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Traditional detection and molecular characterization of salmonella pullorum in broiler chicken in Babylon city- Iraq

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Abstract
Pullorum disease (PD) is one of the most frequent diseases in the world, and it can have terrible effects. There have been money losses in the chicken business. Salmonella enteric subspecies of serovar Gallinarum biovar pullorum causes it. To be sure, you need to culture the bacteria, do biochemical study, and serotype them. The goal of this study was to confirm the presence of bacteria through culture, biochemical and VITEK2 system characterisation, and 16S rRNA gene detection by PCR assay. A total of 100 samples were collected from grill chicken flocks of different ages in 5 districts, including 51 cloacal swabs, 29 visceral organs, and 20 droppings. Sixty-three percent of the samples were found to contain Salmonella colonies, with a higher frequency in visceral organs compared to droppings and cloacal swabs. The samples were identified using selective culture broth and agar with biochemical descriptions. Phylogenetic studies have confirmed the existence of Salmonella pullorum in grill chickens raised in Babylon City, Iraq, according to the latest findings in molecular and genetic research. Twenty isolates, or 100%, of this strain were confirmed using the conventional PCR 16SrRNA gene at 1500 bp. Salmonella pullorum was found in grill flocks in this investigation, which raises concerns about the potential health consequences to other birds that are free-range and not affected.

Keywords: Salmonella, broiler, chicken, 16SrRNA, PCR

Introduction
One of the world's most important food and money generators comes from poultry, the biggest segment of the livestock industry. When compared to other livestock animals, it is crucial for a country's economy that it is cheap, manageable, protein rich, and grows quickly **(**Abdela and Markos; Sulieman). Nevertheless, its global development is impeded by a number of obstacles, including ineffective management, illness prevalence, vaccine ineffectiveness, and limited financial resources. The bulk of the approximately 2600 Salmonella serotypes can adapt to different hosts, one of which is humans. Pathogens such as S. pullorum, which causes pullorum disease (PD), and Sallmonella gallinarum, which causes fowl typhoid (FT), infect both young and adult chicks, respectively, and are rarely associated with human infections (Sarba). In chicks that are two weeks old, the mortality rate in PD can reach 90% (Kebede *et al*., 2016). Contact with contaminated poultry, whether through respiratory or faecal means, can cause PD to spread from person to person. Disease control becomes more complicated when vertical transmission occurs. Sub-clinically infected hens can transmit the infection to their developing embryos through the egg (Taddese *et al*., 2019). When Salmonella gets past the stomach's defences and enters the intestine, it interacts with cells that aren't phagocytic, like the intestinal mucosal epithelial cells. Toxin, virulence plasmids, flagella, and fimbriae are just a few of the virulence features found in S. pullorum, which aid in the establishment of disease (Lou *et al*., 2019). According to Rasamsetti *et al*. (2021) [21], Salmonella pullorum can be isolated using either a selective or non-selective enrichment approach. In contrast, PCR to ascertain bacterial genomic DNA and carbohydrate fermentation tests were utilized in biochemical testing to ascertain infection (Ibrahim & Morin, 2018) [17]. To confirm bacterial isolation, biochemical analyses of Salmonella sp. colonies reveal unique features that shed light on the metabolic process of Salmonella pullorum (Zhike *et al*., 2021) [27]. While other Salmonella species ferment glucose, ornithine, and lycine, Salmonella pullorum produces undesirable outcomes when tested with urea, lactose, citrate, indol, and sorbitol (Ahmed, 2016) [16]. The following culture mediums are used: triple sugar iodine, McConkey agar, Mortality indole ornithine, Simmons citrate, Methyl Red-Voges Proskauer, Eosin Methylene Blue, and Salmonella Shigella agar

(Ghazy and Talib, 2023; Dirwal *et al*., 2020) [8]. According to Liebhart *et al*. (2023) [19], PCR tests have demonstrated their worth as diagnostic confirmatory Salmonella detection methods in broiler and layer chickens. These tests not only reduce diagnostic workload but also significantly shorten diagnostic times. The purpose of this study was to isolate Salmonella pullorum bacteria from grill chicken samples (droppings, cloacal swabs, and visceral organs). The bacteria were cultured, biochemically identified, and validated using a PCR analysis for the 16S rRNA gene.

Materials and Methods

Sample Collecting

A total of one hundred samples were collected from broiler chicken fields in Babylon City, with twenty-three samples sent to the Poultry unit at Babylon Veterinary Hospital and sixty-seven samples taken from broiler flocks in five districts of the province, between November 1, 2024, and April 1, 2025. The samples were selected at random based on clinical signs and postmortem findings. Everything was handled with the utmost care throughout collection; 51 cloacal swabs, 29 visceral organs, and 20 drops were preserved in a sterile plastic container and kept in a refrigerated box until they were sent to the lab.

Identification and Isolation of Salmonella

Using a non-selective Peptone water broth at 37 °C for 24 hours and a 10 ml Tetrathionate broth at 41.4 °C for the same amount of time, the samples were grown (Aljuhaishi and Albawi, 2024) [5]. After that, the cells were cultured in a particular broth (Selenite F broth from Himedia) and kept at 37 °C for 24 hours. According to Aljuhaishi and Albawi (2024) [5], a portion of each broth was streaked over XLD agar (Oxoid) and Salmonella and Shigella agar (Himedia) for additional incubation at 37 °C for 24 hours. According to the manufacturer of the Gram's stain kit, the staining features of the isolated bacteria and the purity of the culture were determined by Gramme staining. The VITEK-2 Compact system (Biomerioux, France) was used to achieve microbial species-level identification (Markey *et al*., 2013) [22].

Molecular identification

A stool DNA kit (Bioneer, Korea) was utilized for the extraction of genomic DNA from the samples. The kit was utilized in accordance with the guidelines provided by the company (8). Prior to being stored at -20ºC until the following tests were conducted, the DNA was verified and measured with a Nano Drop. Primers were used to detect the 16S rRNA gene, which is an essential target of Salmonella pullorum (Table 1). We used the PCR conditions listed in table 2. After running the PCR on a 1% agarose gel stained with ethidium bromide, the gel was electrophoresed, and the results were examined under a UV light.

Table 1: In order to identify the germs, the Salmonella pullorum primer sequence was used.

Gene name	Sequence of primer (5'—3')	Product size (bp)
16S rRNA	F- AGAGTTTGATCCTGGCTCAG	1500
	R- GGTTACCTTGTTACGACTT	

Results

Of the 100 samples analysed, 63 (or 63% of the total) were found to be infected with Salmonella spp. In the early stages of bacterial isolation, the peptone water culture medium was hazy and smelt terrible. Due to hydrogen sulphide formation, the bacterial colonies on the SS surfaced as small, transparent spheres with a black center (Fig.1); on the XLD, little colonies of pink and black were visible. The gram-positive bacteria were seen as solitary or paired in Gram's staining (Fig.3), and the recovered bacteria exhibited a small rod shape (Fig. 2). In addition, the Vitek2 system was used to identify and characterize the bacteria that were isolated (Fig4). Figure 5 demonstrates that S. pullorum was detected in samples using the PCR approach; 20 out of 63 samples were positive for the bacterium. This was accomplished by recognizing the 16S rRNA gene. Approximately 1500 base pairs of the 16S rRNA gene constituted these products.

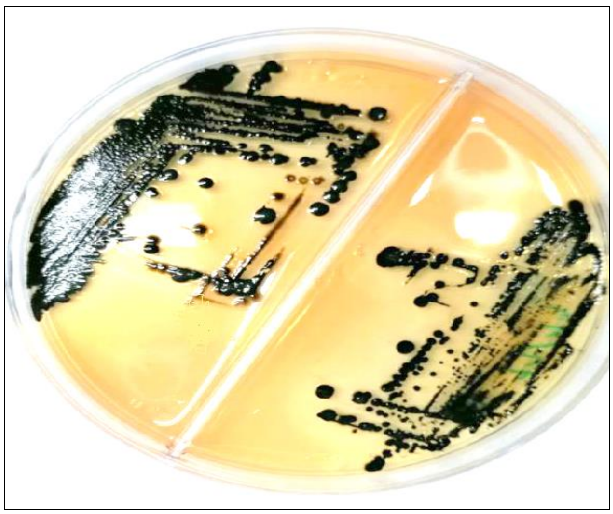

Fig 1: Isolated of Salmonella Spp. on SS agar.

Fig 2: The positive Salmonella isolation XLD agar.

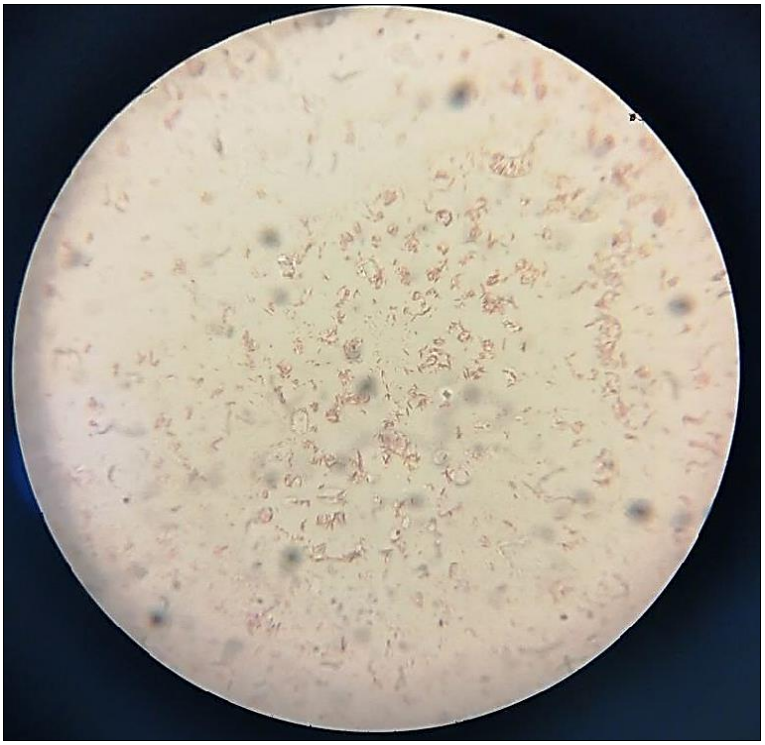

Fig 3: Microscopic Examination of Salmonella (gram stain).

bioMérieux Customer: **Microbiology Chart Report** Printed November 8, 2024 1:02:20 PM AST

Patient Name: Mohammed, Jabbar Hassan
Location: GAMA tec
Lab ID: 2411055

Patient ID: 2411055
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Salmonella enterica ssp diarizonae
BP Infection Site:

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	3.85 hours	**Status:**	Final
Selected Organism	**98% Probability**	**Salmonella pullorum**		
	Bionumber:	0417410741446611		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	+
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	-	18	dMAL	-	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	+
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	-	47	ODC	+	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	+	64	lLATa	-			

Page 1 of 1

Fig 4: Vitek 2 system of S. Pullorum

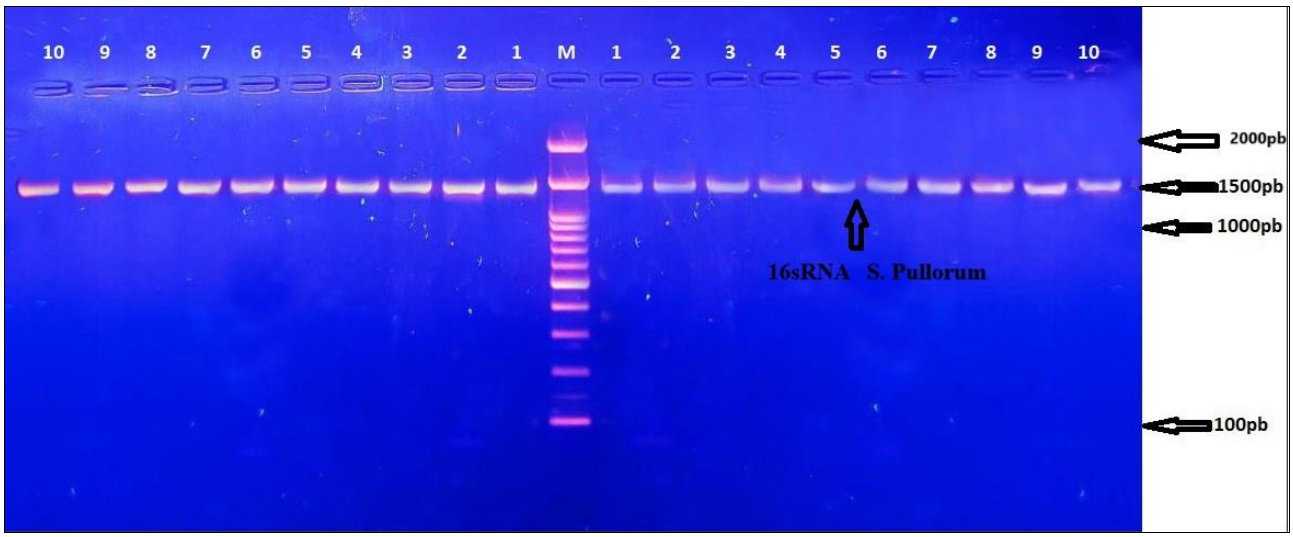

Fig 5: S. Pullorum PCR results targeting the 16S rRNA gene on an agarose gel. lane 2 to 20, positive results. Lane 1: 1500bp marker (Ladder).

Discussion

Poultry serves as both a reservoir for and a source of Salmonella, which can cause a range of acute and chronic illnesses in chickens. Humans can contract bacteria from ingesting this. No other animal species has had more Salmonella infections than chickens (Gast *et al*., 2003; khalil & Ali) [13]. A total of 63 Salmonella infections, or 63% of the total, were detected in a recent analysis of 100 chicken samples from broilers and layer farms. This percentage does not come close to the 31.94% that Hannon (2013) [16] found in Syria. The percentage in Jordan was 11.67%, which is lower than this (Al-Matar *et al*., 2005) [4]. It was found in Iran (Bidhendi *et al*., 2015) [11]. However, according to Mahmood *et al*. (2022) [20] in Bangladesh and Ayesha Khan *et al*. (2014) [10] in India, the prevalence was 55.5% and 56.3%, respectively. Possible explanations include variations in regional variance and management conditions, which could explain why these publications reveal different incidences than the current study. There was an increase in illness cases throughout the winter, which is consistent with earlier reports. Comparing two studies in Iraq, one in Baghdad found a prevalence of 75% for Salmonella, while the other in the Basrah region (AL-Iedani, Khudor, 2014) [3] found a prevalence of 80% and 92%, respectively. Al-Niema and Youkhana (2012) [7] surveyed the most frequent digestive illnesses in laying hens and parent stock in Mosul, and 70% of the cases were salmonellosis. Salmonella spp. was also found in grill flocks from Karbala City, according to Hamzah *et al*. (2020) [15]. Clinicians diagnosed enteritis, laminas with arthritis, and emaciation using the polymerase chain reaction (PCR) method. Environmental and management variables may have contributed to these differences. Geographical dispersion due to the fact that the survey included the most frequent gastrointestinal ailments in addition to Salmonella infection. Rumi *et al*. (2011) [24], Ghazy and Talib, and Ayesha Khan *et al*. (2014) [10] all found the same thing when it came to biochemistry and culture. All Salmonella isolates that were considered suspicious showed colonies of red and white bacteria with black centers on SS agar media. El-Ghany *et al*. (2012) [12] and Ashraf *et al*. (2013) [9] demonstrated the identical outcome. The Salmonella spp. Habitats It may be necessary to use advanced tools, such as PCR techniques, for a detailed diagnosis. In this example, the presence of this bacterium in the chickens' faces was determined using the PCR technique. Consistent with these results, Xiong *et al*. (2016) [26] developed a PCR method to identify these bacteria alongside more conventional laboratory techniques. In addition, replacing serological tests with a fast and inexpensive PCR approach that targeted the O and H antigen alleles yielded positive results (Saud and AL-Zuhariy, 2020) [25]. In addition, a PCR method that may differentiate Salmonella serovars was detailed by Majchrzak *et al*. (2014) [21]. Consistent with prior findings, this investigation confirms that the 16S rRNA gene is an appropriate method for differentiating S. Pullorum in grill chicken (Ghazy and Talib).

Conclusion

Grill chickens in the studied location (Babylon City, Iraq) had S. pullorum, according to recent conventional and molecular studies. Thus, it is important to think about salmonella prevention procedures when dealing with grill chickens.

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