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Antibiotic resistances of *Salmonella spp*. in rectal samples from farm animals in Al-Diwaniyah City, Iraq

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Abstract

The principles of this work were to isolate the bacterium *Salmonella* and evaluate its antibiotic resistance capability from rectal fecal samples of some types of animals from Al-Diwaniyah City, Iraq. A total of 38 randomly collected samples (13, 16, and 9) from healthy cows, sheep, and goats, respectively, were employed to take samples via the use of commercially-available sterile cotton swabs. The samples were cultivated using different agar media. Then, the suspected *Salmonella* growth from the nutrient agar was subjected to biochemical identification. Later, antibiotic susceptibility assay was performed on the pure *Salmonella* isolates using Mueller-Hinton (M-H) agar and via the use of Kirby-Bauer (K-B) method. The cultivation and biochemical tests resulted in 7 (53%) for cows, 5 (31%) for sheep, and 5 (55.5%) for goats) pure isolates of *Salmonella* spp. Antibiotic resistance was varied among the isolates with MAR index of 0.39 for cows, 0.32 for sheep, and 0.24 for goats. Most isolates were resistant to almost all antibiotics, especially streptomycin, ampicillin, kanamycin, and novobiocin. The current findings reveal that fecal samples from cows, sheep, and goats have *Salmonella* spp with antibiotic resistance traits.

Keywords: Antibiotic resistance, farm animals, *Salmonella*.

Introduction

As a serious public health risk, salmonella illnesses have been linked to gastro-intestinal health problems. Antibiotics are routinely employed to cure and manage food-animal related salmonellosis, leading to the rising frequency of antibiotic resistance rates in *Salmonella* that has attracted international concern (1). Despite the fact that antibiotic resistance has existed for a long time, the "resistome" is a growing concern. Antibiotic usage in animal industries and human health care facilities, lack of sanitation, the migration of wildlife animals, and an inadequate sewage system are major factors in the rise of the global resistome. In healthcare, antibiotics are one of the most often utilized treatments for infections. During the 'golden age' of antibiotics, which spanned the 1930s to 1960s, a large number of antibiotics were developed. Antibiotic development slowed due to the emergence of drug-resistant organisms, and as a result, this period came to an end. The establishment of antibiotic resistance is predisposed by a lack of new antibiotics being developed or discovered, as well as overuse of existing antibiotics (2 ,3, 4). As the problem of antimicrobial resistance grows, so does concerns for human, animal, and environmental well-being. Multidrug-resistant (MDR) bacteria, sometimes known as "superbugs," are to blame for this antimicrobial resistance. Five MDR bacteria reside in the animal, human, and environmental niches. Growing antibiotic usage in people and animals, antibiotics marketed over-the-counter, expanded worldwide travel, inadequate sanitary conditions and manure/feces discharge of

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nonmetabolized antibiotics or their residue are all possible factors for "the global resistome" or antimicrobial resistance. These elements put the emergence of MDR pathogens in the population under genetic selection pressure (5,6). Latest information on the utilization of antibiotics in livestock has shown where the greatest concentration of antibiotic usage is taking place throughout the world, which impacts both economic sectors and public health. Cattle, chicken, and pigs are among the most widely antibiotic-given food animals, and antibiotic usage is expected to rise by up to 67% by 2030 in the globe 's most inhabited nations (7). The aim of this work was to isolation of *Salmonella* from rectal fecal samples of farm animals and evaluate its antibiotic resistance capability in Al-Diwaniyah City, Iraq.

Materials and methods

Sampling

A total of 38 animals (13 cows, 16 sheep, and 9 goats) were employed to take samples via the use of sterile cotton swabs dipped in sterile saline solution before they were used to take rectal fecal samples and put them in containers, which pre-loaded with 5ml sterile saline solution. Then, these containers were icebox-transported within two hours to the Laboratory of Microbiology, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah City, Iraq, for immediate Lab processing.

Salmonella cultivation and identification

The samples were cultivated using different agar media, such as 10ml-RAPPAPORT-VASSILIADIS-Soya broth for enrichment (incubated for 24hrs at 37°C), MacConkey agar, Brilliant Green agar, Salmonella-Shigella agar, deoxycholate citrate agar, and Xylose Lysine Deoxycholate agar, which were streaked from the enrichment growth and incubated for 24hrs at 37°C. After that, the growth was sub-cultivated onto nutrient agar plates for 24hrs at 37°C. Then, the suspected Salmonella growth from the nutrient agar was subjected to biochemical identification using Triple Sugar Iron agar, Synthetic Complete agar, methyl red test, and indole test, which were performed by using methods from Coles (8,9).

Antibiotic susceptibility test

antibiotic susceptibility assay was performed on the pure *Salmonella* isolates using Mueller-Hinton (M-H) agar and via the use of Kirby-Bauer (K-B) method. The M-H plates were incubated for 24hrs at 35-37˚C with the utilization of 13 antibacterial agents followed by calculating the multiple antimicrobial resistance (MAR) index (8,10). Determination of MAR index: Determination of MAR index followed the procedure described by (4), in which the number of antibiotics an isolate is resistant is divided by the total number of the antibiotics used in the study.

Ethical approval

The study protocol was approved by the College of Biotechnology, University of Al-Qadisiyah, Iraq.

Statistical analysis:

For statistical analysis, a Chi square test (X^2) was performed to assess the significancy, with the IBM Statistical Package for Social Sciences (SPSS), version 27. Values less than or equal to 0.05 were considered statistically significant.

Results

The cultivation and biochemical tests resulted in 17 (7 (53%) for cows, 5 (31%) for sheep, and 5 (5.5%) for goats) pure isolates of *Salmonella* spp. (Table 1 and 2). Antibiotic

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resistance was varied among the isolates with MAR index of 0.39 for cows, 0.32 for sheep, and 0.24 for goats (Table 2 and 3 and Figure 1).

Table 1: *Salmonella* occurrence in rectal fecal samples.

Type of animals	Number of samples	Number of *Salmonella* positive samples	Percentage (%)
Cows	13	7	53
Sheep	16	5	31
Goat	9	5	55.5

Table 2: *Salmonella* spp. based biochemical features

Features	Outcomes
Capsule	-
Catalase	+
Citrate	-
Flagella	+
Gas	-
Gelatin hydrolysis	-
Gram staining	-
Urease	-
H_2S	+
Indole	-
Motility	+

Negative, +: Positive.

Table 3: Antimicrobial susceptibility test of *Salmonella* spp isolates from cows, sheep, and goats

					Antibiotic Sensitivity									
No.	Isolate code	Tet 30µg	ST 20 µg	Cho 30 µg	Bac 10 µg	Gen 10 µg	Pol 10 µg	Pen 10 µg	Met 5 µg	Amp 10 µg	Nit 200 µg	Kan 30 µg	Nor 5 µg	Van 30 µg
1.	SC4	RES	RES	RES	SEN	SEN	RES	RES	SEN	RES	RES	RES	RES	RES
2.	SC5	RES	RES	SEN	RES	SEN	SEN	RES	RES	RES	RES	RES	RES	RES
3.	SC8	SEN	RES	SEN	RES	RES	RES	SEN	SEN	RES	SEN	RES	RES	SEN
4.	SC10	SEN	RES	RES	SEN	RES	RES	SEN	RES	RES	SEN	RES	RES	SEN
5.	SC11	SEN	RES	SEN	SEN	RES	RES	SEN	RES	RES	SEN	RES	RES	SEN
6.	SC12	SEN	RES	SEN	RES	RES	RES	RES	RES	RES	RES	RES	RES	SEN
7.	SC13	RES	RES	SEN	RES	I	RES	RES	RES	RES	RES	RES	RES	RES
8.	SS1	SEN	RES	SEN	SEN	RES	RES	RES	SEN	RES	RES	RES	RES	RES
9.	SS3	RES	RES	SEN	RES	RES	RES	RES	SEN	RES	SEN	RES	RES	RES
10.	SS12	RES	RES	I	SEN	RES	RES	RES	RES	RES	SEN	RES	RES	RES
11.	SS14	RES	RES	RES	SEN	RES	RES	RES	SEN	RES	SEN	RES	RES	RES
12.	SS8	SEN	RES	RES	RES	RES	SEN	RES	SEN	RES	SEN	RES	RES	SEN
13.	SG3	SEN	RES	RES	RES	RES	SEN	RES	SEN	RES	SEN	RES	RES	SEN
14.	SG4	RES	RES	RES	RES	RES	RES	SEN	SEN	RES	SEN	RES	RES	RES
15.	SG5	RES	RES	SEN	RES	SEN	RES	SEN	RES	RES	SEN	RES	RES	RES

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16.	SG7	RES	RES	SEN	RES	SEN	RES	RES	RES	RES	RES	RES	RES	RES
17.	SG9	SEN	RES	SEN	RES	RES	RES	RES	RES	RES	RES	RES	RES	RES

TE: Tetracycline TE, ST: Streptomycin, Cho: Chloramphenicol, Bac: Bacitracin, Gen: Gentamicin, Pol: Polymyxin-B, Pen: Penicillin-G, Met: Methicillin, Amp: Ampicillin, Nit: Nitrofurantoin, Kan: kanamycin, Nor: Novobiocin, and Van: vancomycin.

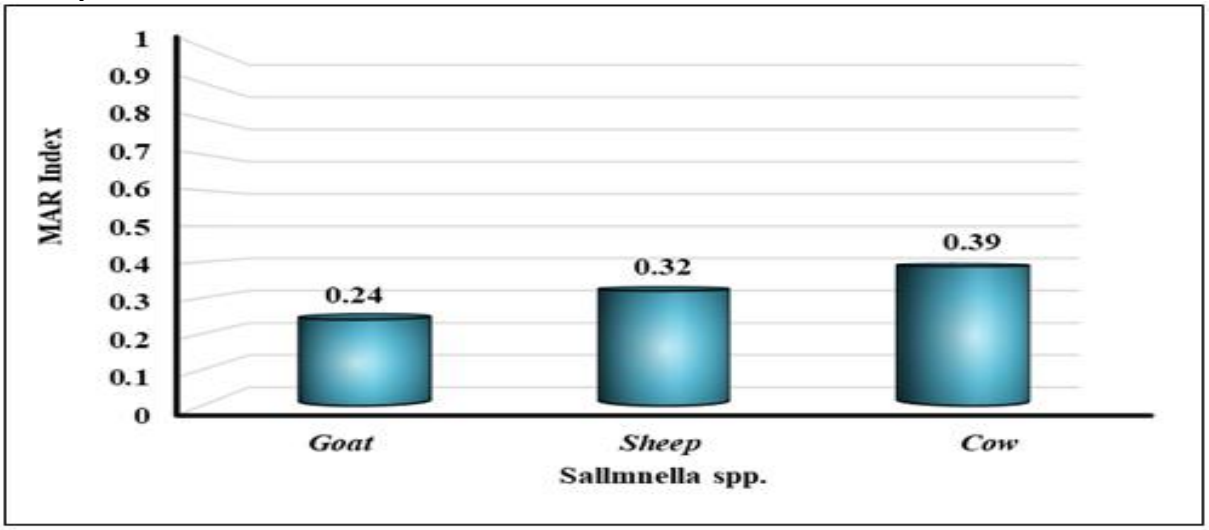

Figure 1: Multiple antimicrobial resistance (MAR) Index of antibiotic susceptibility test for *Salmonella* spp. isolates from cows, sheep, and

Discussion

Among foodborne pathogens, *Salmonella* species rank as the third highest cause of mortality from diarrheal infections in the whole globe. Food derived from animals is the principal way through which this disease is transmitted to humans (11). The current findings revealed that antibiotic resistance varied among the current study isolates with MAR index of 0.39 for cows, 0.32 for sheep, and 0.24 for goats, which agree with those by Ramatla *et al* (12), who mentioned that *Salmonella* spp. recovered from different animal and environmental niches showed antibiotic resistant in (92.0%), (89.3%), (77.4%), (72.6%), (67.4%), and (52.2%) of the isolates against sulphonamides, enrofloxacin and erythromycin, oxytetracycline, imipenem, tetracycline, and trimethoprim, respectively. Tetracyclines decrease protein production by adhering to the small ribosomal subunit and inhibiting aminoacyl-tRNA from adhering to the protein manufacturing machinery. Tetracycline resistance may be acquired by the complex synthesis of several systems such as efflux pumps, enzyme inhibition, and mutations. Tetracyclines are the main frequently utilized or excessively utilized antibiotics in food animal industry. This is attributable to the truth that over-the-counter veterinary drugs are both reasonably priced and extensively accessible (13 , 14, 16). Eagar et al. (17) reported that the most frequently used antibiotic agents in animals at 16.7% and 12.4% were tetracyclines and sulphonamides, respectively. The analysis by Ramatla *et al* (12) revealed that streptomycin was resisted by about 38% of *Salmonella* isolates. However, high rates of 80%, 95%, 65.5%, and 20.1% of resistance against streptomycin were detected from Saudi Arabia, Italy, and Egypt. Due to the fact that streptomycin is on the top-listed of the most utilized antibiotics in food animal industry, frequent rates of antibiotic resistance by

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Salmonella isolates can be highly recorded (16,18 ,19, 20).

Conclusion

The current findings reveal that fecal samples from cows, sheep, and goats have *Salmonella* spp with antibiotic resistance traits. This problem should be faced with specific strategies to decrease antibiotic resistance in farm animals via the use of controlled antibiotic utilization and only in critical needs to use these medicines.

Conflict of interest

The study has no conflict of interest.

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