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Original article

Antimicrobial resistance in *Escherichia coli* and *Salmonella spp.* isolated from table eggs shells and its contents sold at different markets in Peshawar, Pakistan

Rooh Ullah *[1,2], Manzoor [3], Mati Ur Rehman [1,4], Tarique Noorul Hasan [1,5], Muhammad Tahir [6], Hamid Ali Khan [7] , Ziaullah Shah [8], Qurat Ul Ain [1], Shabana Perween [1], Narmeen Arshad [1], Muhammad Yousaf [1], Muhammad Ammad [1], Amjad Hussain [1], Sajjad Hussain [9]

1- *Pure Health Laboratory, Mafraq Hospital, Abu Dhabi, United Arab Emirates.*
2- *Centre of Postgraduate Studies, Lincoln University College, Malaysia.*
3- *Department of Microbiology, Sarhad University of Science and Information Technology, Peshawar, Pakistan.*
4- *Ascencia Business School, College de Paris, France.*
5- *School of Life Science, Manipal Academy of Higher Education, Dubai, United Arab Emirates.*
6- *Federal General Hospital, Islamabad, Pakistan.*
7- *Bacha Khan University, Charsadda, KP, Pakistan.*
8- *CECOS University of Information Technology and Emerging Sciences, Peshawar, Pakistan.*
9- *Department of Microbiology, Harbin Medical University, China.*

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ABSTRACT

Background: The current study was carried out with the aim to reveal the presence of *Escherichia coli (E. coli*) and *Salmonella* species (*spp.)* in table eggs sold for human use in Peshawar, and their antimicrobial resistance to different drugs used in Pakistan for poultry and human practices. **Methods:** A total of 80 eggs were bought from different shops. *E. coli* and *Salmonella spp*. were isolated from 85.36% (70) and 14.63% (12) of the total collected egg samples (80), respectively. The antibiotics susceptibility test was done using the disk diffusion method. **Results:** The shells of eggs showed significantly higher contaminations as compared to egg internal contents for both *E. coli* and *Salmonella spp*. The isolated organism presented a significant resistance to multiple drugs. They were found to be completely resistant to penicillin G, nitrofurantoin, oxacillin, and sulfamethoxazole/trimethoprim. Furthermore, *Salmonella spp*. was also completely resistant to tetracycline. The susceptibility pattern of both these isolates was quite different for some antimicrobials, as *Salmonella* isolates were completely sensitive to neomycin, whereas 100% susceptibility was observed in *E. coli* isolates to amikacin, amoxicillin-clavulanic acid, levofloxacin, and neomycin. In addition, resistance frequency among isolates of *E. coli* against amoxicillin (77.14%), minocycline (54/28%), streptomycin (80%), and tetracycline (80%), and in *Salmonella spp.* against amikacin (50%), amoxicillin (66.66%), amoxicillin-clavulanic (66.66%), levofloxacin (33.33%), minocycline (50%) and streptomycin (83.33) were also high. **Conclusion:** Hence it can be concluded from the present study that the table eggs sold in the markets of Peshawar Pakistan are infected with multidrug resistance (MDR) strains of *E. coli* and *Salmonella spp*.

Introduction

In Pakistan, poultry farming is adopted widely, for consumption and business purposes. Science and technology are behind the poultry industry's successful expansion [1]. There are about 25000 poultry farms present in Pakistan, which

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* *Corresponding author:* Rooh Ullah
E-mail address: roohullah@bs.qau.edu.pk

provide jobs to about 15000 individuals. In Pakistan about 400 hatcheries are present. In addition, the country has feed mills (150), about 8.5 million broiler breeders, 0.428 million layer breeders, and 5.51 million metric tons consumed on their feed every year [2].

Egg from poultry farming is one of the most nutritious food that contain a high amount of proteins [3]. It is an economical source of food obtained from livestock and widely consumed as part of the diet, across the world [4]. Not only it is consumed directly but several other commercial products are prepared from it including sandwiches, hamburger, cakes, etc. Poultry is the 2nd most nutritive food [5]. Egg consumption is useful for the function of the brain and vision, as the egg's yolk contain huge amounts of Omega-3 Fatty acids. Eggs are also rich in different types of vitamins including vitamins A, B2, B9, B12 and D, E, K. In addition important minerals such as phosphorus and iron are present in eggs [6], but the high nutritive value of the egg makes it susceptible to different types of pathogenic bacteria [5].

It is possible for the egg to get contaminated horizontally and transovrially through the shell. There are different mechanisms by which egg can become contaminated such as exposure to contaminated environment [6]. Recently it has been documented that different types of bacteria such as *Salmonella*, *E. coli*, *Listeria* etc. can enter and contaminate the egg, which ultimately give rise to different types of food borne diseases [2]. Eggs act as a vector for pathogens like extra intestinal infectious *E. coli* [7]. *Escherichia coli (E. coli*) normally lives in the guts of human, poultry and animals. Most of the *E. coli* do not cause any harm to their host however some of the strains have been found linked to different types of food borne infections [8]. Such pathogenic species having adverse effects on human health and include, enterohaemorrahgic, enteropathogenic, shiga toxin (STX)-secreting, enteroinvasive, enterotoxigenic, enteroaggregative (EAEC) and cyto-lethal distending toxin *E. coli*. *Escherichia coli (E. coli*) with positive STX, infections leads to hemorrhagic colitis, hemolytic uremic syndrome that leads to thrombocytopenia and renal injuries [6].

Salmonella is also one of the main bacteria that is responsible for causing food borne diseases around the world [4, 9]. In Europe salmonellosis has been held responsible for causing 40% of the food borne diseases [10]. Therefore it is a major problem for human's health and causes a huge loss to the economy of countries worldwide. An association has been observed between the outbreaks of *S. enteritidis* and the consumption of contaminated egg [11]. According to reports of Egg Safety Action Plan of the US, 796 *S. enteritidis* outbreaks were documented from 1985 and 1998, which caused illness, hospitalization and deaths of 28,689, 2,839 and 79 peoples respectively. Most of the outbreaks (more than 75%) were found to be associated with commercial foods product prepared from eggs [12].

Increase in the emergence of antimicrobial resistant bacteria has raised a concern worldwide over the last few years [13]. The antimicrobial have been utilized in poultry industries as growth enhancers and also for the prevention of different types of diseases [14]. Wide array of antimicrobial are used including penicillin, sulfa, macrolides, tetracyclines, cephalosporins, and ionophores etc. Resistance to antimicrobial therapy, used for the treatment of food-borne diseases has been developed due to the over and unselective use of drugs in the poultry [15]. On the other hand the high level of disinfectant in poultry is another reason for antimicrobial resistant especially co- or cross-resistance [16]. Widespread bacteria have been reported to be isolated from egg like *E. coli, Salmonella, S. dysenteriae and* other species of *Enterobacteriaceae, Pseudomonas aeruginosa, Neisseria gonorrhoeae, Hemophilus influenza* etc. [15].

The present work was carried out with the purpose to study the prevalence and characterization of antimicrobial susceptibility pattern in *E. coli* and *salmonella spp.* isolated from the table eggs sold for human consumption.

Materials and methods

Samples collection

Eighty egg samples were bought for microbiological study from different shops in Landi Akhun Ahmad Peshawar. Though the eggs were stored at room temperature in the shops, the use of freshly laid (not older than a day) eggs for the study were ensured. Egg samples with feces contaminated shell were avoided. The egg samples were collected in sterile bags and care was taken to avoid the breaking or contamination of these egg samples. After collection, the eggs were carried to Microbiology lab, Sarhad University of Science and Information Technology, Peshawar. Samples were stored in

refrigerator at 4°C till processing. Eggs shell, white and yolk of these eggs were continuously examined for microbial contamination.

Isolation of *E. coli* and *salmonella* spp. from egg shell surface and from egg contents

To isolate *E. coli* and *Salmonella* spp., different culture media such as nutrient agar (OXOID, UK), Salmonella Shigella agar (OXOID, UK), eosin methylene blue agar (OXOID, UK) and macConkey agar (OXOID, UK) media were prepared. These media were prepared following manufacturer's instruction. All the media were autoclaved at 121^0C for 20 minutes for sterilization.

Bacterial species were cultured from the egg shell on to media. For this purpose, the surface of the eggs were swabbed with a cotton swab stick and then was transferred to culture media. The cotton swab was dipped in 0.1% peptone water each time before obtaining the bacterial species from the shell surfaces. All the plates were then incubated for bacterial growth at 37^0C for 24 hours.

Before culturing the inside content, each egg was surface sterilized using 70% ethanol. Then a hole was made in all the collected samples with the help of a sterile spatula. The content were mixed thoroughly inside till complete homogenization was achieved. After that 0.1 mL of the homogenized content or mixture (egg white and yolk) was obtained from each egg, by using a sterile needle and syringe. It was then dropped on different media plates and spread with a sterile cotton swab stick. A water proof PH meter (HANNA, HI 98/30) was used to determine the PH of all the collected samples.

Egg shell rinsete of 0.1mL and 1 ml of the egg content mixture of each egg were inoculated into 9 mL pre-enrichment-buffered peptone water medium separately, which after that were incubated at for 18-24 h at 37°C. For isolation of *Salmonella* spp., about 1mL from both the pre-enriched egg content and shell rinsete were inoculated into 10 ml each of nutrient broth. The media were incubated for 24-48 h at 37°C. Subcultring was performed by transferring enriched egg shell rinsete and egg content onto SS agar using a loop. All the plates were incubated at 37°C for 24 h. After the appearance of unconfirmed colorless and transparent colonies *Salmonella* spp., they were transferred to nutrient agar slants and were stored at 4°C. Furthermore, Gram staining and different biochemical tests were performed for identification.

For isolation of *E. coli*, pre-enriched egg shell rinsete and content were transferred onto separate macConkey media using a loop. All the plates were incubated at 37°C for 18-24 h. Pink color colonies was appeared, which were then inoculated into EMB agar. Further biochemical tests were performed to confirm the greenish metallic sheen producing *E. coli*.

Biochemical characterization of isolates

Biochemical identification of *E. coli* and *Salmonella spp.* was done using standard microbiological techniques [17]. The suspected *E. coli* and *Salmonella spp.* were subjected to different biochemical tests (citrate, triple sugar iron and urease test) after Gram staining. Following the biochemical characterization, confirmed *E. coli* and *Salmonella spp.* isolates were kept at 4°C in nutrient agar slants for determination of antimicrobial susceptibility tests.

Antimicrobial susceptibility of the isolates

After confirmation of *Salmonella spp.* and pathogenic *E. coli,* The antibiotic susceptibility test were performed by Kirby Bauer's disc diffusion method using 12 different antibiotics including; amikacin (AK 30µg), amoxicillin (AML 10µg), amoxicillin-clavulanic acid (AMC 30µg), levofloxacin (LEV 5µg), minocycline (MH 30µg), neomycin (N 30µg), nitrofurantoin (F 300µg), oxacillin (OX 5µg), penicillin G (P 10µg), streptomycin (S 20µg), sulfamethoxazole/trimethoprim (SXT 25 µg), tetracycline (TE 30µg).

Results

Prevalence of *E. coli* and *Salmonella spp.*

PH for examined samples ranged from minimum 7.48 to maximum 9.80 with the mean value 8.64. After identification it was observed that out of the 80 samples, 70 (85.36%) were positive for *E. coli,* in which 50 (60.97%) of isolates were from shell while 20 (24.38%) were isolated from the egg content mixture. Additionally, 12 (14.63%) samples produced *Salmonella spp*. in which 10 (12.19%) isolated were obtained from the shell while 2 (2.43%) isolates were from the egg white and yolk mixture **(Table 1)**.

All *E. coli* isolates were positive for TSI test and negative for both citrate utilization and urease tests while all *Salmonella spp.* isolates were positive for both TSI and citrate utilization tests but negative for urease test.

Antimicrobial susceptibility testing

Antimicrobial susceptibilities of *E. coli* isolates to the selected 12 antimicrobials agents were carried out. All *E. coli* isolates were sensitive to amikacin, amoxicillin-clavulanic acid, levofloxacin and neomycin, whereas, they presented resistance to nitrofurantoin, sulfamethoxazole-trimethoprim, penicillin G, and oxacillin. Out of 70 *E. coli* isolates, 80% offered resistance to both streptomycin and tetracycline, while 77.14% and 54.28% of the isolates presented resistance to amoxicillin and minocycline respectively **(Table 2)**.

The susceptibility patterns of *Salmonella spp.* to 12 different antimicrobial drugs have been illustrated in **(Table 3).** The susceptibility pattern showed that *Salmonella* species were 100% sensitive to neomycin and were resistant to penicillin G, oxacillin, tetracycline, nitrofurantoin and sulfamethoxazole/trimethoprim. 83.33% of the *Salmonella spp*. offered resistance to streptomycin, 66.66% of the isolates presented resistance to amoxicillin and amoxicillin-clavulanic acid, resistance to amikacin and minocycline was shown by 50% isolates, 33.33% isolates were observed to be resistant to levofloxacin.

Table 1. Prevalence of *Salmonella spp.* and *E. coli* in egg shell and content.

Organism	Number of samples	Egg shell (%)	Egg content (%)	Total (%)
Escherichia coli	80	50 (60.97)	20 (24.38)	70 (85.36)
Salmonella spp.		10 (12.19)	2 (2.43)	12 (14.63)

Table 2. Antibiotics susceptibilities of *E. coli* (*n*=70) isolates against 12 antibiotics.

Antibiotics	Antibiotic susceptibility (*n* =70)			
	Sensitive – S		Resistant – R	
	n	%	n	%
Amikacin (AK 30µg)	70	100%	0	-
Amoxicillin (AML 10µg)	16	22.85%	54	77.14%
Amoxicillin-clavulanic acid (AMC 30µg)	70	100%	0	-
Levofloxacin (LEV 5µg)	70	100%	0	-
Minocycline (MH 30µg)	32	45.71%	38	54.28%
Neomycin (N 30µg)	70	100%	0	-
Nitrofurantoin (F 300µg)	0	-	70	100%
Oxacillin (OX 5µg)	0	-	70	100%
Penicillin G (P 10µg)	0	-	70	100%
Streptomycin (S 20µg)	14	20%	56	80%
Sulfamethoxazole/trimethoprim (SXT 25 µg)	0	-	70	100%
Tetracycline (TE 30µg)	14	20%	56	80%

Table 3. Antibiotics susceptibilities of *Salmonella* isolates (*n*=12) against 12 antibiotics.

Antibiotics	Antibiotic susceptibility (n =12)			
	Sensitive – S		Resistant – R	
	n	%	n	%
Amikacin (AK 30µg)	6	50%	6	50%
Amoxicillin (AML 10µg)	4	33.33%	8	66.66%
Amoxicillin-clavulanic acid (AMC 30µg)	4	33.33%	8	66.66%
Levofloxacin (LEV 5µg)	8	66.66%	4	33.33%
Minocycline (MH 30µg)	6	50%	6	50%
Neomycin (N 30µg)	12	100%	0	-
Nitrofurantoin (F 300µg)	0	-	12	100%
Oxacillin (OX 5µg)	0	-	12	100%
Penicillin G (P 10µg)	0	-	12	100%
Streptomycin (S 20µg)	2	16.66%	10	83.33%
Sulfamethoxazole/trimethoprim (SXT 25 µg)	0	-	12	100%
Tetracycline (TE 30µg)	0	-	12	100%

Discussion

The contamination of the poultry products is a main concern for the poultry industry especially when it comes to international trade. Another concern that is linked with egg contamination is the transmission of disease to human population.

Our study confirmed that the eggs sold in Landi Akhun Ahmad, Peshawar for consumption were contaminated with *Salmonella spp.* and *E. coli.* Both these pathogens were isolated from the shell as well as the inside content of the egg. The above findings were in agreement with [18], which showed that microorganism are present both on the outside and inside of the egg. This is because of the common inside and outside environment shared by the egg and the feces of the laying hen. In addition, both the feces and egg passes through cloaca which can result in the contamination of egg. The pathogens found in the inside content is due the fact that the microorganism could be present in the oviduct or ovary of the hen. These pathogens are obtained from the food the birds feed on, or due to contact with contaminated environment. Furthermore, the microorganism can enter the egg from the shell through pores and crakes [19].

In our study we reported that contamination of the shell was greater than egg content. 60.97% of isolates from the shell were *E. coli* and 12.19% were *Salmonella*, while the *E. coli* and *Salmonella* obtained from the content of the egg were 24.38% and 2.43%, respectively. A study conducted in Enugu state of Nigeria documented that the contamination on the outside of the egg was greater than inside. They reported that the *E. coli* and *Salmonella spp.* isolated from egg shell were 38.2% and 8.8% respectively, while the isolation rate of *E. coli* and *Salmonella spp.* from the content were 16.2% and 1.5% respectively [5]. The rate at which *E. coli* is obtained from the egg shell is high and it is due to the fact that, *E. coli* normally live in the intestine of humans and birds and thus the chances of egg to get contaminate with it is high [20].

However our finding are not consistent with the study carried out by **Salihu et al.** [21] in which the isolation rate of *Salmonella* from content was higher than the shell, which is 22(6.11%) and 27(13.50%) from the egg shell and content respectively. This may due to the presence of

salmonella in bird's ovary or oviduct before the shell synthesis around it [21, 22]. In addition, the findings of **Mai et al.** [12] revealed that rate of isolation of *E. coli* from the egg shell was 27.5%, which is lower than isolation rate of *E. coli* (60.97%) reported in our study.

The emergence of resistance pathogens at high rate has raised concerns in the developing countries [23]. The misuse or overuse of the drugs, biological traits that bacteria exploit to confront antimicrobials and poor economic situations are the reasons behind the spread of antimicrobials.

Our findings revealed that the isolated *E. coli* and *Salmonella spp.* were completely resistant to antimicrobials like oxacillin (OX), penicillin G (P), nitrofurantoin (F) and sulfamethoxazole-trimethoprim (SXT). However 100% resistance in *Salmonella spp.* to tetracycline was also observed. The unselective use of antibacterial agents in Pakistan in veterinary and clinical practices is the main reason behind this. Study carried out by **Okorie-Kanu et al.** [5] showed similar results. They revealed that the *E. coli* and *Salmonella* showed 100% resistance to oxacillin, penicillin G, nitrofurantoin and sulfamethoxazole-trimethoprim and 100% resistance was reported in *Salmonella* to tetracycline. Our study is also consistent with **Khan et al.** [2] study, which documented that the isolated *E. coli* were weakly sensitive to amoxillin, and they were almost completely resistant to tetracycline. The high level of antibacterial resistance was similarly observed in SXT and tetracycline among *Salmonella* isolates by **Ekundayo et al.** [24].

In our study *E. coli* isolates were observed to be 100% sensitive to amikacin, amoxicillin-clavulanic acid, levofloxacin and neomycin, whereas *Salmonella* isolates were found to be completely sensitive to neomycin. Study carried out by **Okorie-Kanu et al.** [5] showed similar results, which revealed that *E. coli* were 100% sensitive to gentamicin, neomycin, ciprofloxacin and AMC acid while *Salmonella* showed 100% susceptibility to erythromycin, neomycin, and rifampicin. The reason for this may be the high cost of all the mentioned drugs, which farmers cannot afford and thus they choose cheap drugs as an alternative for the prevention of diseases and for other benefits from poultry. Thus the indiscriminate and misuse of these drugs result in the development of antimicrobials.

Hence it can be concluded from the present study that the table eggs that are sold in markets of Peshawar Pakistan are contaminated with infectious agents such as *E. coli* and *Salmonella spp.*, which are highly responsible for causing food borne diseases. In addition, it was also reported that both these pathogens were completely resistant to nitrofurantoin, oxacillin, penicillin and sulfamethoxazole/trimethoprim. The transmission of these resistant microorganism through food chain to human population could create problems in treating these pathogens. Therefore, it is very much necessary to aware people about the consequences of egg contaminations and educate them to properly handle table eggs to prevent unnecessary contaminations and avoid the utilization of poorly cooked eggs or products of the egg.

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No potential conflict of interest was reported by the authors

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