate reading of the three plates, was 24.25h. In general, *Salmonella* spp. detection was the conventional method with the longest TAT average (96 h), *Shigella* spp. (72 h) then *Campylobacter* spp. (53 h), and the shortest procedure was *S. aureus* detection. (24.7 h). The automated system significantly reduced the time required to detect *Salmonella* spp. and *Shigella* spp. 76 and 52 h, respectively. While the automated system lengthened the time required to detect *S. aureus* is 28 hours old. In comparison to the conventional method, these differences in TAT were statistically significant at $P < 0.05$. The TAT for *Salmonella spp.* and *S. aureus* using the immunoassay method ranged between 48.25, 25, and 24.25 min, for *Campylobacter* spp., respectively. In the interim, the method of automated identification was the most expensive, followed by the immunoassay method. Conventional detection was the cheapest method, but it took the longest detection time compared to

other methods. Consequently, national control strategies are required to prevent the spread of bacterial pollutants in all phases of poultry production through the use of mechanical controls and/or the addition of natural substances to improve sanitation.

CONCLUSIONS

Indicators for laboratory methods performance and outcome measurements of the study recommend the use of the conventional detection method combined with a rapid confirmation device (BD Phoenix M50) for bacterial detection. Which increases the test's accuracy, sensitivity, and specificity with minimal laboratory worker intervention and reduces the detection time. This harmonization between the two methods will result in a higher detection rate for bacterial contaminants that affect poultry products. Certainly, national control strategies are needed to avoid the spreading of pathogenic bacteria in all poultry production phases using either mechanical controls and/or adding natural substances at a certain point to improve sanitation. Consciously, more comprehensive studies are needed to better determine the prevalence and hazards of poultry products' pollutants and using of natural substances such as herbs and spices' essential oil. As well as evaluate the accumulation effect of adding these substances to poultry products.

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Table 1. Slaughterhouses sampling distribution

Sampling area	Sampling points description	Type of collected sample	Number of collected samples	
			Site 1	Site 2
CCP 1	Receiving the live bird area.	Chicken feces and cages swabs	13	11
CCP 2	Scalding, de-feathering and leg separation area.	Rinsing water and table swabs	7	10
CCP 3	Evisceration, head and feet removal area.	Parts	14	12
CCP 4	Liver and gizzard collection and processing area.	Liver and gizzard	11	9
CCP 5	Monitoring the carcasses, and other parts for fecal contamination, washing, and pre-chilling area.	Parts	26	20
CCP 6	Final product	Parts	20	21
Total collected samples			91	83
			174	

Table 2. Average turn-around-time for each microorganism and methodology in the laboratory

Microorganism	Conventional identification (h)	Automated system identification (h)	Immunological identification (h)	LSD
S. aureus	24.66[c]	28.02[a]	25.01[b]	0.02
***Salmonella* spp.**	96.05[a]	75.98[b]	48.09[c]	0.02
***Shigella* spp.**	72.04[a]	52[b]	NA	0.02
***Campylobacter* spp.**	53.05[a]	NA	24.24[b]	0.01

Means in the same row with the same letter (a, b, c) are not significantly different at $P < 0.05$
LSD: least significant difference
NA: Not applicable

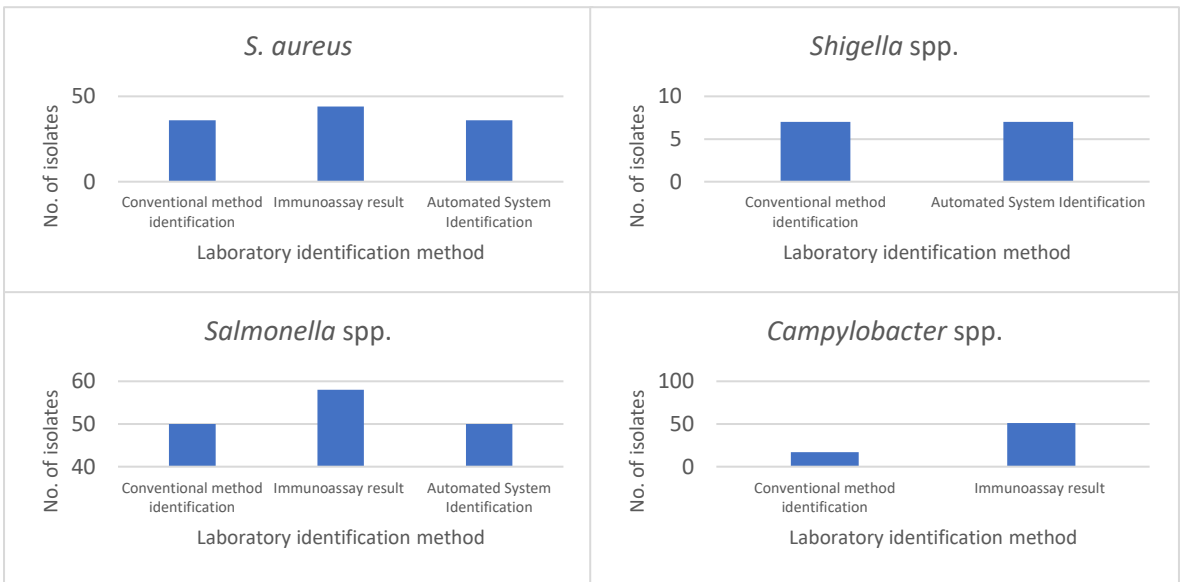

Fig 1. *The total number of detected pathogens by different methodologies*

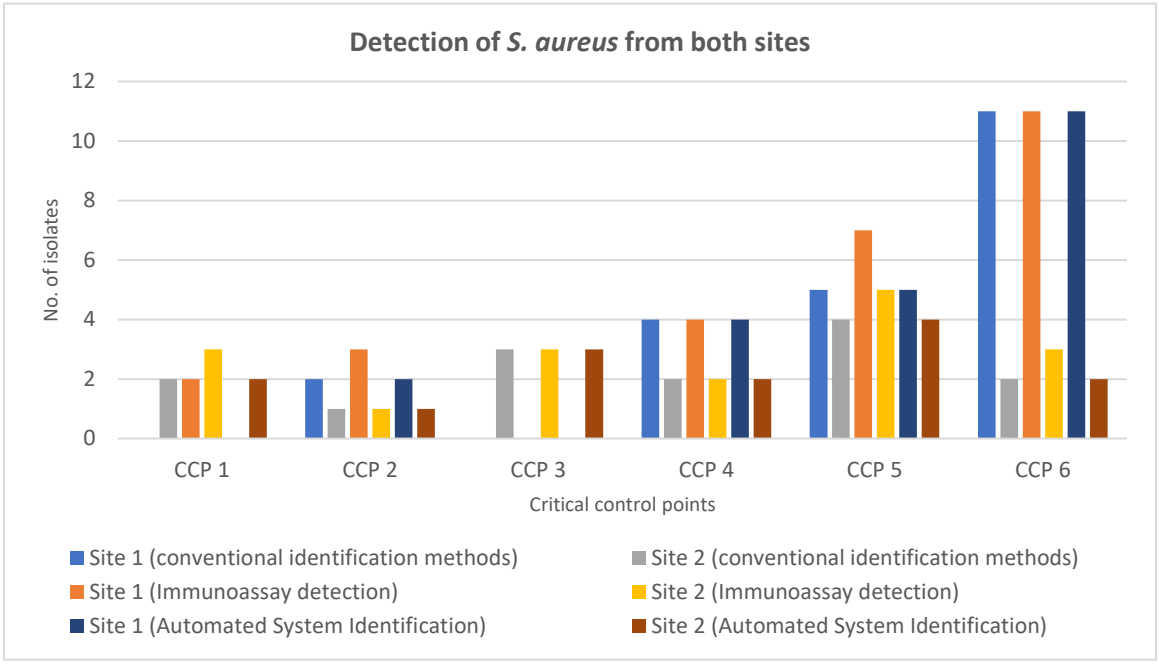

Fig 2. *Detection of S. aureus among the critical control points in both sites*

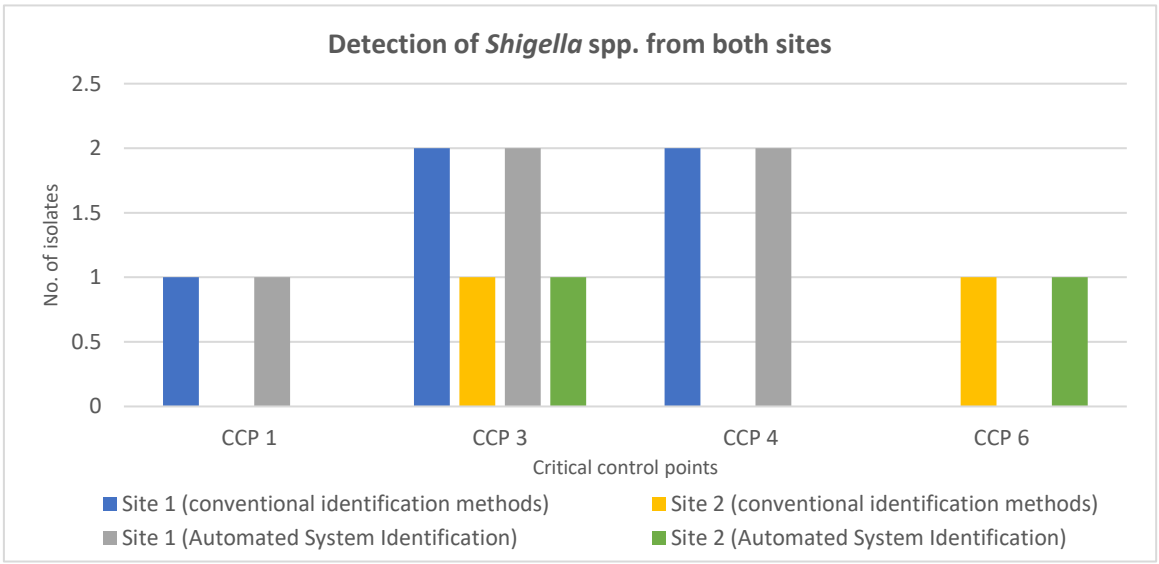

Fig 3. *Detection of Shigella spp. among the critical control points in both sites*

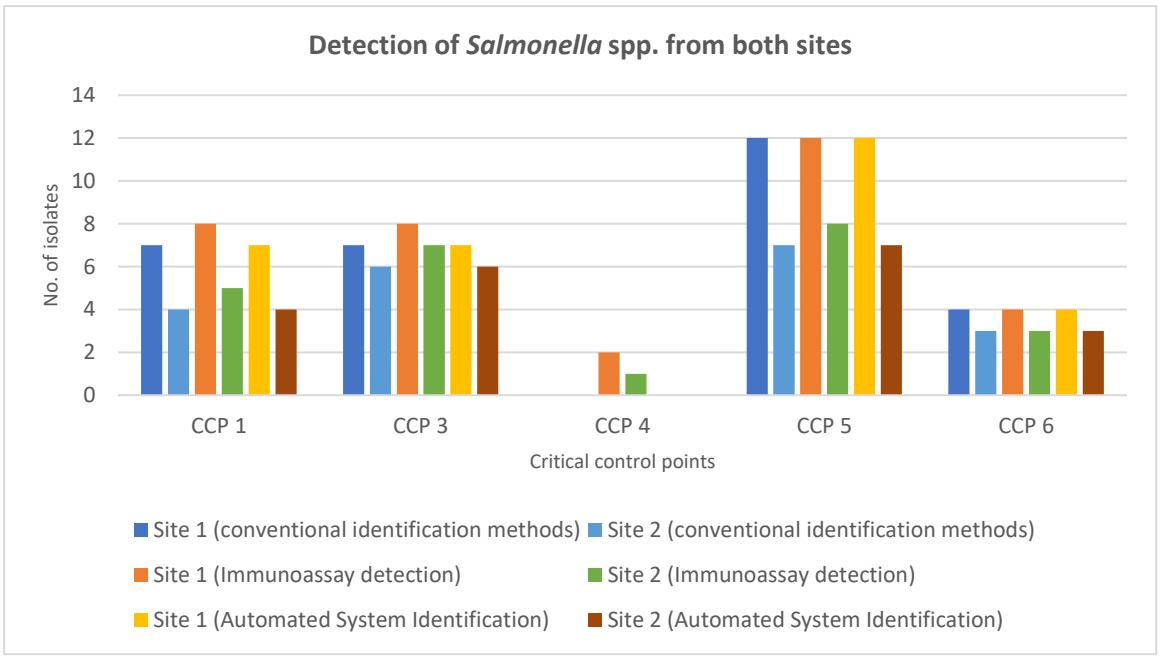

Fig 4. *Detection of Salmonella spp. among the critical control points in both sites*

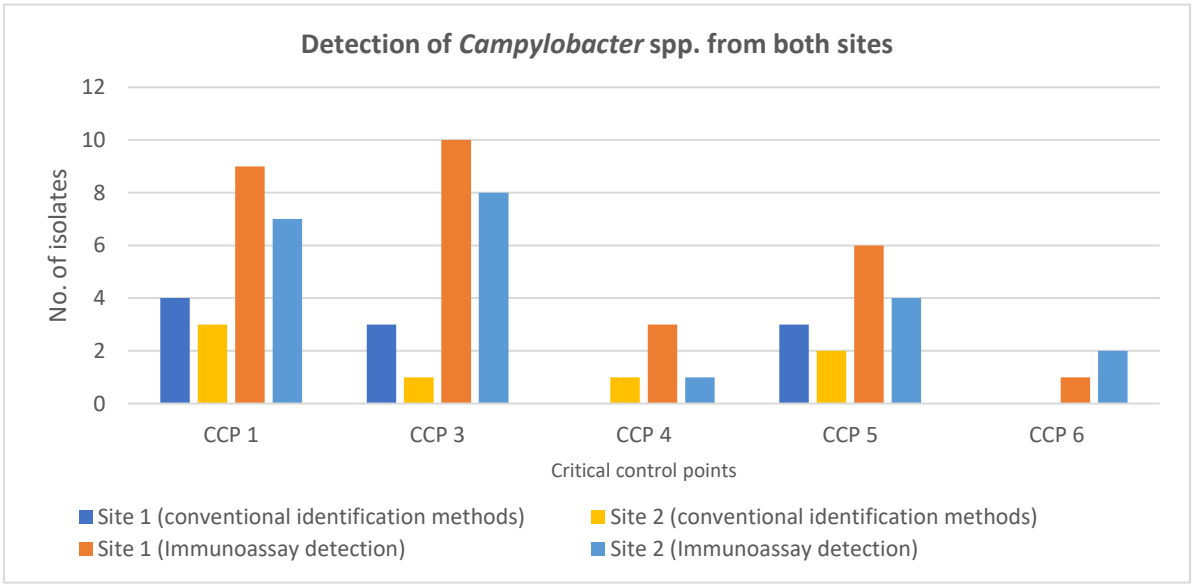

Fig 5. *Detection of Campylobacter spp. among the critical control points in both sites.*

تقييم طرق الكشف المعملية عن بعض مسببات الأمراض البكتيرية فى خط إنتاج لحوم الدواجن

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الملخص العربى

يمكن أن تؤدي الكائنات الحية الدقيقة التي تنتقل عن طريق الأغذية إلى تفشي الأوبئة الشديدة. يعد الاكتشاف السريع ضروريًا لمنع انتشار المرض. تبحث هذه الدراسة في التقنيات المعملية المختلفة، بما في ذلك الكشف التقليدي والتقنية المناعية باستخدام VIDAS وأنظمة التعريف الآلية باستخدام .BD Phoenix M50 وتم أستهداف أربعة مسببات للأمراض التي تنتقل عن طريق الأغذية والأكثر انتشارًا Staphylococcus aureus و .Shigella spp و .Salmonella spp إلى جانب .Campylobacter spp وذلك من خلال تحليل 174 عينة من الدواجن. تم استخدام نظام التحليل الإحصائي (SPSS) لكل الدراسة. وتم العثور على Staphylococcus aureus في 20.7٪ من العينات باستخدام نظام الكشف التقليدي والنظام التعريف الآلي ، بينما تم الكشف عنها في 25.3٪ من العينات باستخدام التقنية المناعية. بالنسبة إلى .Shigella spp تم التعرف على Shigella flexneri بواسطة Phoenix M50 في 4٪ من العينات وبالمثل تم اكتشافها باستخدام الطرق التقليدية. في المقابل 33.3٪ من .Salmonella spp باستخدام VIDAS ، تم الكشف عن 28.7٪ بالطريقة التقليدية ، 70٪ من العزلات تم التأكد من أنها Salmonella enterica و 30٪ تم التأكد من أنها بكتيريا .Salmonella spp والتى تحتاج إلى عمل تأكيد سيرولوجى. تم التعرف على Campylobacter jejuni في 9.8٪ من العينات بطرق الكشف التقليدية و 29.3٪ بالتقنية المناعية. وكذلك تم تقليل الوقت المتغرق للكشف عن .Salmonella spp و .Shigella spp بواسطة جهاز BD Phoenix M50 مقارنة بالطرق التقليدية ، بينما كانت الطرق التقليدية أقصر عن غيرها في الكشف عن .Staphylococcus aureus

الكلمات المفتاحية:

طرق الكشف التقليدية – مجازر الدواجن – سلالات من البكتيريا الممرضة