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Antibiotic resistance profile of *Salmonella typhimurium* isolated from poultry chicken and domestic goat in Punjab, Pakistan

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Abstract

Introduction: Salmonella is the main source of foodborne diseases and internal microorganisms as the species *S. typhi* and *S. enteritidis*. From poultry and animals, it is transferred in different ways like poultry feed, air, debris, unhealthy environments and vectors such as insects, rodents, and human beings. Objectives: This study focuses on determining the prevalence of *S. typhi* in goats and chickens. and to assess antibiotic susceptibility patterns for different antibiotics used against *S. typhi*. Methods: Numerous stool samples were collected from various locations on animal and poultry farms for Salmonella isolation, Salmonellosis emerges as a highly impactful disease-causing significant mortality in goats and chickens. The study employs various culture-based methods for the pathogenicity of Salmonella. Antibiotics including Oxacillin, Ceftazidime, Nitrofurantoin, Ofloxacin, and Nalidixic acid are employed to measure the zone of inhibition, susceptibility, and resistance patterns. Results: It was found that salmonella typhi was obtained during the experimental trial. Different antibiotics show different results including Nitrofurantoin, Ceftazidime, and Ofloxacin (5 µg) are identified as effective antibiotics for treating bacterial infections in chickens. Nitrofurantoin and Ofloxacin are deemed suitable for treating bacterial infections in goats, while caution is advised against the use of Oxacillin and Ceftazidime due to observed resistance patterns. Conclusions: The study concludes that antibiotics like nitrofurantoin and ofloxacin are effective in treating bacterial infections in goats, while nitrofurantoin, ceftazidime, and ofloxacin (5 µg) are recommended for chickens. Furthermore, the overuse of medicines in the field of animal husbandry emerges as a significant concern for human health.

Keywords: Goat, Chicken, *Salmonella typhimurium,* Antibiotics, Resistance, Pakistan

Introduction

Enterobacteriaceae is a huge family consisting of gram-negative microbes many of which are disease-causing microorganisms like the group *Salmonella*. It is a gram-negative, oxidase negative and lactose-negative moving bacterium and has worldwide distribution. It is a very strong pathogen and can live in every place and every condition as can survive in water and may also live in hard places for a long period. It has several species which cause diseases in humans as GITs infections and diarrhea in livestock and poultry [1]. Salmonella is the main source of foodborne diseases and internal microorganisms as the species *S. typhi* and *S. enteritidis*. Currently, the names serotypes have been added to salmonella species. Few microbes of the salmonella family are host-specific like in cattle Salmonella Dublin lives in pigs. Salmonella choleraesuis lives. So, in humans, when these pathogens create infection, they are very dangerous and fatal. But some others have many hosts and can live everywhere [2].

In undomesticated animals and also in pet animals, salmonella broadly prevails. In food animals, they are dominant as in poultry, pigs and cattle and in domesticated animals like cats, dogs, birds etc. So, salmonella can easily pass from food animals to humans and can also disturb the entire food chain. Salmonellosis is a disease caused by salmonella. In humans, it occurred due to the taking of polluted food such as eggs, mutton, poultry and milk. Green vegetables polluted by feces of animals also cause Salmonellosis. Sometimes it occurs due to direct contact with pet diseased animals [3].

From the start of the 1990s, salmonella microbe is insusceptible to the scope of antibiotics have appeared and now it has become a major public problem. It has a broad prevalence in every place. The rising antibiotic insusceptibility of salmonella, as well as its commonness, harmfulness, and its stability in all types of environments, becomes a big dare at the global level. For the cure of animals and treating diseases, people use different medicines and drugs in animals and poultry as well as for increasing the growth rate of animals. Although these drugs aid in the medication of animals and improve their physical state their overuse causes antibiotic resistance and their power becomes inadequate for the growth of pathogens [4].

At the global level Salmonellosis is a big cause of gastroenteritis infection yearly as it causes

2.8 billion infections and as well as causes great losses economically It is caused by animal food and its related products such as chicken, eggs, dairy elements [5, 6]. From poultry, Salmonella is transferred in different ways like poultry feed, air, debris, unhealthy environments and vectors such as insects, rodents, and human beings. Commonly, it is very difficult to stop salmonella prevalence in poultry as the unhygienic conditions may occur at any stage of chicken processing. And the use of antibiotic drugs causes antimicrobial prevalence [7, 8]. In the European Union 2006, officially stopped the extra use of medicines like some medicines are used in the feed of animals to enhance milk or meat production or to improve the growth rate level [9]. The purpose of this study was to determine the antibiotic resistance and susceptible patterns of non-typhoid salmonellae of chicken and goat, from district Okara Punjab Pakistan.

Materials and Methods

Study duration and sample collection

The study was conducted from August 2021 to August 2022 in various village areas and livestock zones within District Okara. Sampling days were randomly assigned, with bi-monthly field visits. This study was conducted with the help of Higher Education Commission under project Ref. No. 473/IPFP-II(Batch-I)/SRGP/NAHE/HEC/2020/121, Islamabad, Jan 15, 2021.

Consent to publish and Ethical concern

The study adhered to the Declaration of Helsinki, and ethical approval was obtained from the university. Consent for the study and publication was obtained from animal owners.

Sample collection and placement

Fecal samples were collected aseptically from animal holders using sterile zipper bags, containers, and gloves and transported to the molecular biology laboratory of University of Okara. A total of 80 samples were collected, with 2 from goats and 3 from chickens testing positive for bacteria. Positive samples were retained for further analysis, and the rest were discarded after being collected in uncontaminated bags. Samples were promptly transported to the microbiology laboratory using insulated ice bags, ensuring purity during collection and preventing contamination during transportation and storage

Bacterial isolation and biochemical tests

The collected samples underwent routine microbiologic and biochemical tests to detect various enteropathogenic bacteria. Cultures were supplemented in nutrient broth and incubated at 37°C for 20 hours. Stool samples were streaked on Salmonella-Shigella agar for Salmonella spp. Biochemical tests, including Gram's stain, catalase, and indole tests, were performed for bacterial confirmation, gram staining [10], catalase test as Positive results were indicated by bubble production when bacterial colonies were submerged in 3% H2O2 [11] and Indole test as Positive results were confirmed by the production of a red color using Kovac's reagent

Disc diffusion method for antimicrobial susceptibility testing

Antimicrobial sensitivity testing was conducted using the Kirby Bauer disc diffusion method as per the National Committee for Clinical Laboratory Standards. Mueller Hinton agar medium was used for all disc diffusion tests. The zone of inhibition around the antimicrobial agent discs determined the antibiotic's efficacy [12-14].

Antibiotic discs used against bacteria

The following antibiotics were used for susceptibility testing: Oxacillin (OX, 1µg/disc), Ceftazidime (CAZ, 30µg/disc), Nitrofurantoin (F, 300µg/disc), Ofloxacin (OFX, 5µg/disc), and Nalidixic acid (NA, 30 µg/disc) [15].

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 20.0. Data, expressed in frequencies and percentages, were collected from different situations and subjected to analysis.

Results

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Biochemical tests for bacterial isolation

The microbiological identification of bacterial colonies, presumed to be Salmonella, was conducted through various biochemical tests, including Gram staining, catalase test, and indole test. The results are visually presented in Figure 1. The Gram staining revealed that the bacteria exhibited characteristics typical of gram-negative organisms, appearing as rod-shaped structures with a pink color under the microscope (Figure 1a). Furthermore, the catalase test demonstrated bubble formation (Figure 1b), affirming the presence of catalase enzyme. The indole test, depicted in Figure 1c, displayed a distinct color change, with the red coloration indicating a positive result. These biochemical tests collectively supported the preliminary identification of the bacterial isolates as Salmonella, showcasing their characteristic morphological features and biochemical responses

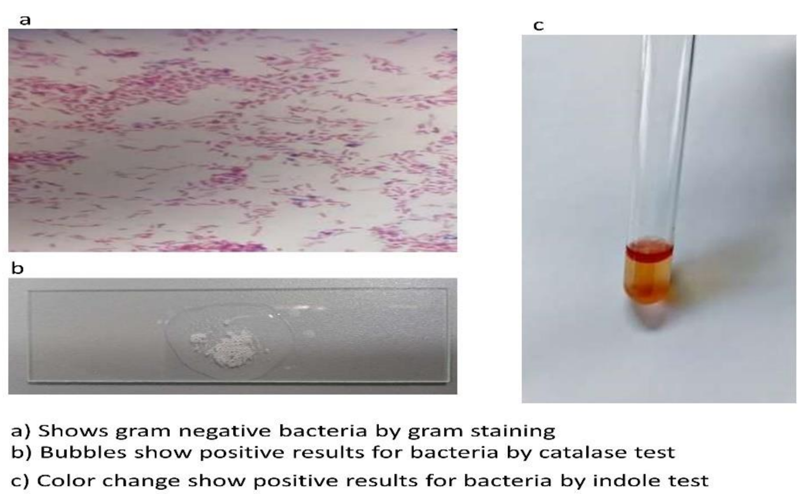

Figure1: Confirms gram-negative bacteria on different samples

Antibiotic resistance disc chart for chicken and goat

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In evaluating the antibiotic resistance of *S.typhi*, five antibiotics including Oxacillin (OX, 1µg), Ceftazidime (CAZ, 30µg), Nitrofurantoin (F, 300µg), Ofloxacin (OFX, 5µg), and Nalidixic acid (NA, 30µg) were applied to samples from both goats and chickens. The antibiotic resistance disc chart specifically focuses on chicken samples.

Antibiotic resistance disc chart for chicken

For chicken samples, three positive isolates underwent testing against the mentioned antibiotics. Table 1 displays the antibiotic susceptibility patterns, indicating susceptible, intermediate, and resistant values for each antibiotic. Notably, Ofloxacin (OX) with a concentration of 1µg showed a zone of inhibition of 10mm, falling into the resistance category according to Kirby disc diffusion chart standards. Ceftazidime (CAZ) with a concentration of 30µg displayed no zone of inhibition, categorizing it as resistant. Nitrofurantoin (F) with a concentration of 300µg demonstrated a zone of inhibition of 20mm, falling within the susceptible range. Conversely, Ofloxacin (OFX, 5µg) and Nalidixic acid (NA, 30µg) displayed no zones of inhibition, indicating resistance according to standard values. In conclusion, Nitrofurantoin, Ceftazidime, and Ofloxacin (5 µg) are identified as effective antibiotics for treating bacterial infections in chickens. However, the study suggests refraining from using all other antibiotics tested for chicken treatment due to observed resistance patterns.

Table 1: Shows antibiotic resistance patterns in chicken

Sr. no	Antibiotics	Symbols	Conc.	Susceptible	Intermediate	Resistance	Findings (1)	Findings (2)	Findings (3)	Results (1)	Results (2)	Results (3)
1	Ofloxacin	OX	1µg	≥13	11-12	≤10	10mm	11mm	10mm	Resistance	Susceptible	Resistance
2	Ceftazidime	CAZ	30µg	≥21	18-20	≤17	0mm	0mm	0mm	Resistance	Susceptible	Susceptible
3	Nitrofurantoin	F	300µg	≥17	15-16	≤14	20mm	17mm	22mm	Susceptible	Susceptible	Susceptible
4								32mm	25mm	Resistance	Susceptible	Susceptible
5	Ofloxacin	OFX	5µg	≥16	13-15	≤12	0mm					
6	Nalidixic acid	NA	30µg	≥19	14-17	≤18	0mm	0mm	15mm	Resistance	Resistance	Resistance

Antibiotic resistance disc chart for goat

In the assessment of antibiotic resistance for *S.typhi* in goat samples, two positive isolates were examined against a range of antibiotics, and the results are summarized in Table 2. Oxacillin, with a concentration of 1µg, exhibited a 10mm zone of inhibition, categorizing it as resistant based on the Kirby disc diffusion chart standards. Ceftazidime at a concentration of 30µg

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displayed no zone of inhibition, indicating resistance. Nitrofurantoin, with a concentration of 300µg, demonstrated a 16mm zone of inhibition, suggesting susceptibility. Ofloxacin, at 5µg, showed a substantial 29mm zone of inhibition, classifying it as susceptible. Conversely, Nalidixic acid at 30µg showed no zone of inhibition, indicating resistance. Consequently, Nitrofurantoin and Ofloxacin are deemed suitable for treating bacterial infections in goats, while caution is advised against the use of Oxacillin and Ceftazidime due to observed resistance patterns. This underscores the significance of judicious antibiotic use to ensure effective treatment and mitigate antibiotic resistance in goat populations

Table 2: Shows antibiotic resistance patterns in goat

Sr. no.	Antibiotics	Symbols	Conc.	Susceptible	Intermediate	Resistance	Zone (1) (mm)	Zone (2) (mm)	Results (1)	Results (2)
1	Oxacillin	OX	1µg	≥13	11-12	≤10	10mm	10mm	Resistance	Resistance
2	Ceftazidime	CAZ	30µg	≥21	18-20	≤17	0mm	0mm	Resistance	Resistance
3	Nitrofurantoin	F	300µg	≥17	15-16	≤14	16mm	16mm	Susceptible	Susceptible
4	Ofloxacin	OFX	5µg	≥16	13-15	≤12	29mm	29mm	Susceptible	Susceptible
5	Nalidixic acid	NA	30µg	≥19	14-17	≤18	0mm	0mm	Resistance	Resistance

Discussion

This research delineates resistance patterns observed in chickens and goats from the Okara district. Within the sampled population, 34% of instances were identified as contaminated with Salmonella spp., and a striking 97% (66) of isolates displayed multidrug resistance. The emergence of multidrug resistance in various Salmonella serovars linked to poultry has been documented [16]. The prevalence of multidrug resistance in foodborne Salmonella isolates poses a significant challenge to public health, underscoring the imperative for continuous surveillance and judicious antibiotic usage. Notably, the study identified a 100% resistance to penicillin, followed by high resistance rates to oxacillin and clindamycin (97%), vancomycin (92.6%), erythromycin (89.7%), and ampicillin (85.2%). Similar findings were reported by [17] who also observed 100% penicillin-resistant Salmonella isolates. Additionally, [18] reported substantial resistance to ampicillin (81.25%) among Salmonella isolates. The investigation further revealed resistance percentages to tetracycline (67.6%), streptomycin (61.7%), neomycin (55.8%), and cephalothin (52.9%). Throughout the study, Nitrofurantoin, Ceftazidime, and Ofloxacin (5 µg) exhibited efficacy in treating bacterial infections in chickens, while other antibiotics used during the study were found ineffective. A study by [15] also indicated that isolates from chickens exhibited the highest resistance to tetracycline, followed by resistance to nalidixic acid, sulphamethoxazole, streptomycin, ampicillin, and cephalothin.

In goats, chickens, and pigs, all Salmonella isolates exhibited sensitivity to cefotaxime, enrofloxacin, florfenicol, and polymyxin B. However, resistance was prevalent among most Salmonella isolates, with trimethoprim showing the highest resistance, followed by ampicillin, oxytetracycline, and kanamycin [19]. A comprehensive analysis revealed that 18 Gram-positive and 13 Gram-negative bacterial species from goats and sheep displayed resistance to ten antibiotics: penicillin, ampicillin, amoxicillin, chloramphenicol, streptomycin, tetracycline, cephalothin, gentamicin, ciprofloxacin (CIP), and sulfamethoxazole [20]. In the context of this study, it was determined that Nitrofurantoin and Ofloxacin could be effectively utilized for treating bacterial infections in goats, while the remaining antibiotics exhibited resistance against bacteria.

A relevant study conducted in Canada highlighted a substantial level of resistance in animals such as goats and poultry chickens, which serve as primary sources of meat for human consumption. The pathogen exhibited resistance to antibiotics including Ampicillin, Streptomycin, Sulphamethaoxazole, and tetracycline [21] similarly, in Ethiopia, a related study revealed a notably high level of Salmonella resistance to various types of medicines, particularly when analyzing the resistance patterns of the pathogen isolated from stool specimens of chicks [22]. In Alberta, similarities with the present study were identified, indicating a significant prevalence of resistance against antibiotics such as Ampicillin, Kanamycin, Sulphamethoxole, Streptomycin, and tetracycline [23]. Additionally, findings from other researchers concerning *S.typhi* isolated from stool samples of livestock, including cattle and goats, showed higher resistance compared to chicks. The antibiotics to which the highest resistance was observed included Ampicillin, Nalidixic acid, Streptomycin, Tetracycline, and Chloramphenicol.

Conclusion

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In conclusion, our findings highlight the significant impact of the excessive and improper use of medicines in the field of animal husbandry on human health. The indiscriminate use of medications in livestock farming poses a serious threat to human health. Specifically, this study underscores the efficacy of antibiotics such as nitrofurantoin and ofloxacin for treating bacterial infections in goats, and nitrofurantoin, ceftazidime, and ofloxacin (5 µg) for chickens. The ineffectiveness of all other antibiotics is evident. This study emphasizes the urgent necessity for additional research in the field of microbiology to better understand the antimicrobial resistance of Salmonella. The pathogen's presence in livestock and poultry poses a concealed risk and underscores its adverse effects on global public health. Further investigations are warranted to comprehensively explore and address this issue.

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Authors Contribution

All authors contributed equally

Conflict of interest

None

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