Research article

Isolation and identification of *Salmonella* serotypes in poultry

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(Received 21/8/2017, Accepted 26/12/2017)

Abstract

Our study was designed to investigate the prevailing serotypes of *Salmonella* in chickens and the rate of isolation of each serotype. Between 27 September 2014 and 15 February 2015, a total of 200 samples were collected from cloacal swabs and cecal contents from different sources and ages at Al-Diwaniyah Province. The bacterial culture was carried out by using different culture media such as *Salmonella* Shigella agar (SS), Xylose Lysine Deoxycholate Agar (XLD) and Chrome agar *Salmonella* (CAS), the serotype was determined by confirmative bio-chemical essays using the Vitek-2 system. The current study was able to identify three serotypes as following: *S. enteritidis*, *S. Paratyphi B* and *S. typhimurium* with isolation rate 10%, 6.5% and 4.5% respectively.

Keywords: chicken, Identification, Infection, *Salmonella*, Serotypes.

Introduction

Approximately 2.500 different serotypes have been isolated of *Salmonella* genus, which considered as an important animal health issue, which cause a serious common illness of humans and animals, with 3 million recorded deaths among people and large losses in poultry (1, 2, 3). Avian Salmonellosis is an acute or chronic infectious disease of many types of birds caused by many different highly specialization serotypes such as *S. pullorum*, *S. gallinarum*, and *S. arizonae*, or as zoonotic serotypes such as *S. typhimurium*, *S. enteritidis* and other paratyphoid *Salmonella* serotypes (4). Moreover, (5) found a close genetic relationship between *Salmonella* isolates of chicken meat and patient with food poisoning. Chicken is infected or contaminated with *Salmonella* serotypes either by infected birds, or cross contamination with water, feces, tools, and workers (5). Recently the most common serotype is *S. enteritidis*, which cause a lot of economic losses to poultry producers (6), and are still a major challenge to public health as

one of the most dangerous serotypes that causes human food poisoning (7, 8). About 75% of these cases are due to the consumption of chicken's meat, and eggs contaminated with this serotype (9), although *S. typhimurium* came second after *S. enteritidis* as causative agent of food poisoning (10, 11), while previously *S. typhimurium* was associated most frequently with diarrheal disease in United States (12) and Europe (13). Hence, this study was aimed to isolate and identify the most prevalent *Salmonella* serotypes in chickens in Al-Diwaniyah province.

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 413

Bacterial Isolation and Identification:

A. Collection of Samples:

A total of 200 samples of cloacal swabs and cecal contents of live chickens were collected with different ages and sources for the between the 27/9/2014 to 15/2/2015.

B. Cloacal swabs:

Samples were taken using sterile cotton swabs and each sample was placed in 10 ml of selenite broth (Himedia India Lab, PVT Ltd.) to encourage growth of *Salmonella*, despite low number or damaged cells and to prevent other contaminated bacteria and incubated at 37 C° for 24 hrs. (14).

C. Caecal content:

After the dissection of the chicken, the cecum was cut with sterile scissors, and then the contents of the cecum 1-0.5 gm were placed (squeezed) in standard universal tubes, each containing 4.5-10 ml of selenite broth, and incubated at 42 C° for 24 hrs. (15).

D. Isolation :

In vitro culturing was carried out in the different media such as SS agar (Himedia India Lab, PVT Ltd.), XLD (Oxoid, England) and CAS (CHROM agar, France), respectively, for repeated culturing

(subculture) and for purification of selected colonies, and incubated at 37- 42C° for 24-48 hrs., all media were prepared in accordance with the manufacturer's recommendations, and subjected to quality control.

E. Identification:

Salmonella was identified by the Vitek-2 system at the postgraduate research lab. College of Veterinary Medicine / University of Al-Qadisiyah using the French GN Card (Biomerieux, France), which contains a number of bio-chemical testing and control fossils, specialized for the diagnosis of G⁻ fermented and non-fermented bacteria, one pure colony was taken immediately after removing media from the incubator and measuring (0.5 MCF) and inserting it in the Vitek-π system, the results are read after 5 - 10 hrs., the procedure for serotype identification in accordance with the manufacturer and (16).

Statistical analysis:

The analytical assay was used one way ANOVA and determined less significant difference below P <0.05 (17).

Results

The total number of positive samples of *Salmonella* genus are 52 samples of 200, with a total percentage of 26%. The shape of the developing colonies was circular with a colorless containing a black center on the SS agar as shown in Figs. (1) and (2), while the growing colonies on XLD were circular with a red color containing a black center as shown in Figs. (3), (4), (5) and (6), with a totally different appearance of *Salmonella* colonies on the CAS appeared as Red mauve colored colonies as shown in Figs. (7) and (8). Of the 52 positive samples of *Salmonella*, genus three species are identified included *S. enteritidis*, *S. paratyphi B*, *S. typhimurum*. The results in Table (1) showed isolation or prevalence rate of three detected serotypes listed above were as follows: 10%, 6.55% and 4.5% respectively, with significant difference (P<0.05) in isolation rate of *S. enteritidis* compared to other

serotypes. Only 42 serotypes of 52 samples were recognized, the Vitek system has no portability to identified 10 of 52 positive samples, although it was diagnosed as *Salmonella* bacteria on SS agar, XLD and CAS.

Table (1): prevalent *Salmonella* serotypes and isolation rates from chickens

Identified Spp.	Positive samples	Percentage of positive samples
<i>Salmonella enteritidis</i>	20/200	10% A
<i>Salmonella paratyphi B</i>	13/200	6.5% AB
<i>Salmonella typhimurium</i>	9/200	4.5% B
Unrecognized <i>Salmonella</i> serotypes	10/200	5%
Total	52	26%

Figure (1): Salmonella colonies on SS, was identified as *S. enteritidis* on Vitek-2 system.

Figure (2): Salmonella colonies on SS, was identified as *S. paratyphi B* on Vitek-2 system.

Figure (3): Salmonella colonies on XLD, was identified as *S. typhimurium* on Vitek-2 system

Figure (4): Salmonella colonies on XLD, was identified as *S. enteritidis* on Vitek-2 system.

Figure (5): Salmonella colonies on XLD, was identified as *S. S. paratyphi B* on Vitek-2 system.

Figure (6): Salmonella colonies on XLD, was identified as *S. S. typhimurium* on Vitek-2 system.

Figure (7): Salmonella colonies on chrome agar, was identified as *S.S. typhimurium* on Vitek-2.

Figure (8): Salmonella colonies on chrome agar, was identified as *S. S. enteritidis* on Vitek-2 system.

Discussion

Salmonella Shigella (SS) Agar is moderately selective and differential media for the isolation, cultivation, and differentiation of *Salmonella* spp. *Salmonella* will not ferment lactose but produce hydrogen sulfide (H₂S) gas (18). The resulting bacterial colonies appeared colorless with black centers Fig.1 and 2. Xylose Lysine Deoxycholate Agar (XLD) is a selective differential media, practiced for the detection of *Enterobacteria* through fermentation and acidification of the media, turning the medium to yellow Figures (3, 4). Hydrogen sulfide production from thiosulfate is easily detected because colonies become dark or gloomy, due to the precipitation of ferric sulfide (19), cadaverine may be observed due to decarboxylation of lysine, where it produces alkalisation, accordingly the indicator turns to red (incubation for more than 24 hrs.) Figure (5) and 6. Chrome agar *Salmonella* (CAS) is relatively a newer selective, high specific chromogenic media, i.e., fewer false-positive results, *Salmonellae* colonies appeared as red mauve Figures (7, 8). While other members of the *Enterobacteriaceae* appeared as blue or uncolored colonies, (20, 21), all standard morphological features of *Salmonella* colonies that described by (18, 19, 20 and 21) are in compatible with our results. Our current study shows that these three serotypes are the most common at the time of investigation, Carli and his colleagues (22) proved that *S. gallinarum* and *S. typhimurium* were prevalent in the 1980s of last century, while in the 1990s *S. enteritidis* prevails or exceeds any other serotypes and considers as the main cause of poultry infections. However, the serotypes and prevalence rates are varied, in one study carried out in Jordan showed surprisingly dominance of *S. enteritidis* with 42% followed by 33% of *Salmonella* B and 19% *Salmonella* Group C (23) which is incompatible with our results, while in another study in Jordan also one year later, the isolation rate of *S. enteritidis*

was 8.09% while *S. typhimurium* was 13% (24). Close to our results in the south and east of Iran, the rate of isolation or prevalence was 8.5% of *S. enteritidis* (25). But in another study in Kermanshah, Iran (26) mentioned that the prevalence rate of *S. typhimurium* was 62% of 50 samples collected from poultry meat. While another study conducted in eastern Turkey (27) showed that the isolation rate was 4% of chicken's liver samples. In our study only three serotypes were recognized, although in Denmark 20 serotypes belong to *S. enterica* were isolated and identified (28). In Belgium, the isolation rate was 17% for *S. enteritidis* in 1993 and 27% in 1994 and 20% in 1995, in the Netherlands the proportion of *S. paratyphi* B variant Java was less than 2% before 1996, rising to 60% in 2002, these results in the Netherlands come in parallel with the high level of infections in Germany compared with European countries (29), although (30) reported that many *Salmonella* serotypes have been isolated in European Union (EU) countries such as *S. infantis*, *S. enteritidis*, *S. typhimurium*, and *S. Kentucky* at rates of 29.2%, 13.6%, 4.4%, and 6.2% respectively, while in Brazil, (31) appointed the total *Salmonella* isolation rate of 90 samples collected from live chickens by cotton swabs and cecal contents was 25%, included many serotypes such as *S. enteritidis* with isolation rate of 12% and 3% for *S. typhimurium*, these results again are close to our results with respect to the *S. enteritidis* and *S. typhimurium*, although the number of samples being different than ours. In Iraq (32) used PCR technique for *Salmonella* identification she concluded that the following serotypes of *Salmonella* are the most common and are as follows, *S. enteritidis*, *S. good wood*, *S. anatum*, *S. Emek*, and *S. typhimurium*, while our study is compatible with this study in terms of agreement on the existence of two serotypes, *S. enteritidis* and *S. typhimurium*. In addition, (33) reported that the total rate of infection in

a domesticated pigeon in the city of Al-Diwaniyah was 33.8% of the following serotypes *S. paratyphi* A, B and C. In Bangladesh, a total of 160 samples, the isolation rate of *S. gallinarum* was 64%, *S. pullorum* was 22.3% and other *S. paratyphoid* was 13.3% (34). In Nigeria, (35) clear up that *S. paratyphi* A had an isolation rate of 12.5% from poultry droppings, *S. typhimurium* 6.7%, *S. enteritidis* 5%, *S. gallinarum* 6.7%, and *S. pullorum* 4.1%, while in our study *S. gallinarum*, *S. pullorum* could not be identified. When the results of previously mentioned studies are compared with the results of the current study, it is concluded that there is an agreement of some our results and disagreement of others. From our study and others it obvious that the serotypes of *S. enteritidis* is prevailing on other serotypes, but at the same time many other serotypes are isolated and there is also a difference in the isolation rates among them and among different studies, these

differences may be due to several factors contributing to the emergence of varying proportions of *Salmonella*, since the samples belonged to different species and ages in addition to the health status of the bird and other environmental factors or may be due to the source of chicks, the type of feed and medicines that used during the period of rearing in addition to geographical location (36). period of sample collection, different organs under examination also may be due to the quality of the taken samples or due to different laboratory procedure's assay on which the different studies relied or based on. The high rates of *Salmonella* isolation or prevalence in some studies may indicate a serious problem in the biosecurity of the poultry industry and in all joints of these industry i.e from hatching to the processing, this problem requires realistic solutions to protect human health, poultry industry investment and the welfare of birds.

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