Research Article
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Comparative Analysis of Antimicrobial Resistance Patterns in *Staphylococcus aureus* and *Escherichia coli* Across the one Health Interface

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Abstract: The livestock sector is a key contributor to Pakistan's economy, accounting for 14.36% of GDP and supporting rural livelihoods. This study investigated the prevalence and antimicrobial resistance profiles of *Staphylococcus aureus* and *Escherichia coli* from animal, human, and environmental sources in District Bahawalpur. A total of 420 samples were collected, including sheep (n=100), goat (n=100), environmental samples (n=120), and livestock handlers (n=100). Isolation and identification were performed using standard microbiological techniques, followed by biochemical confirmation. Antimicrobial susceptibility testing was conducted using the disk diffusion method. The overall prevalence of *S. aureus* was 29.17% in sheep milk, 31.67% in goat milk, 30% in humans, and 23.33% in environmental samples. The prevalence of *E. coli* was 17.5% in sheep, 23.33% in goats, 16% in humans, and 26.67% in the environment. Statistical analysis showed no significant differences in prevalence among sample sources ($p > 0.05$), indicating a uniform distribution across the animal–human–environment interface. Antimicrobial susceptibility profiling revealed widespread resistance among both pathogens. High resistance was observed against commonly used antibiotics, including ampicillin and oxytetracycline ($P<0.05$), which showed complete resistance across isolates. Ofloxacin exhibited moderate to high resistance depending on the source, particularly in environmental isolates. In contrast, fosfomycin and spiramycin showed comparatively better activity against *E. coli*, especially in goat-derived isolates. Overall, approximately 30% of samples were positive for *S. aureus* and 21% for *E. coli*, demonstrating their widespread occurrence. The study highlights the emergence of antimicrobial-resistant strains across animal, human, and environmental reservoirs. These findings emphasize the need for rational antibiotic use, continuous surveillance, and integrated One Health strategies to limit the spread of resistant pathogens across interconnected ecosystems.

Keywords: *E. coli*; *S. aureus*; Prevalence; One Health; AST

1. Introduction

Pakistan is an agricultural country, and it is the major sector of Pakistan′s economy. This sector directly assists the population of the country and contributes 22.9% of GDP with 1.55% of annual growth. Share of

livestock in GDP is 14.36% in the year of 2022-23 (Pakistan economic survey 2022-23). Livestock sector provides milk, meat and eggs. Pakistan is the world's third-largest producer of milk. More than 8.0 million rural families earn their livelihood by raising livestock [1]. The rate of population of main farm species (sheep, buffaloes, goats, and cattle) has constantly enlarged. Pakistan contains 55.5 million cattle, 45.0 million buffaloes, 32.3 million sheep and 84.7 million goats (Pakistan economic survey 2022-23). Small ruminants have been the major role in agricultural industry and it has major contribution in productivity, economic importance and food production [2].

Livestock is essential for guaranteeing food security and providing Pakistan's population with a sustainable source of animal protein. Livestock products including meat, milk, and eggs are nutrient-dense and crucial in the fight against malnutrition, especially in children [3]. Additionally, the industry helps to meet the demand for premium animal products, lowering the nation's dependency on imports and boosting self-sufficiency.

Staphylococcus aureus is one of the zoonotic organisms which spreads through interaction among people, livestock and its products [4]. *S. aureus* is a gram-positive bacterium. It is facultative anaerobe with circlet shape and mostly present in "grape-like" clusters. It grows in the media with up to 10% salt concentration and shows golden or yellow color colonies (aureus means golden or yellow). It is catalase and coagulase positive [5].

Antibacterial resistance has been reported to *Staphylococcus aureus* including resistant to the antibiotic methicillin, penicillin, vancomycin and many others [6]. The majority of *S. aureus* strains have resistance to three or more antibiotics, including vancomycin, ciprofloxacin, erythromycin, clindamycin, and trimethoprim-sulfamethoxazole [7].

Escherichia coli, is a typical bacterium that normally lives in both people and animals' guts [8]. Some *E. coli* strains can, however, result in infections that range from minor diarrhea to serious conditions like urinary tract infections (UTIs) as well as sepsis [9]. The species of the infecting bacterial pathogen can influence the outcome of a mammary glands' infection. Coliform bacteria which are Gram-negative often cause severe acute swelling with clinical sings [10]. One Health is a theory that emphasizes the value of an integrative approach to illness control by acknowledging the linkages between humans, animals, and environmental health [11]. Understanding the prevalence and antibodies of *E. coli* at one healthcare facility can shed light on how this bacterium is disseminating as well as help to inform control measures.

E. coli is distinguishing integrant of both human and animal gastrointestinal flora (8). Through contaminated water and food, it spreads to those with impaired immune systems, including kids, pregnant women and people with chronic diabetes in particular [12]. Many different strains of *E. coli* have become important zoonotic foodborne pathogens [13]. Foodborne illnesses are frequently associated with diarrheagenic pathotypes of enterotoxigenic *E. coli* (ETEC), enteropathogenic, Shiga toxin-producing, enteroaggregative and enterohemorrhagic *E. coli* [14].

Antimicrobial resistance is a worldwide problem that poses a serious risk to both human and animal health [15]. Although utilizing antimicrobials in excess of the allowable limit and abusing them were the root causes of this issue, many other factors also played a role in its emergence.

Several factors contribute to the increase in antibiotic resistance, including continued antimicrobial drug use and antimicrobial drugs misuse. There is an increase in resistance to the oldest antibiotics, which have been utilizes the longest in human or veterinary medicine [16]. Antibiotics are used in chicken production and to combat infectious illnesses. Bacteria are known to develop resistance to antimicrobials as a result of antimicrobial exercise, particularly misuse [17]. Antibiotic therapy is regarded as the most critical factor supporting the creation and dissemination of antibiotic resistance bacteria in both human and veterinary medicine [18].

A major global medical issue that is receiving more attention is antimicrobial resistance (AMR). Given that the great number of antibiotics in the livestock industry contributes to the development of resistance, the class of antimicrobials that are effective towards bacteria is being closely examined (WHO, 2015). The fact that it is used so frequently in livestock is due to a variety of preventive and growth-promoting uses, largely as a feed addition [19]. In human medicine, similar practices are uncommon. Several instances have described the transmission of animal-resistant germs to humans. It is uncertain how frequently this occurs or how considerably the livestock industry shared to overall human AMR [20].

The One Health is a worldwide perspective conceded that the public health is connected with animal health and the environment [21]. It concerns collaboration of different fields between physicians, veterinarians, environmental scientists, public health professionals, wildlife experts and many others. Public health concerns can be better tracked and managed using a multisectoral and interdisciplinary strategy. In order to achieve the best possible health outcomes, the resulting synergism advances our understanding of the way these diseases, known as zoonotic infections, might be transmitted between animals and people [22]. Although the idea of one health is not new, it has gained increased significance since 2006 as a result of newly and reemerging diseases.

According to the species and production methods, AMR can be spread to people by contact with animals, eating undercooked food, or consuming agricultural goods contaminated with resistant organisms [23]. Antibiotic-resistant bacteria from animals may also spread through animal waste by eliminating unmetabolized antibiotics and disseminating faeces and urine from farms that utilize livestock as fertilizer. Notably, there are still information gaps on how widespread this zoonotic transmission is.

According to the World Health Organization (WHO), antibiotic resistance is named as 3rd major public health concern (WHO, 2022). Methicillin resistant *Staphylococcus aureus* (MRSA) infection is one of the origins of ailment in animals and humans [24]. The rate of appearance of multi drug resistant strains of MRSA are increasing due to which its treatment is becoming demanding.

Several therapeutic agents have been developed for treating infectious diseases including antibiotics. This becomes important to know which antibiotics are more effective on specific bacteria [25]. Increasing prevalence of *S. aureus* and *E. coli* blood stream infections is a serious public health concern. Therefore, the current study has been designed to find out the prevalence rate of *S. aureus* and *E. coli* and their antibiograms at one health interface, connecting the health of humans, animal and the environment [26].

2. Materials and Methods

The present study was designed to explore the prevalence and antibiogram analysis of *Staphylococcus aureus* and *Escherichia coli* in the urban and peri-urban areas of district Bahawalpur by using the convenient sampling technique, total of n=320 samples were collected from the ears, nose and skin of livestock farmers in the shed and grazing areas. The soil samples were also collected in the close vicinity of the shed/farm areas. The milk samples of animals and samples of farmers were placed in ice pack cooler and by maintaining cold chain were transported to Microbiology laboratory, Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur [27].

2.1. Isolation and Identification of *Staphylococcus aureus* and *Escherichia coli*

Isolation and identification of *Staphylococcus aureus* and *Escherichia coli* were performed using conventional microbiological and biochemical techniques [28]. Samples (10 µL) were aseptically inoculated onto 5% sheep blood agar plates and incubated aerobically at 37°C for 24 h. Following primary cultivation, representative colonies were subcultured onto selective and differential media, including Mannitol Salt Agar (MSA) and MacConkey agar, for purification and preliminary differentiation of bacterial isolates [29], [30]. Media were prepared according to the manufacturer's instructions and sterilized by autoclaving at 121°C and 15 psi.

Purified isolates were subjected to morphological characterization based on colony appearance on selective media. Gram staining was subsequently performed on freshly prepared bacterial smears, and microscopic examination was carried out under oil immersion (100× objective) to determine Gram reaction and cellular morphology [31], [32].

Biochemical characterization of presumptive isolates was conducted using standard diagnostic assays. Catalase and coagulase tests were performed for the identification of *S. aureus* using 3% hydrogen peroxide and plasma, respectively. Identification of *E. coli* was carried out using indole and methyl red (MR) tests following inoculation into tryptone broth and MR-VP broth. Indole production was assessed using Kovac's reagent [33], [34]. All biochemical assays were performed under standardized incubation conditions at 37°C for 24–48 h in accordance with established microbiological procedures.

2.2. Antibiotic Susceptibility Test

Antimicrobial susceptibility testing of bacterial isolates was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar, in accordance with standard laboratory protocols. Fresh pure

colonies of each isolate were selected and used to prepare a standardized bacterial suspension in sterile normal saline, adjusted to match the turbidity of a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). The standardized inoculum was then uniformly swabbed across the entire surface of Mueller–Hinton agar plates using a sterile cotton swab to ensure even distribution of bacterial growth.

Following inoculation, antibiotic-impregnated discs representing different antimicrobial classes were aseptically placed on the agar surface using sterile forceps, ensuring appropriate spacing to prevent overlapping of inhibition zones. The plates were subsequently incubated aerobically at 37°C for 24 hours.

After incubation, the diameter of the zones of inhibition around each antibiotic disc was measured in millimeters using a calibrated ruler or digital caliper. The results were interpreted as susceptible, intermediate, or resistant according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (2015). All tests were performed under aseptic conditions to maintain culture purity and ensure reproducibility of results.

2.3. Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 26.0. Zone of inhibition data (mm) from the disk diffusion assay were analyzed as continuous variables. Differences among antibiotics and bacterial isolates were assessed using one-way ANOVA, with post hoc comparisons where applicable. Data were expressed as mean ± standard deviation, and statistical significance was set at $p < 0.05$.

3. Results

The current study found 29.17% prevalence of *S. aureus* isolated from sheep milk. The lowest %age found from environment 23.33% and from human 30% found. The current study shows there is no significant association *p-value* 0.789 between sheep human and environment. A total of 35 *S. aureus* were isolated from the 120 milk samples of sheep with the prevalence rate of 29.17% while the *S. aureus* from human isolated 15 samples out of 50 with the prevalence rate of 30% and from the environment *S. aureus* isolated 7 out of 30 with the prevalence rate of 23.33%. In the present study the overall, prevalence results showed significant changes that, the prevalence rate in sheep were found 5.84% higher than those of the environment while as compared to human it has 0.83% of lower prevalence rate. As contradictory with human found has 0.83% higher prevalence then the sheep while 6.67% higher from the environment.

3.1. Colony Characteristics and Microscopic Morphology of Bacterial Isolates

Presumptive *Staphylococcus aureus* isolates produced smooth, circular, convex yellow colonies on Mannitol Salt Agar, indicating mannitol fermentation. Gram staining revealed Gram-positive cocci arranged predominantly in grape-like clusters. Presumptive *Escherichia coli* isolates exhibited smooth pink lactose-fermenting colonies on MacConkey agar. Microscopic examination demonstrated Gram-negative short rod-shaped bacilli occurring singly or in pairs (Figure 1).

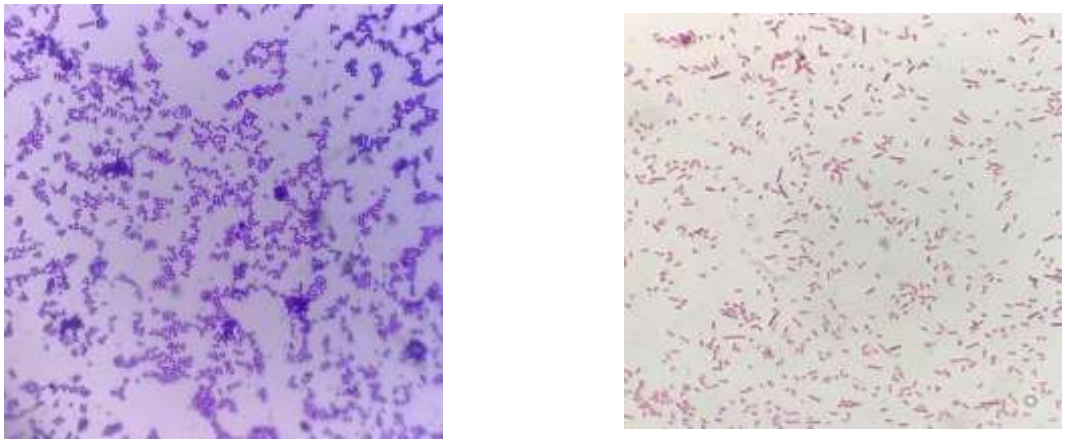

Figure 1. Gram-stained microscopic morphology of bacterial isolates under oil immersion (100×): (a) *Staphylococcus aureus* showing Gram-positive cocci arranged in grape-like clusters; (b) *Escherichia coli* showing Gram-negative short rod-shaped bacilli.

3.2. Antibiotic susceptibility Test

Antimicrobial susceptibility testing using the disk diffusion method demonstrated variable resistance profiles among *Staphylococcus aureus* and *Escherichia coli* isolates recovered from sheep milk, goat milk, human and environmental samples across different classes of antimicrobial agents. The antimicrobial susceptibility profiling of *Staphylococcus aureus* isolates demonstrated variable resistance patterns across sheep milk, goat milk, human, and environmental samples. High resistance rates were predominantly observed against ofloxacin, tetracycline, fosfomycin, and spiramycin, whereas comparatively greater susceptibility was recorded against ampicillin, piperacillin, and oxytetracycline in selected sample categories. Intermediate susceptibility responses were also frequently observed among the tested isolates. The overall mean susceptibility pattern showed moderate variability among antibiotics (33.33 ± 27.22). Statistical analysis revealed no significant differences in antimicrobial susceptibility profiles among isolates recovered from different sample sources ($p > 0.05$), indicating a comparable distribution of resistance traits across animal, human, and environmental reservoirs (Table 1)

Whereas, the antimicrobial susceptibility analysis of *Escherichia coli* isolates revealed heterogeneous resistance profiles against the tested antibiotics among sheep milk, goat milk, human, and environmental samples. Elevated resistance was mainly detected against ofloxacin, tetracycline, piperacillin, and gentamicin, while relatively higher susceptibility was observed against spiramycin, enrofloxacin, and oxytetracycline in specific sample groups. The mean antimicrobial response pattern was recorded as 33.33 ± 27.22, reflecting variability in isolate susceptibility across different antimicrobial classes. Statistical evaluation demonstrated no significant association between sample origin and antimicrobial resistance pattern ($p > 0.05$), suggesting a similar dissemination of resistant *E. coli* strains among livestock, humans, and environmental sources (Table 2) and inhibition diameter zones are illustrated in figure 2, 3 &4.

The *S. aureus* was identified in the samples collected from sheep, human and the environment. Among 120 samples of sheep, there were 35 (29.17%) positive and 85 were negative. Out of 50 human samples, 15 (30%) were positive and 35 were negative. On the same note, 7 of 30 environmental samples (23.33%) were positive and 23 samples were negative. The statistical analysis indicated that there were no significant differences between the groups ($p = 0.789$; $p > 0.05$).

In the case of *Escherichia coli*, 120 goat samples, 50 human samples and 30 environmental samples were sampled. The positive isolates were observed in 38 (31.67%) goat samples, 18 (36%) human samples, and 7 (23.33%) environmental samples with the rest being negative. The statistical difference in prevalence between the groups was insignificant ($p = 0.97$; $p > 0.05$).

(a) **(b)**

Figure 2. (a) Zones of inhibition produced by different antibiotics against *Escherichia coli*, including ampicillin, piperacillin, ofloxacin, gentamicin, and tetracycline. (b) Zones of inhibition produced by different antibiotics against *Staphylococcus aureus*, including enrofloxacin, spiramycin, piperacillin, oxytetracycline, ampicillin, gentamicin, and tetracycline.

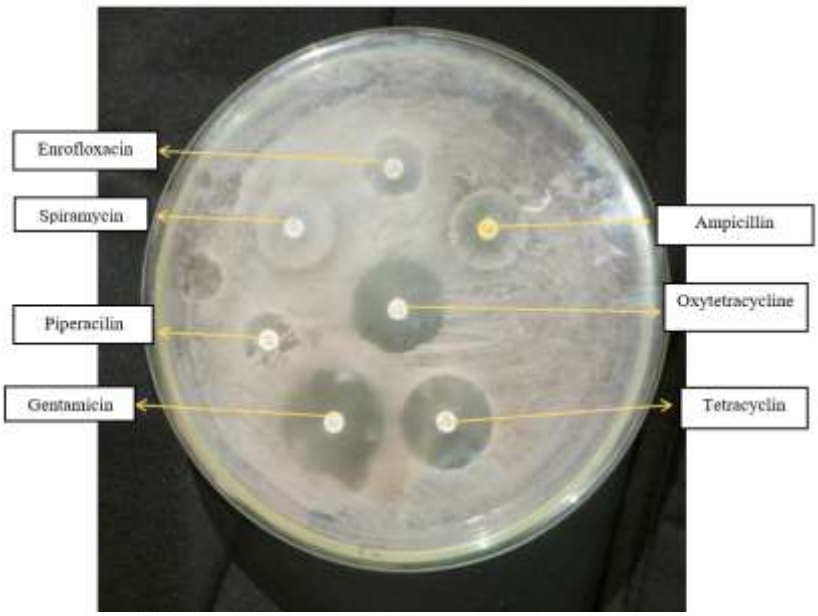

Figure 3. Representative antimicrobial susceptibility pattern showing the maximum zone of inhibition produced by gentamicin against *Staphylococcus aureus* isolates in the disk diffusion assay.

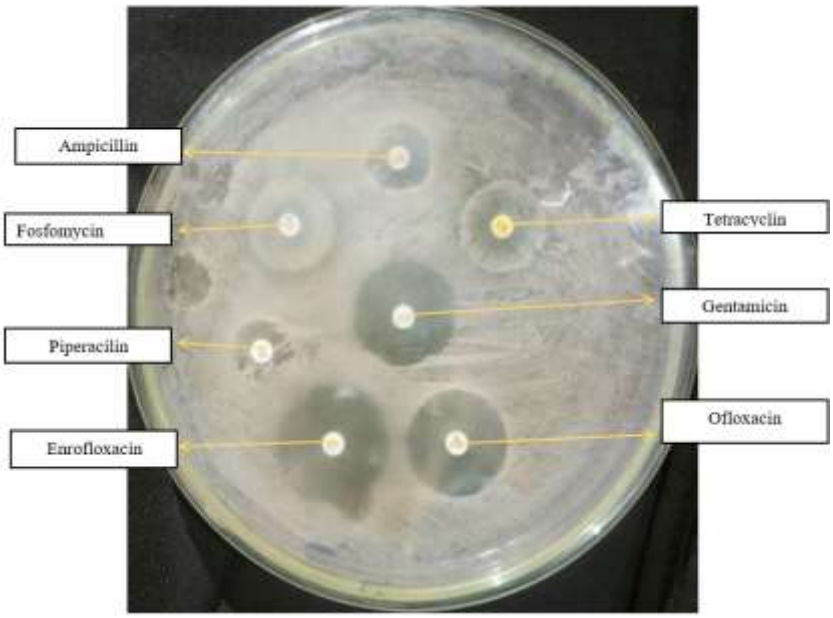

Figure 4. Representative antimicrobial susceptibility pattern showing the maximum zone of inhibition produced by enrofloxacin against *Escherichia coli* isolates in the disk diffusion assay.

The distribution pattern demonstrated a consistently higher proportion of negative isolates compared to positive isolates across all examined sample types. Environmental samples showed the highest contribution to the overall negative isolates, followed by human, goat, and sheep samples. In contrast, positive isolates were comparatively lower across all categories, with a relatively uniform distribution among animal and human sources. The cumulative analysis further indicated that the total negative isolates predominated over positive isolates, suggesting a greater prevalence of the measured negative outcome across the studied sampling sources. (Figure 5).

The present study demonstrated the occurrence of *Staphylococcus aureus* and *Escherichia coli* in sheep milk, human, and environmental samples, highlighting their potential role in zoonotic transmission and antimicrobial resistance dissemination. The prevalence of *S. aureus* was recorded as 29.17% in sheep milk, 30% in human samples, and 23.33% in environmental samples, whereas *E. coli* prevalence was 31.67%, 36%, and 23.33%, respectively. Although comparatively higher prevalence rates were observed in animal and

human samples than environmental samples, statistical analysis revealed no significant association among the studied groups ($p > 0.05$). These findings suggest possible epidemiological linkage between animal and human reservoirs, particularly under close-contact farming systems.

Phenotypic characterization showed that *S. aureus* produced yellow mannitol-fermenting colonies on Mannitol Salt Agar and appeared as Gram-positive cocci in clusters, while *E. coli* exhibited pink lactose-fermenting colonies on MacConkey agar with Gram-negative rod-shaped morphology. These microbiological characteristics were consistent with standard identification criteria reported for both bacterial species.

Antimicrobial susceptibility testing demonstrated variable resistance patterns against multiple antibiotic classes. Both *S. aureus* and *E. coli* isolates exhibited resistance to several commonly used antibiotics, indicating the emergence of multidrug-resistant bacterial populations in both animal and human sources. Notably, gentamicin showed comparatively greater inhibitory activity against *S. aureus*, whereas enrofloxacin demonstrated the highest activity against *E. coli*, as evidenced by larger inhibition zone diameters. The observed resistance profiles may be associated with the indiscriminate or prolonged use of antimicrobial agents in veterinary and human medicine, contributing to selective pressure and resistance development.

Overall, the study emphasizes the public health significance of antimicrobial-resistant *S. aureus* and *E. coli* circulating among livestock, humans, and the farm environment. Continuous surveillance, prudent antimicrobial use, and improved hygienic management practices are essential to minimize the spread of resistant pathogens within the animal-human-environment interface.

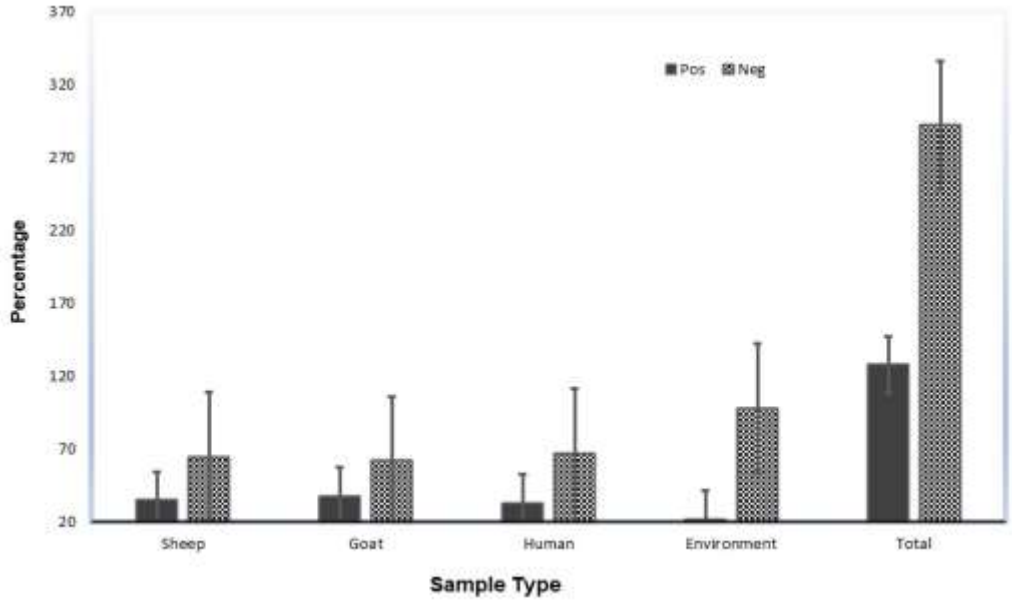

Figure 5. Distribution of positive and negative bacterial isolates among different sample types (sheep, goat, human, and environmental samples), expressed as percentage prevalence. Error bars represent standard deviation.

Table 1. Combined antibiotic susceptibility profile (%) of *Staphylococcus aureus* isolates recovered from sheep milk, goat milk, human, and environmental samples

Antibiotic	Sheep Milk (R/I/S)	Goat Milk (R/I/S)	Human Samples (R/I/S)	Environmental Samples (R/I/S)	Mean ± SD
Ampicillin	0/66.67/33.33	0/66.67/33.33	0/33.33/66.67	0/33.33/66.67	30.23 ± 27.20
Ofloxacin	66.67/33.33/0	66.67/33.33/0	66.67/33.33/0	66.67/33.33/0	33.33 ± 27.19
Tetracycline	66.67/0/33.33	66.67/0/33.33	0/0/33.33	0/0/33.33	25.00 ± 28.86

Enrofloxacin	33.33/66.67/0	0/33.33/66.67	0/66.67/33.33	0/66.67/33.33	33.33 ± 27.22
Piperacillin	33.33/0/66.67	0/66.67/33.33	66.67/33.33/0	66.67/33.33/0	33.29 ± 27.20
Fosfomycin	66.67/33.33/0	33.33/66.67/0	66.67/0/33.33	66.67/0/33.33	33.33 ± 27.22
Oxytetracycline	33.33/0/66.67	33.33/66.67/0	66.67/0/33.33	66.67/0/33.33	33.30 ± 27.19
Spiramycin	66.67/33.33/0	66.67/0/33.33	33.33/66.67/0	33.33/66.67/0	31.23 ± 27.18
Gentamicin	33.33/66.67/0	33.33/66.67/0	66.67/33.33/0	66.67/33.33/0	33.33 ± 27.22

R: Resistant, **I:** Intermediate, **S:** Susceptible

Table 2. Combined antibiotic susceptibility profile (%) of *Escherichia coli* isolates recovered from sheep milk, goat milk, human, and environmental samples

Antibiotic	Sheep Milk (R/I/S)	Goat Milk (R/I/S)	Human Samples (R/I/S)	Environmental Samples (R/I/S)	Mean ± SD
Ampicillin	0/66.67/33.33	0/66.67/33.33	33.33/66.67/0	33.33/66.67/0	33.33 ± 27.31
Ofloxacin	66.67/33.33/0	66.67/33.33/0	66.67/33.33/0	33.33/0/66.67	33.21 ± 27.22
Tetracycline	66.67/0/33.33	66.67/0/33.33	66.67/0/33.33	66.67/0/33.33	31.27 ± 25.22
Enrofloxacin	0/66.67/33.33	66.67/33.33/0	33.33/66.67/0	0/66.67/33.33	33.33 ± 27.22
Piperacillin	66.67/33.33/0	33.33/66.67/0	66.67/33.33/0	66.67/33.33/0	29.33 ± 24.21
Fosfomycin	66.67/0/33.33	66.67/0/33.33	0/66.67/33.33	66.67/0/33.33	33.33 ± 27.22
Oxytetracycline	33.33/66.67/0	0/66.67/33.33	0/33.33/66.67	33.33/66.67/0	32.33 ± 27.22
Spiramycin	33.33/0/66.67	66.67/33.33/0	33.33/66.67/0	33.33/0/66.67	31.23 ± 27.22
Gentamicin	66.67/33.33/0	66.67/0/33.33	0/33.33/66.67	66.67/33.33/0	33.33 ± 27.22

R: Resistant, **I:** Intermediate, **S:** Susceptible

4. Conclusions

This study demonstrates a notable prevalence of *Staphylococcus aureus* and *Escherichia coli* across animal, human, and environmental sources with no significant differences among sampling groups ($p > 0.05$), indicating widespread circulation of these pathogens. Both organisms exhibited concerning levels of antimicrobial resistance, particularly against commonly used antibiotics such as ampicillin and oxytetracycline. However, some antibiotics, including fosfomycin and spiramycin, retained comparatively better efficacy against *E. coli*. Overall, the findings highlight the emergence of multidrug-resistant strains across the animal–human–environment interface, emphasizing the need for strict antibiotic stewardship, continuous surveillance, and integrated One Health strategies to mitigate further spread.

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Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest

The authors declare that there is no conflict of interest. This work did not get any external funding; therefore, no funding agency had a role in the whole plan of the study, the collection, the analysis or the interpretation of data, not even in the writing of the manuscript.

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