Isolation and Identification *Salmonella ssp* from Local and Imported Chicken Meat by using VIDAS® -Up *Salmonella*

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Abstract

This study was carried out to isolate *Salmonella* species from Chicken meat, comparing the conventional method with rapid methods VIDAS ® -Up salmonella SPT (BioMérieux), 142 meat samples (Chicken) from the Local and Imported meat From local markets in the city of Baghdad within the period Septembers 2013 -march 2014. nineteen isolates were diagnosed as genus *Salmonella* and ensured by National center of Salmonella in Baghdad. nineteen *Salmonella* serotype (15.8 %) were diagnosed from chicken meat that includes *S.typhimurium* (% 31.5) S.*braenderup, S. dublin S. antum,* and *S. hadar* (10.5 %) for each of them and finally *S.entertidis , S.livingstone and S.give* at 5.2 % for each of them . The study recorded the serotypes of *S.give* and *S. living stone* as the first time in Iraq from chicken meat These results showed high significant differences at (P <0.01) between food samples. The results revealed that the contamination of imported meat was more than local meat and recorded of *Salmonella* isolates from imported meat that higher compared with local meat , The results Sensitivity test eight antibiotics (Ampicillin , Amoxycillin , cefitizdime, Chloramphenicole ,Impinem, trimethprime, Ciprofloxacin and Ceftriaxone) were observed resistant of the serotypes isolated from food samples towards Trimethprime Cetazidime, Ampicillin, Chloromphenicol, Ceftriaxone, and Amoxicillin at (100 , 86.6 ,40,30 , 33.3 and 16.6 %) respectively, and showed that the sensitivity for each of Imipenem and Ciprofloxacin 100%. VIDAS ® UP *Salmonella* showed high values of specificity and accuracy. VIDAS ® significantly decreased the time to obtain results compared to the conventional method and this is an important factor in the selection of an analytical method.

Keyword: Salmonella, Vidas UP Salmonella (SPT).

عزل وتشخيص بكتريا السالمونيلا من اللحوم الدجاج المحلية والمستوردة باستخدام تقنية VIDAS® - *Up Salmonella (SPT)*

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الخلاصة

أجريت الدراسة لغرض التحري عن بكتريا السالمونيلا من اللحوم ، ومقارنة الطرق التقليدية مع تقنية Vidas UP *Salmonella (SPT)* في التحري عن السالمونيلا ، جمعت 142عينة لحم (دجاج) محلي ومستورد من اسواق مدينة بغداد خلال الفترة من (1/11/2013– 2014/1/3). شخصت19 عزلة تعود الى جنس السالمونيلا ، وتم تاكيدها مختبر الصحة العامة المركزي – مركز الوطني للسالمونيلا لمعرفة الانماط المصلية، حيث شخص 19 نمط مصلي 15.8% من لحم الدجاج تضمن *S. antum S.ohoi S.braenderup* (%31.5),*S. dublin S.typhimurium* و *S . hadar* بنسبة 10.5% لكل منها ، وأخيرا لكل من *S.entertidis*، *S.livingstone* و *S.give* بنسبة 5.2% لكل منها.

سجلت الدراسة عزل النمطين المصليين *S.give* و*S .living stone* لاول مرة في العراق من الدجاج المستورد. وكانت نسبة التلوث في اللحوم المستوردة اكثر مما في اللحوم المحلية بنسبة 46.6% و23.3% على التوالي.

أظهرت نتائج فحص حساسية العزلات اتجاه ثمان انواع من المضادات الحيوية ، وكانت الأنماط المصلية المعزولة من العينات الغذائية مقاومة لكل من rimethprime Cetazidime, Ampicillin, Chloromphenicol, Ceftriaxone، وAmoxicillin بنسبة، (100، 86.6، 30، 33.3، 40 و16.6) % على التوالي واظهرت حساسية لكل من Imipenem و Ciprofloxacin بنسبة 100 % لكل منهما. اكدت النتائج ان تقنية Vidas افضل من الطرق التقليدية لسرعتها ودقة في الكشف عن السالمونيلا في العينات الغذائية.

الكلمات المفتاحية: السالمونيلا ،اللحوم ، Vidas UP *Salmonella* (SPT).

Intoduction

Salmonella is a food-borne pathogen influencing on food safety and public health around the world. According to, Salmonella is the second major cause of food borne disease acquired in the United States and leads episodes of hospitalization and death. Salmonella detection in foods by Conventional culture methods consist of a series of steps that include nonselective enrichment, selective enrichment, and selective/differential plating and, finally, [1] morphological, biochemical and serological confirmation as described in ISO 6579:2005 method. [2]

This standardized classical culture method is rather sensitive and quite inexpensive, but it requires at least, three working days to produce a negative result and five to ten working days for a confirmed positive result. Nevertheless, it is still in use by many labs, especially by regulatory agencies, because it is harmonized method, looked at as the "gold standards" in food diagnostics[3]. The conventional methods for the detection of microorganisms in foods require a long period of time to obtain results. Moreover, due to environmental factors, variations in gene expression of microorganisms can occur and may affect the discriminatory power of biochemical tests. Furthermore, viable but non culturable cells are not detected by the conventional methodology [4]. Alternative and rapid methodologies for the detection of *Salmonella* in foods are attractive and allowing convenience, flexibility and potential for automation included those immunoassay-based techniques, such as, the VIDAS ®Salmonella assay (bioMérieux, France) that can produce results in one to three days.[5] Despite of require onerous commercial kits and sophisticated equipment, the alternative methods present high productivity and quality of results and the cost effective should be considered mainly by testing laboratories that analyze samples routinely for food borne pathogens.[6]

Materials and Methods

Isolation and identification of Salmonella from meat:

Samples collection*:* one hundred fourty two chicken meat samples were collected from from local markets in the city of Baghdad and transmitted to the laboratory with cooling box.

Culturing of samples: 25 (gm) from meat samples were put immediately in a sterile tube contained 225 (ml) nutrient broth contained was incubated into at 37C° for 24 hours, and then transmitted 1(ml) from tube to each Selenate Cysten broth SCB, Tetrathionate broth TTB was incubated into at 37C° for 24 hours, and then subculture streaked on Xylose lysine deoxycholate agar, , MacConkey agar incubated at 37C° for 24 hours The growing colonies were examined by naked eye concerning the color, shape and size and bacterial cells examined by gram stains According to. [7]

VIDAS® Up Salmonella (SPT)

the sample is inoculated into Tryptone soya broth and incubated for 18-24 hours at 37°C and then transmitted 3 ml of broth is placed in a tube, which is heated for 15 min at 100°C. Following the boiled test suspension is placed into the reagent strip and is cycled in and out of the Solid Phase Receptacle. Salmonella antigens, if present, bind to the monoclonal antibodies coating the interior of the SPR. All other unbound material is washed away. Antibodies conjugated with alkaline phosphatase are cycled in and out of the SPR, binding to any Salmonella antigen bound to the SPR wall. The final wash step removes unbound conjugate. The substrate, 4-methyl umbelliferyl phosphate, is converted by the enzyme on SPR wall to the fluorescent product, 4-methyl umbelliferone. The intensity of fluorescence is measured by the optical scanner in VIDAS. The fluorescence intensity is measured twice at 450 nm. The first result is related to the background, the second is the value after incubation of the substrate with enzyme. Based on that, the apparatus calculates the result of the test and interprets it as a positive or negative one. The assay is automated and

carried out in the VIDAS instrument following the manufacturer's instructions According to

Gram stain: bacterial cells examined by gram stains According to. [8] [9]

Biochemical tests: Important biochemical Tests (Triple Sugar Iron (TSI) , Catalase test, Oxidase test, Lactose fermentation, Urease test ,Indole test , Citrate utilization test) were conducted according to. [10]

Api-20E system (Analytical profile index for Enterobacteriaceae test):

It was done according to [11], this test (Api-20E system) is used clinically for the rapid identification of the bacterial isolates.

***Slide agglutination test*:** all isolates were examined with polyvalent O and H antisera by using slide agglutination test as follows:

1- One drop from physiological normal saline was placed on each of the glass slides at each side, and then a loopful from bacterial culture was mixed with each drop.
2- One drop from each O, H polyvalent antisera was added to one of the previous drop and then mixed by plastic rod and rocked. The other drop was used as control.
3- The clear agglutination occurred within 1-2 minute indicated a positive result According to. [12]

Serotyping diagnosis: Suspected *Salmonellae* genus was sent to the Central Public Health Laboratories (National Center of *Salmonellae* in Baghdad) on Kligler iron medium for final serotyping diagnosis.

Antimicrobial resistance test:

The antimicrobial resistance of the isolates was determined by the agar diffusion method with Mueller Hinton agar and use 8 antimicrobials, There are: Ampicillin 10 (mg), Amoxycillin 10 (mg), cefitizdime 30 (mg), Chloramphenicole 30 (mg), Impinem 10 (mg), trimethprime 25 (mg), Ciprofloxacin 5 (mg) and Ceftriaxone 30 (mg). At least 3-5 well isolated colonies were selected from the agar plate culture. The top of each colony was touched with a loop, and the growth was transferred into a tube containing 4 ml nutrient broth. The turbidity broth culture was comparable to the point of the 0.5 McFarland standards and then use a sterile cotton swab was dipped into the suspension. The swab streaking on the surface of a Mueller-Hinton agar plate was inoculated and then antimicrobial disks were dispensed onto the surface of the inoculated agar plate. Each disk was pressed down individually to ensure complete contact with the agar surface.

The plates were inverted and placed in an incubator set to 37 °C within 24hour After incubation, each plate was examined. The resulting zone of inhibition was measured .The sizes of zones of inhibition were interpreted reported as susceptible, intermediate or resistant to the agents that have been tested According to. [13]

Results and Discussion

Isolation and identification of *Salmonellae* spp:

Results showed nineteen *Salmonella* serotype 15.8 % were diagnosed from 142 chicken meat the different morphology characteristics of *Salmonellae spp* which grow on different media table (1) and figure (1)

Microscopic examination:

Results of microscopic examination has showed that these isolates were gram negative rod, non-spore forming after (18–24) hours post incubation at 37 ºC.

Biochemical identification:

The results of the biochemical tests showed that these isolates gave negative results for oxidase, urease, and indole tests, while gave positive results for catalase and citrate utilization test, Kligler – Iron agar and Lysine– Iron agar gave Red /Yellow with H_2S production as shown in table (2).

Api-20E system identification:

The result of Api-20E test has revealed the numerical profile (6704752) as confirmed diagnostic test for *Salmonella* isolate in table (3) and figure (2).

VIDAS® Up *Salmonella (SPT):*

The results of isolation of *Salmonella spp* from 142 meat samples (Chicken) (19) *Salmonella spp* isolates.

Culturing Salmonella on chromogenic media:

Culturing on several chromogenic media such as Hicrome MM agar, Chrom agar TM *Salmonella*, *Salmonella* differential agar as confirmed diagnostic test for *Salmonella* isolate in table (1) and figure (1)

Slide agglutination test (Polyvalent):

The genus *Salmonella* detected by using polyvalent O and H antisera showed that clear agglutination indicated a positive result in comparison to clear homogeneity of the control reaction in each one.

Serotyping of Salmonella (Monovalent):

The result of the serotyping of the bacteria in the Central Public Health Laboratories/ Ministry of Health has ensured that these bacteria are nineteen *Salmonella* serotype (15.8 %) were diagnosed from chicken meat that includes *S.typhimurium* (%31.5) *S.braenderup S. dublin S.Ohi S. antum, and S. hadar* (10.5 %) for each of them and finally *S.entertidis , S.livingstone and S.give* at 5.2 % for each of them . The study recorded the serotypes of *S.give* and *S. living stone* as the first time in Iraq from chicken meat as shown in table (4)

Antimicrobial resistance test:

A total of 19 isolates were tested against eight commonly used antimicrobials. The results of the antimicrobial sensitivity test showed resistance towards Trimethprime Cetazidime, Ampicillin, Chloromphenicol, Ceftriaxone, and Amoxicillin at (100, 86.6,40, 30, 33.3 and 16.6 %) respectively, and showed that the sensitivity for each of Imipenem and Ciprofloxacin 100% shown in the table (5).

Discussion

VIDAS® *Up Salmonella (SPT):*

Although traditional methods are used to detect bacteria, salmonella is being investigated recently using the Vidas UP Salmonella technique, Several validation studies have been reported that the detection rate of VIDAS systems were comparable to that of culture method because VIDAS ® UP *Salmonella* showed high values of specificity and accuracy. VIDAS ® significantly decreased the time to obtain results compared to the conventional method and this is an important factor in the selection of an analytical method. [14]

Report on the use of VIDAS ® for screening raw meat and beef showed that the number of positive samples detected was two-fold higher than that by culture method.[15]

Culturing *Salmonella* on chromogenic media:

Several chromogenic media have been developed in order to speed up the detection and diagnostics of *Salmonella* [16].The conventional media for the detection of *Salmonella* in some cases have poor specificity and false positives (such as *Citrobacter, Proteus*) hinder identification of positive *Salmonella* colonies. In addition, examination of potential *Salmonella* colonies growing on conventional media is time consuming. Chromogenic media provide a rapid, accurate means of isolating and enumerating target microbes based on the detection of specific enzymatic activities. [17]

Serotyping of Salmonella (Monovalent):

The contamination of chicken meat by *Salmonella* is commonly reported and its occurrence varies, the level of contamination in this study is consistent with the recent literature reporting 14.5% from Nepal [18], 19.2% from South Africa [19], 17.36 % from Saudi[20], 11.6% from Jordon [21] and 12% from Turkey.[22]

This result is consistent with [23] who recorded that isolations *S. anatum, S. typhimurium* from meat in al-Basra province. In contrast, study of [24] on *S.livingstone* isolations from chicken while the level of contamination beef meat in this study is consistent with the recent literature reporting 5.9% from Baghdad. [25]

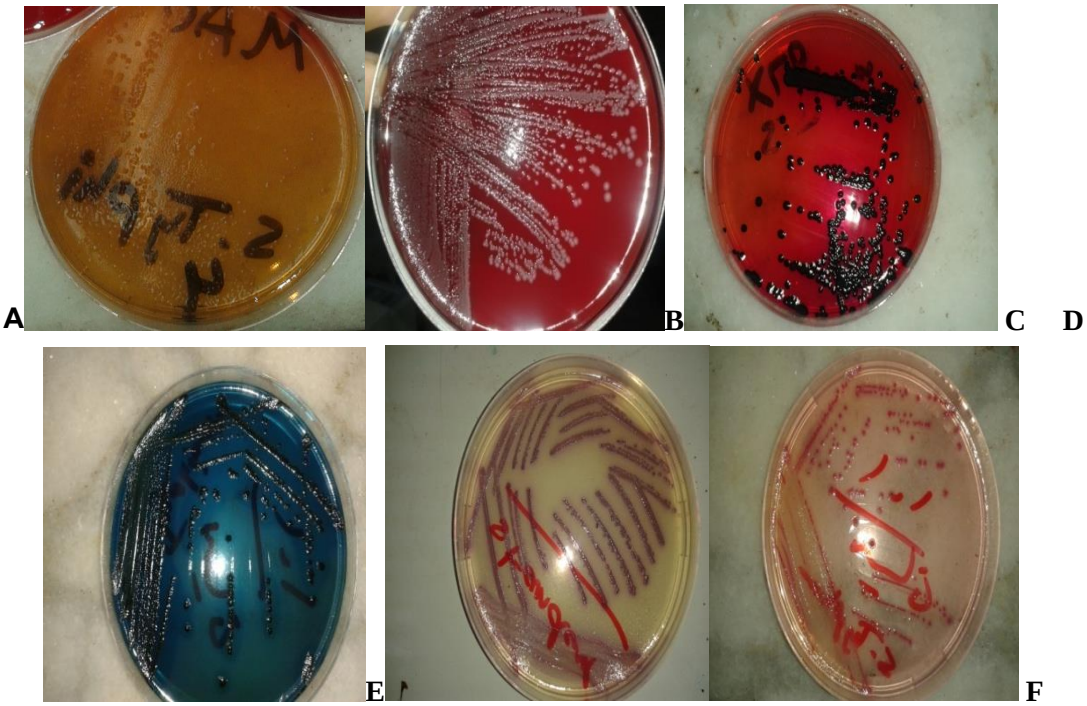
Antimicrobial resistance test:

The use of antimicrobial in food animals has resulted in the development of antimicrobial resistance, [26] through mutation and acquisition of resistance encoding genes [27]. The Salmonella isolates from poultry were reported Ciprofloxacin sensitive. [28]

Consistent with study in Thailand poultry isolates were resistance Chloramphenicol 18.18% [29]. *S.typhimurium* is sensitive to Chloramphenicol 28٪ and Ampicillin 57%,[30] also reported *S.typhimurium* and *S. anatum* is sensitive Ciprofloxacin. [31]

Table (1) The Results of Culture Characteristics of *Salmonellae spp*.

Culture Media	Morphology of colonies
Hicrome agar	small, rounded, black
Salmonella differential agar	small, smooth, rounded, pink to red color
MacConkey agar	small, smooth , and pale
CHrom agar TMSalmonella	small, smooth, rounded, mauve color
Xylose-Lysine Deoxycholate agar	small, smooth, rounded, red in color with black center



A.MacConkey agar, B.Blood agar, C.XLD, D- Hicrome agar
E. Chrom agar TMSalmonella and F- Salmonella differential agar

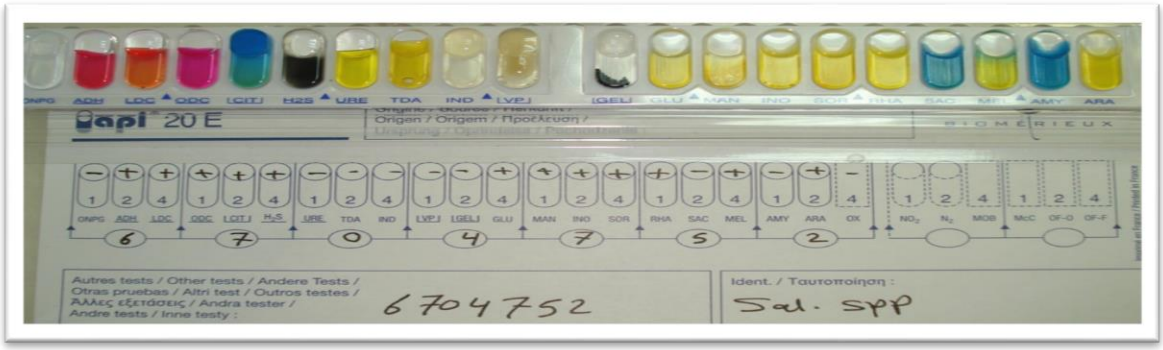
Figure (1) Characteristics of Morphology of Colonies *Salmonellae spp*

Table (2) The Results of Some Biochemical tests of *Salmonellae spp.*

Biochemical test	Result
Oxidase test, Indol , Urease test	-
Catalase test, simmon citrate test	+
KI and TSI	Red/Yellow with H_2S production

Table (3) The Results of Api-20 E tests of *Salmonellae spp*.

Biochemical test	Results	Biochemical test	Results
(LDC) lysine decarboxylase, (ODC) ornithine decarboxylase , (CIT) citrate utilization , (H_2S) H_2S production , (ADH) arginine dehydrolase, fermentation / oxidationglucose (GLU) ,mannitol (MAN), inositol(INO), sorbitol(SOR), rhaminose(RHA), melibiose(MEL) & arabinose(ARA)	+	(ONPG) β-galactosidase (ADH) arginine dehydrolase (URE) urease,(GEL) gelatinase (TDA) tryptophane deaminase fermentation/oxidation of these sugars (SAC) sucrose, (AMY) amygdaline indol(IND), acrtoin production(VP), & cytrochrome-oxidase(OX)	–



(+): The test positive. **(-)**: The test negative. **(6704752):** The numerical profile.
Figure (2) Calculate the Numerical Profile in Api-20E System

Table (4) Different Serotypes of *Salmonellae* Isolated from Poultry Meat

Samples (number)	Number of isolation (%)	Species	Number (%)
Local poultry meat (50)	5 (10)	*S.typhimurium*	2 (6.7)
		S. hadar	1 (3.3)
		S. anatum	1 (3.3)
		S. dublin	1 (3.3)
Important poultry meat (92)	14 (15.2)	*S.typhimurium*	4 (13.3)
		S.braenderup , S.ohio	2(6.7)
		S. enteritidis , S . hadar	1 (3.3)
		S. anatum , S. dublin	1 (3.3)
		S . living stone , S . give	1 (3.3)
Total (142)	19 (13.3)		

Table (5) Level of Antimicrobial Resistance by Serotypes

Serotypes (NO)	Number of Serotypes sensitive or resistance (%)							
	AMP	CIP	CAS	C	IMP	CRO	AMC	TS
S.typhimrium (6)	5 R (83.3) 1 S (60)	S(100)	5 R (83.3) 1 S (60)	4 R (66) 2 S (33)	S(100)	5 R (83.3) 1 S (60)	3 R (50 3S (50)	R (100)
S.livingstone (1)	R(100	S(100	R(100)	S(100)	S(100)	S(100)	S(100)	R (100)
S.anatum (2)	R(100	S(100	1R(86.6) 1S(13.3)	S(100)	S(100)	S(66.6)	S(83.3)	R(100
S.hadar (2)	R(100	S(100	R(86.6)	S(100)	S(100)	S(66.6)	S(83.3)	R(100
S.entertidis (1)	R(100	S(100	R(100)	S(100)	S(100)	S(100)	S(100)	R (100)
S. brandrup (2)	1R(50 1 S (50)	2S(100	1R(50) 1S(50)	2S(100)	S(100)	2S(100)	S(100)	R(100
S.dublin (2)	2R(100	2S(100	1R(50) 1S(50)	S(100)	2S(100)	2S(100)	S(100)	R(100
S.give (1)	R(100	S(100	R(100	S(70)	S(100)	R(100	S(70)	R(100
S.ohio(1)	R(100	S(100	S(70)	R(100	S(100)	S(70)	R(100	R(100
	R(84) S(15)	S(100)	R(68) S(13)	R(84) S(15)	S(100)	R(84) S(15)	R(84) S(15)	R(84) S(15)

R: Resistance S: Sensitive I:Intermediat CIP: Ciprofloxacin TS: Trimethprim C: Chloromphenicol CAS: Cetazidime AMP: Ampicillin Amox: Amoxicillin CRO: Ceftriaxone IMP: Imipenem

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