Bacteriological Survey on *Salmonella Enterica* from Rabbit and Poultry with Special Reference to Its Antibiotic Resistance Patterns

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Abstract

Salmonellosis has been considered as one of the most important infectious diseases in humans and animals. The present study reports traditional methods for characterization of *Salmonella enterica* from different sources. Two hundred seventy samples were collected from different poultry farms at Sharkia Governorate during the period from November 2015 to May 2016, and subjected to traditional bacteriological examination for isolation of Salmonella. Incidence of Salmonella from fecal swabs and internal organs revealed the isolation of Salmonella with an overall incidence 14.07% (38/270). Absolute resistance was observed for amoxicillin/clavulanic acid, ampicillin, doxycycline and ceftriaxone On the other hand, sensitivity to ciprofloxacin (81.6%), gentamicin (78.9%) ,sulphamethoxazol/trimethoprim (73.7%),amoxicillin (65.8%) and chloramphenicol(63.2%), Serotyping of Salmonella revealed two Salmonella serogroups. The first is *Salmonella* Typhimurium with antigenic formula 1,4,5,12:i:1,2 as (6/8) by (75 %) and *Salmonella* Lagos with antigenic formula 1,4,5,12:i:1,5 as (2/8) by (25%). These findings illustrated that all serotypes were resistant to ceftriaxone, doxycycline, ampicillin and Amoxicillin/clavulanic acid. while, ciprofloxacin gave high degree of sensitivity, the outbreak of infection in poultry is not only by common type such as *S.* Typhimurium but also by other serotypes such as *S.* lagos.

Introduction

The genus Salmonella is Gram-negative rod shaped bacteria, and it is a member of the family *Entrobacteriaceae,* a facultative intracellular microorganism that able to cause different disease syndrome in a different kinds of hosts. Salmonellosis has been considered as one of the most important infectious diseases in humans and animals ***(Keusch, 2002).***

Salmonella enterica serovare Typhimurium and Enteritidis are the most frequently isolated serovars throughout the world leading to severe economic losses, especially in poultry industry ***(Herikstad et al., 2002)***. Pathogensis of Salmonella is depending on many factors controlled by several genes that play the main role in virulence and these genes are clustered on SPI ***(Murugkar et al., 2003)***.

Controlling of Salmonella in poultry and food products of animal origin is problematic and it has relies historically on a combination of farm use biosecurity and the use of antibiotic ***(White et al., 2001)***. This work was designed for characterization of antibiotic resistant patterns of Salmonella serotypes isolated from poultry.

Material and Methods

Sampling and isolates characterization

Two hundred and thirty five fecal samples were collected aseptically using sterile cotton swabs including rabbits (50), ducks (50) and chicks aged from7 to 10 days (135). Moreover, 35 samples were collected aseptically from internal organs of broiler chickens including (liver (15),spleen (10) and intestine(10)). All samples were subjected to conventional methods for isolation and identification of Salmonella species ***(FDA, 1998)***. *Salmonella* species were further identified with API identification kits (BioMérieux, Mary l'Etoile, France) and serotyped in the Serology Unit, Animal Health Research Institute, Dokki, Giza, Egypt using commercial antisera (Difco, Detroit, MIUSA) according to the manufacturer's instructions.

Antimicrobial susceptibility testing

Antibiotic susceptibilities were determined by the standard disk diffusion method according to the guidelines of Clinical and Laboratory Standards Institute **(CLSI, 2011)**. *Salmonella* species isolates were tested using gentamicin (CN:10mg), ciprofloxacin (CIP:5 mg), amoxicillin-clavulanic acid (AMC: 20/10 mg), ceftriaxone (CRO:30mg), doxycycline (DO:30 mg), chloramphenicol (C:30 mg), sulfamethoxazole/trimethoprim (SXT:25 mg), ampicillin (AM:10 mg) and amoxicillin (AX: 20 mg) **(Oxoid).**

Results

Bacterial incidence

Incidence of Salmonella from fecal swabs and internal organs revealed the isolation of Salmonella with an overall incidence 14.07% (38/270) from chicks only. Salmonella isolates gave pale colonies on MacConkey's agar and gave slightly transparent zone of reddish color with black center on XLD. Regarding the biochemical identification by traditional method, Salmonella isolates were lysine decarboxylase positive, urease negative and gave yellow butt and

red slant with hydrogen sulfide production on TSI.

Serotyping of Salmonella isolates

Serogrouping analysis of Salmonella isolates revealed that Salmonella Typhimurium was the most prevalent serovare (75%) followed by Salmonella Lagos (25%).

Drug resistance analysis of bacterial isolates

Absolute resistance was observed for amoxicillin/clavulanic acid, ampicillin, doxycycline and ceftriaxone (100%). On other hand, higher rates of sensitivity to ciprofloxacin, gentamicin, Sulphamethoxazol/ trimethoprim, amoxicillin and chloramphenicol (81.6%, 78.9%, 73.7%, 65.8%, and 63.2%, respectively) were found. Besides, all isolates show multidrug resistance pattern, (Figures 1 and 2).

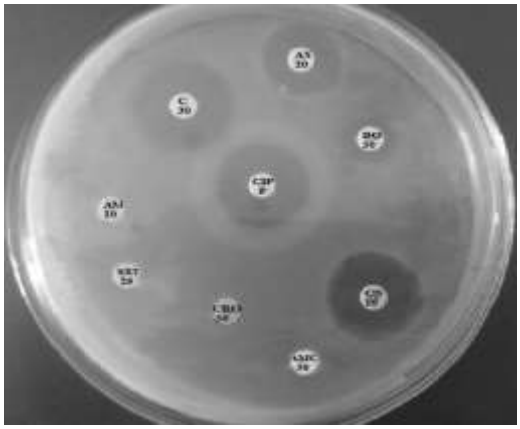

Fig (1): *Antimicrobial resistant profile for a Salmonella Typhimurium using disk diffusion method , the isolate showing sensitivity to ciprofloxacin, chloramphenicol, gentamicin and amoxicillin and resistance to amoxicillin/clavulanic acid, doxycycline, ampicillin, ceftriaxone and Sulphamethoxazol/trimethoprim.*

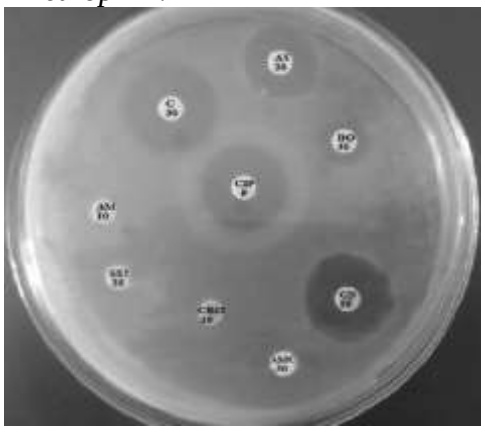

Fig (2): *Antimicrobial resistant profile for a Salmonella Lagos using disk diffusion method , the isolate showing sensitivity to ciprofloxacin, chloramphenicol, gentamicin and amoxicillin and resistance to amoxicillin/clavulanic acid, doxycycline, ampicillin, ceftriaxone and Sulphamethoxazol/trimethoprim.* .

Discussion

Salmonellosis occurs worldwide in many countries and represent a major contributor to morbidity and mortality with resultant economic costs in poultry and food industries ***(Antoine et al., 2008)***.

In this study, examination of 270 samples collected from poultry the overall prevalence of Salmonella from the obtained samples was 38/270 (14.0%).Many studies showed different incidence rates of Salmonella isolates worldwide in Egypt, low prevalence rate reported in Egypt(10%) ***(Taha, 2002)*** and (13.6%) in Ethiopia ***(Addis et al., 2011)***. Other countries (13.0% to 88.2%) in Poland ***(Mikolajczyk and Radkowsk, 2002)*** in Cambodia ***(Lay et al., 2011)***. These differences may be attributed to the difference in environmental conditions.

Poultry and food products are commonly infected by a wide variety of Salmonella serovars; one serovar may be a predominant isolate in a country for several years before it is replaced by another serovar. Serovars vary geographically, but clinically significant S. Typhimurium and S. Enteritidis were identified as the most common serovars reported globally ***(Fsanz, 2005)***. The most prominent serovars identified in our study were S. Typhimurium (75%), followed by S. Lagos (25%).Another study in Egypt reported a predominance of S. Enteritidis and S. Typhimurium from chicken (58.33% and 41.66%, respectively ***(Nagwa Rabie et al., 2012)***. In Saudi Arabia, S. Enteritidis and S. Typhimurium dominated among the recovered Salmonella serovars from chicken (55.56% and 22.22%, respectively) ***(Moussa et al., 2010)***. In Nigeria, *S.* Typhimurium and *S.* Enteritidis are increasingly isolated ***(Akinyemi et al., 2007).*** Interstingly, *S.*lagos was isolated and identified with relatively high proportion rate (25%).These results agree with another study conducted in Egypt (10%) ***(Jakee et al., 2014)***.

With respect to the antimicrobial susceptibility testing of Salmonella serovars to nine different antibiotics, Salmonella isolates showed different degree of sensitivity to antimicrobial agents higher degree of sensitivity were observed to ciprofloxacin, gentamicin and sulphamethoxazole/ trimethoprim with percentages comparable to those found in many developing countries, especially Bangladesh, Nigeria, and Pakistan ***(Boris et al., 2012, Ramya et al.,***

2013 and Umeh and Enwuru 2014). All isolates showed resistance to amoxicillin/clavulanic acid, doxycycline, ampicillin, ceftriaxone. multidrug resistance *Salmonella* spp. had spread due to the extensive use of antibiotics ***(Munawwar et al., 2010).***

In recent years, antibiotic resistance in Salmonella has assumed alarming proportions worldwide ***(Murugkar et al., 2005).*** Monitoring the drug resistance pattern among the isolates not only gives vital clues to the clinicians and farmers regarding therapeutic regime to be adopted against individual cases, but it is also an important tool to devise a comprehensive chemoprophylactic and chemotherapeutic drug schedule on flock basis within a geographical area. Additionally, the recent occurrence of multidrug resistance may lead to treatment failure ***(Yan et al., 2003).***

In this study, absolute resistance detected to ampicillin. Same results were reported in Ethiopia, ***(Reda et al., 2011)***, ***(Olivera et al., 2003)*** *and* ***(Suresh et al.,2006)*** while in Egypt, ***(Bayomi, 2012 and Abdelfatah, 2014)*** resistant to Ampicillin(84.4%) and (70.9%) respectively and in Thailand,***(Chuanchuen and Padungtod,(2009)*** found resistance to ampicillin (87%%).

Resistance of Salmonella to Amoxicillin/clavulanic acid was (100%). These results agree with another report in South India, ***(Suresh et al., 2006).*** And higher than results in Eastern China (80%) ***(Yan et al., 2014).***

Resistance to ceftriaxone, with percentage of 100%. It is near with (78 %)in Malaysia **(Learn *et al.*, 2009)**, resistance to doxycycline was 100%.These results agree with result in Eastern China ***(Yan et al., 2014)***.

This resistance may be attributed to indiscriminate use of antibiotics at recommended doses or at therapeutic doses as feed additives to promote growth and as chemotherapeutic agent to control epizootic diseases on farms.

The most sensitive antimicrobial agent was ciprofloxacin followed by gentamicin and sulfamethoxazole/Trimethoprim as sensitivity to ciprofloxacin was (81.6%) the result agreed with (85.4%) in Croatia ***(Boris et al., 2012)***,*(* 87.88 %) in United Arab Emirates ***(Munawwar et al., 2010).*** Sensitivity to gentamicin was 78.9%.The result is in harmony with (85.4%) in Croatia ***(Boris et al., 2012)*** and(90%) in India ***(Ramya et al., 2013)***.

Resistance to Sulphamethoxazol/Trimethoprim was (13.5%) this result agree with (7.9%) in Ghana ***(Osei and Adu 2015)***, (20%) in Thailand ***(Sunpetch et al., 2005)***, and (31.6%) in *India* ***(Ammini et al., 2011)***.On the other hand the sensitivity to sulphamethoxazole-trimethoprim (SXT) was 73.7% similar results were reported in

Egypt ***(Jakee et al., 2014)*** as (70.6%).

Resistance rate to Amoxicillin was 5.2 %.These results are similar to Poland ***(Dariusz and Andrzej, 2004)***, but lower than (29.5%) in Egypt ***(Enas et al., 2015)***, and resistance rate to chloramphenicol was (7.9 %).These results are similar to the study in India (10%) ***(Ammini et al., 2011)***, (16.7%) in South Korea ***(Roy et al., 2012)***.The widespread usage of antimicrobial reagent in human and animal that may lead to an increase in the incidence of occurance of bacterial resistance to antibiotics in Ethiopia ***(Molla et al., 2003)***.

The development of antimicrobial resistance in zoonotic bacteria (Salmonella) constitutes a public health risk, as it may potentially affect the efficacy of drug treatment in humans ***(Abdellah et al., 2009)***. The differences between these results of the present paper and those of other researchers may be explained when several factors, such as differences in origin, period of collection and sampling procedure. These results indicate that the presence of Salmonella resistances to antimicrobial drugs is common in poultry and meat products. Further studies are needed to identify the sources and causes of this drug resistance.

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الملخص العربى

يعتبر ميكروب السالمونيلا من الميكروبات الخطيرة نظرا لانه يعتبر احدي مسببات الامراض المشتركة التي تنتقل للانسان من الحيوان فله دور مهم في التسمم الغذائي في الانسان.
فغالبا ما تنتقل السالمونيلا للإنسان من خلال تناول الغذاء، فالدواجن هي واحدة من أهم الناقلات لعدوى السالمونيلا معتمدة في ذلك على العديد من عوامل اهمها هي جينات الضراوة.
في هذه الدراسة تم تجميع 270 عينة من الدواجن (الأرانب والبط ودجاج اللحم والكتاكيت) من مزارع اماكن مختلفة بمحافظة الشرقية.
وقد تم عزل الميكروب اولا بالطرق التقليدية المعروفة بداية من عزلها علي شوربة البيتون ثم نقلها الي شوربة الرابابورت فسيليدس ثم زرعها بعد ذلك علي الماكونكي اجار و XLD اجار.
وكانت نتائج العزل علي الماكونكي اجار ظهرو المستعمرات شاحبة اللون او عديمة اللون بينما ظهرات علي XLD اجار كمستعمرات وردية او حمراء اللون وقد تظهر سوداء من المنتصف او لا .
وبعد ذلك تم اجراء الاختبرات البيوكيميائية للتاكد ما ان كانت تلك المستعمرات هي لبكتريا السالمونيلا اما لا. فظهرت في اختبار اليوريز باللون الاصفر حيث ان السالمونيلا تعطي نتيجة سالبة لهذا الاختبار فلا يحدث اي تغيير في اللون.بينما في اختبار تخمر السكر فقد اعطت نتيجة ايجابية وايضا في اختبار الليسين اعطت نتيجة ايجابية .
اظهرت نتائج الاختبارات البيوكيميائية عزل 38 عترة من السالمونيلا بنسبة 14.07 %.
واجريت الاختبارات المصلية لتصنيف عدد 8 عينات وهم يمثلوا جميع المزارع التي تم اخذ عينات منها وكانت النتائج 6 عزلات السالمونيلا التيفميوريم بنسبة 75٪ و 2 لسلالات لوجس السالمونيلا بنسبة 25٪.
اجري اختبار الحساسية لفحص مقاومة العزلات للانواع المختلفة من المضادات الحيوية واستخدم لذلك الاختبار 9 انواع من المضادات الحيوية وهم اموكيسيلين / كلافيولنك اسيد, امبيسيلين, ديوكسيسيكلين, اموكيسيليين, سيبروفلوكساسين,سيفتريكسون , سلفونامید/ ترايميثوبريم, جينتاميسين و كلورامفينيكول.
وكانت نتائج الاختبار مختلفة ولكن جميعها اشتركت في التعدد لمقاومة المضادات الحيوية فظهرت نسبة للمقاومة مع اموكيسيلين / كلافيولنك اسيد, امبيسيلين, ديوكسيسيكلين و ,سيفتريكسون بنسبة 100%. يليهم سلفونامید/ ترايميثوبريم, جينتاميسين بنسبة 13.2% لكلاهما ثم تقل المقاومة الي ان تصل لـــ 7.9% للكلورامفينيكول و 2.6% ل سيبروفلوكساسين.