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Original Article

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Antibiogram Studies of Biofilm Forming *Escherichia Coli* and *Pseudomonas Aeruginosa* Isolated from Clinical Mastitis Cases in Bovines

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Abstract

Mastitis is a common disease of dairy cattle. It is characterized by pathological changes in udder and bacteriological changes in milk, making it unfit for human consumption. In veterinary medicine, antibiotic treatment of mastitis has led to the development of antibiotic-resistant bacteria. To combat the treatment challenge of mastitis caused by Gram-negative bacteria, antibiogram profiling of Escherichia coli and Pseudomonas aeruginosa was done to select effective antibiotics against these bacteria. In this research, samples containing milk and puss were collected from affected quarter of udder of cattle and screening of mastitis was performed by using Surf field mastitis test. Samples tested positive for mastitis were used for culturing of bacteria. E. coli and P. aeruginosa were isolated and identified by using selective media and biochemical tests, respectively. The biofilm formation capacity of E. coli and P. aeruginosa was determined by performing biofilm assay. Both E. coli and P. aeruginosa isolates exhibited varied biofilm-formation capacities. E. coli isolates were classified as strong biofilm-formers (12%), moderate biofilm-formers (50%) and weak biofilm formers (38%). Furthermore, 20% of P. aeruginosa isolates were classified as strong biofilm-formers, 40% as moderate biofilm-formers and 40% as weak biofilm-formers Antibiogram of isolated bacteria was determined using disc diffusion test. Overall, E. coli isolates showed resistance to all the tested antibiotics except meropenem. P. aeruginosa isolates were found resistant to enrofloxacin, linezolid, cefixime and chloramphenicol, and susceptible to meropenem, vancomycin and azithromycin. These results suggest that meropenem could be an effective antibiotic against mastitis infections caused by E. coli and P. aeruginosa. Moreover, vancomycin and azithromycin could be used to treat mastitis infection caused by P. aeruginosa. These results indicate a dire need for the development of alternative therapeutic strategies for antibiotic-resistant biofilm-forming bacteria and surveillance of antibiotic resistance in treatment of mastitis infection in cattle.

Introduction

Mastitis is the inflammation of mammary glands and ducts usually caused by contamination of teat opening by bacterial infection. Mastitis is a major threat to animal health and the quality of milk obtained from animals [1]. There are a variety of causative agents of mastitis. Gram negative bacteria like E. coli can lead to severe clinical symptoms of mastitis including severe inflammation to udder, pain, swelling, redness, high fever, pus or blood in milk and predominately significant reduction in milk production [2]. Modern dairy farms are at greater risk of mastitis caused by Gram negative bacteria. E. coli is said to be the key contributor to Gram-negative infections [3]. The investigations at different farms often show an infection pattern specific to farm where single Gram-negative bacteria are prevalent [4]. Gram-negative bacteria are more resistant to antibiotics as compared to Gram positive bacteria [5]. Water supplies on dairy farms such as troughs, wells and parlor wash hoses are the major source of Pseudomonas aeruginosa on dairy farms [6]. Improving sanitary conditions such as maintenance of hygiene during milking procedure, disinfection of teat after milking, disinfecting the machines used for milking is the principal measure for prevention of new mastitis cases [7].

E. coli and P. aeruginosa are pathogens that can adhere to the surface of udder and produce complex community of bacteria called biofilm. Biofilm formation enables single-celled microbe to presume a transient multicellular habitat. Inside the biofilm, bacteria are enveloped in a self-made extracellular

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matrix and resists the penetration of antibiotic drugs [8]. Biofilm infections are chronic and are known to be difficult to eliminate with antibiotics. This biofilm formation on udder makes them resistant to antibiotics treatment that can lead to persistent infection, reduced milk production and quality [9].

The treatment of mastitis infection includes pain management, supportive care to reduce clinical signs and is dependent mainly on the use of antibiotics. Mastitis is the root cause of antimicrobial usage on dairy farms [10]. Antibiotics used to effectively eliminate mastitis infection have now developed antibiotic resistance towards them, especially towards Gram negative bacterial infections. Implementing the relative efficacy of antimicrobial treatment for mastitis will serve to refine the ability of decision maker to engage in effective stewardship of antimicrobials by eluding excess use of incompetent antibiotics [11]. Prudent use of antibiotics based on identifying pathogens and their drug susceptibility is essential to maintain effectiveness. Banning antibiotic use in dairy farming could negatively impact animal health, welfare, and food production. Therefore, responsible and strategic antibiotic use remains a necessary and valuable practice in the dairy industry [12]. This study was conducted to determine the current trend of antibiotic resistance of E. coli and P. aeruginosa isolated from milk and assess the biofilm formation capacity of these bacteria. To determine the effectiveness of specific antibiotics against E. coli and Pseudomonas aeruginosa we designed an antibiogram studies that depicts susceptibility of proportion of bacteria to certain antibiotics. This antibiogram will aid veterinary medicine in the selection of the most effective group of antibiotics for treatment of mastitis.

Materials and methods

❖ Sampling

Total 50 Samples were collected from various farms located in the district of Faisalabad, Sahiwal, Tandlianwala, and Jaranwala, Pakistan. In this research, 50 samples containing milk and puss were collected from affected quarter of udder of cattle. 5-6 mL of milk was collected from each cow aseptically into a 15 mL sterile Falcon Tubes.

❖ Screening of milk samples

Screening of mastitis was performed by using Surf field mastitis test to confirm if samples were mastitis positive. The procedure was conducted by collecting a small quantity of milk from affected teats of the udder into separate cups or a paddle. An equal amount of surf solution,1–3% detergent solution (such as sodium lauryl sulfate), was added to milk samples. Mixture of milk and detergent was gently swirled, and the reaction was observed within thirty seconds. 32 Samples tested positive for mastitis were used for culturing of bacteria [13].

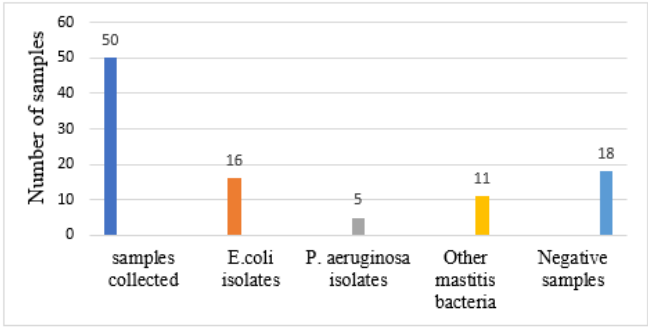

Figure 1. Distribution of Microorganisms Isolated from Mastitis-Affected animal samples.

Isolation and identification of E. coli and Pseudomonas aeruginosa

The isolated bacteria were identified based on their colony characteristics, gram staining reaction and biochemical characterization. 16 E. coli isolates were identified on MacConkey agar as pink lactose-fermenting colonies as shown in Figure 2.1 and on EMB agar as black colonies with characteristic green metallic sheen [14] Figure 2.2. 5 P. aeruginosa isolates were identified on cetrimide agar as characteristic green-pigmented colonies [15] Figure 3.1 and beta hemolysis colonies on blood agar [16] Figure 3.2. Both Escherichia coli and P. aeruginosa showed Gram-negative pink colored rods observed under microscope at 100X [17] as shown in Figure 4.1 and 4.2. Further bacteria were confirmed by performing biochemical tests as performed by Roy et al. (2023) in his studies such as citrate utilization test Figure 5.1 and 5.2, methyl red test Figure 6.1 and 6.2, Voges – Proskauer test Figure 7.1 and 7.2, indole test Figure 8.1 and 8.2, catalase test Figure 9.1 and 9.2, urease test Figure 10.1 and 10.2, Tripple sugar iron (TSI) test Figure 11.1 and 11.2 and cetrimide agar test for P. aeruginosa [18] Figure 12.

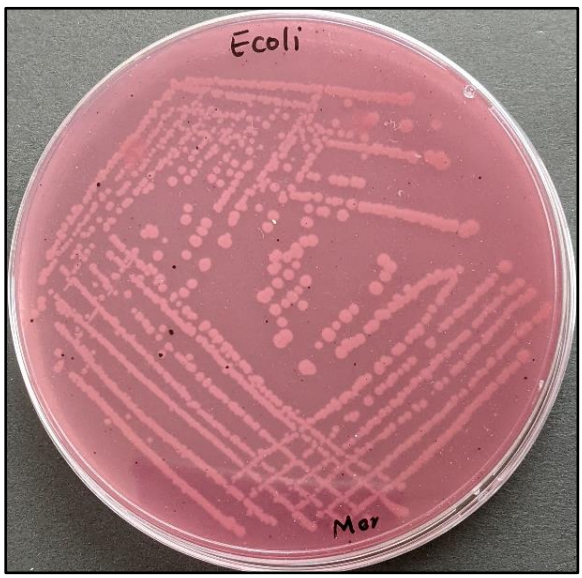

Figure 2.1 Pink, round colonies appeared on MacConkey agar, indicating pure culture of isolated E. coli.

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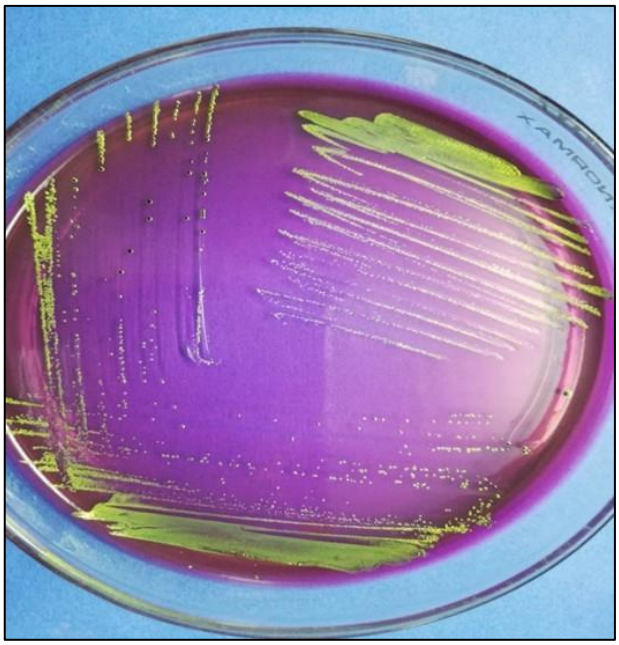

Figure 2.2. E. coli showing isolated blue-black colonies with green metallic sheen on EMB agar

Figure 3.1. P. aeruginosa green pigmented colonies on Cetrimide agar

Figure 3.2. P. aeruginosa showing beta hemolytic colonies on Blood agar

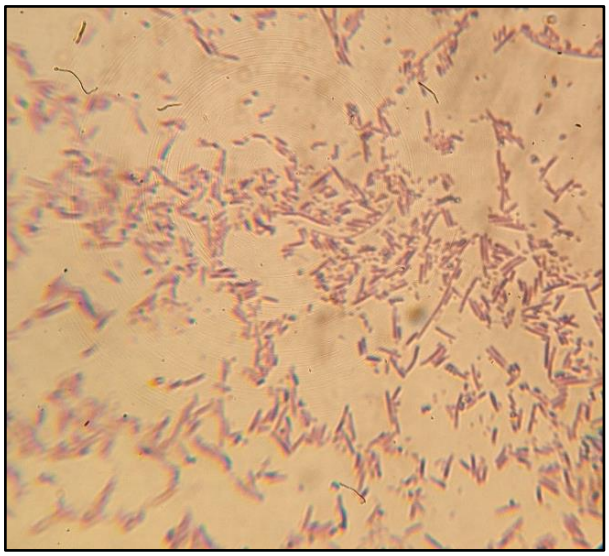

Figure 4.1. Gram-Negative Rods of Escherichia coli Observed Under Microscope at 100X.

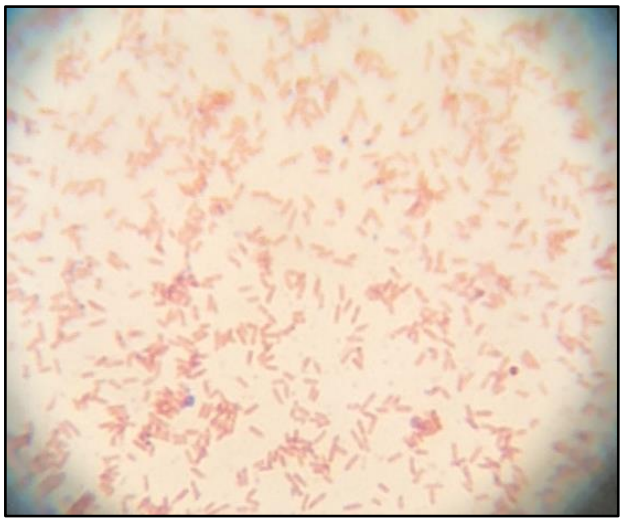

Figure 4.2. Gram-Negative rods of P. aeruginosa observed under Microscope at 100X

Figure 5.1. Citrate utilization test showing result for E. coli

Figure 5.2: Citrate utilization test showing positive result for P. aeruginosa

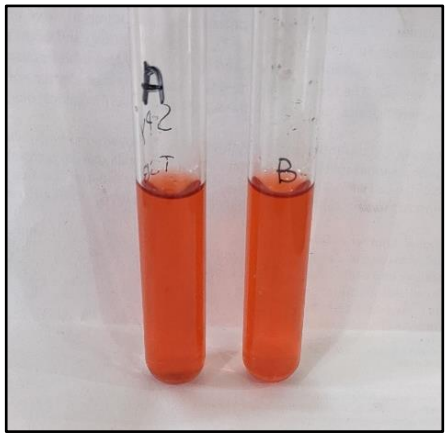

Figure 6.1: Methyl red test showing positive result for E. coli (A: control, B: MR+)

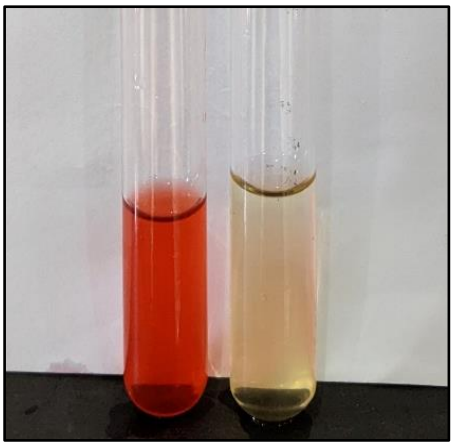

Figure 6.2: MR test showing negative result for P. aeruginosa

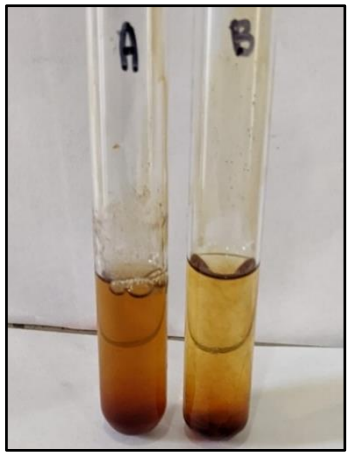

Figure 7.1. VP test showing negative result for E. coli (A: control, B: VP negative)

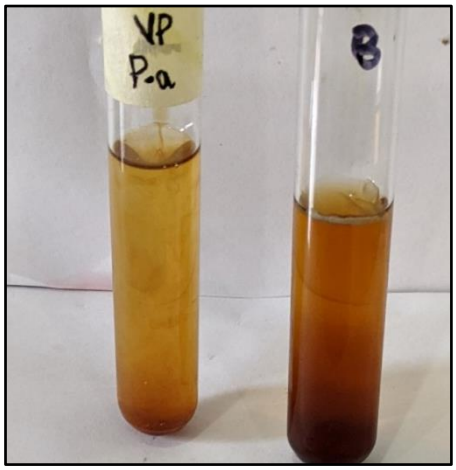

Figure 7.2. VP test showing negative result for P. aeruginosa (B: control)

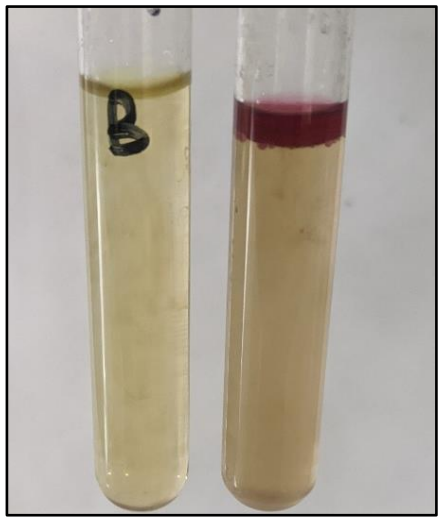

Figure 8.1. Positive indole test of E. coli. (B: control)

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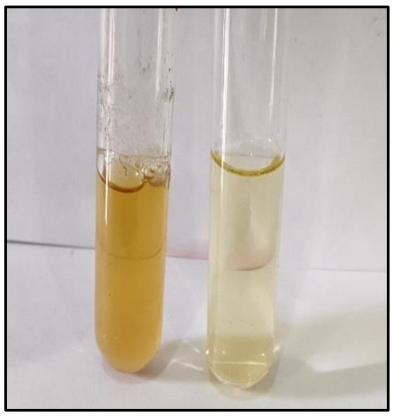

Figure 8.2. Indole test showing negative result for P. aeruginosa (B: control, A: indole negative)

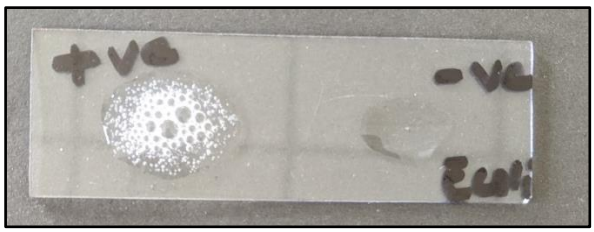

Figure 9.1. Catalase test showing positive result for E. coli

Figure 9.2. Catalase test showing positive result for P. aeruginosa

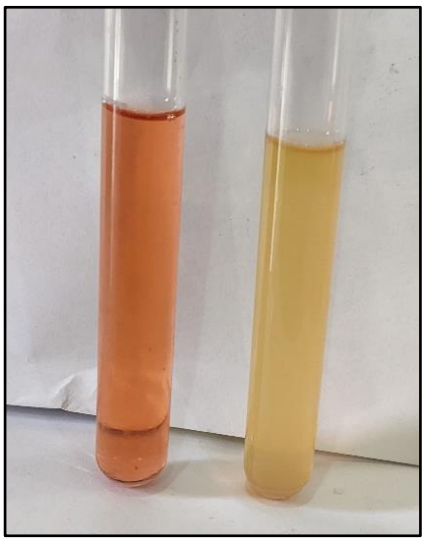

Figure 10.1. Negative urease test of E. coli (B: control, A: Urease negative)

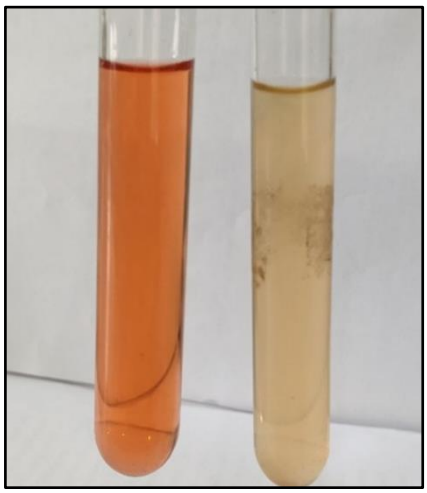

Figure 10.2. Urease test showing negative result for P. aeruginosa (B: control, A: Urease negative)

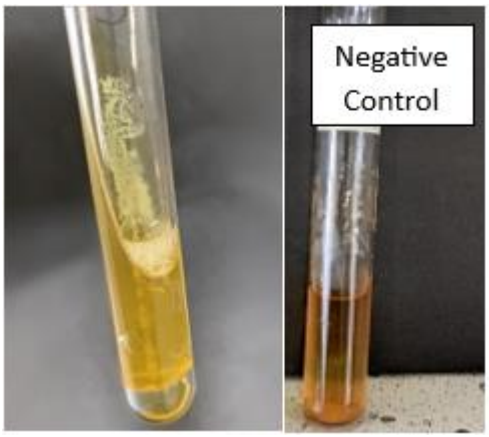

Figure 11.1. TSI test showing positive results for E. coli.

Figure 11.2. TSI test showing negative result for P. aeruginosa

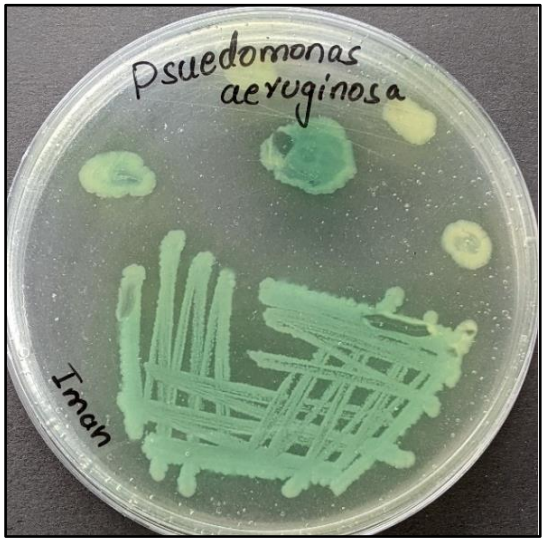

Figure 12. Cetrimide agar test showing positive result (green pigment) for P. aeruginosa

Glycerol Stock Preparation

Each bacterial culture (700 µl) was combined with 300 µl of the 50% glycerol solution under sterile conditions in sterile 1.5 mL microcentrifuge tubes. The mixtures were vortexed until homogeneous and then labeled and stored at -20°C for long-term storage [19].

- **Biofilm assay**

Biofilm formation capacity of E. coli and Pseudomonas aeruginosa was assessed by biofilm assay in 96-well microtiter plate as shown in Figure 13.1 and 13.2. To induce biofilm, 250 µl of the overnight bacterial culture was inoculated to 9.5 ml of fresh TSB supplemented with 0.25% glucose. After this mixture was prepared, 200 µl of aliquot per strain was added to a well of 96-well microtiter plates. The control wells were filled with plain TSB supplemented with glucose. The plates were then incubated at 37°C for 18 hours. After incubation, the non-adherent cells were discarded and wells were rinsed with 200 µl of phosphate-buffered saline (PBS). For fixation of biofilms methanol solution was added in wells and kept for 15 minutes. Then wells were stained using crystal violet solution and kept for 15 minutes. Afterwards the plates were rinsed thoroughly using distilled water to eliminate excess stain prior to air-drying. The crystal violet stained biofilms were dissolved in 95% ethanol. OD values were observed at 590 nm in spectrophotometer. The wavelength absorbance in each well was used to quantify the levels of biofilm formed by different isolates [20].

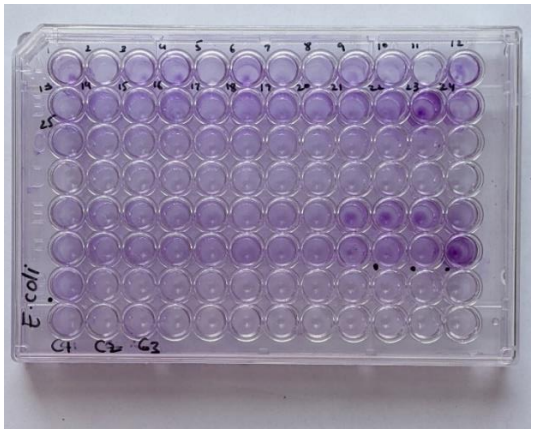

Figure 13.1: Biofilm assay microtiter plate for E. coli

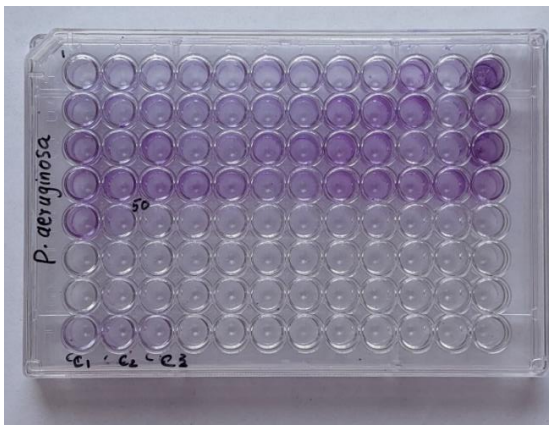

Figure 13.2: Biofilm assay microtiter plate for P. aeruginosa

Optical densities of isolates (OD) and negative controls (OD avg) were obtained, from these values the OD cut was calculated by using formula: Optical density cut-off (OD cut) = Average Optical density (OD avg) of negative control + 3 × Standard deviation (S.D) of OD avg of negative control [20].

Biofilm formation capacity of E. coli:

$$OD_{cut} = OD_{avg} + 3 \times S.D \text{ of ODs of negative control}$$

$$= 0.12 + 3 \times 0.08$$

$$OD_{cut} = 0.36$$

OD cut obtained for E. coli was 0.36. The criteria used for biofilm classification of isolates [20] is shown in table 1.

Table 1: Classification of biofilm forming capacity of E. coli isolates

Criteria for the classification	Optical density values

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OD ≤ OD $_{cut}$ = non-biofilm-former (NBF)	OD < 0.36
OD $_{cut}$ < OD ≤ 2 × OD $_{cut}$ = Weak biofilm-former (WBF)	0.37 < OD ≤ 0.72
2 × OD $_{cut}$ < OD ≤ 4 × OD $_{cut}$ = Moderate biofilm-former (MBF)	0.72 < OD ≤ 1.44
OD >4 × OD $_{cut}$ = Strong biofilm-former.	OD > 1.44

E. coli Isolates having OD ≤ OD cut that is OD < 0.36 are classified as non-biofilm-former. There were zero non-biofilm formers. 6 E. coli isolates having OD cut < OD ≤ 2 × OD cut that is between 0.37 < OD ≤ 0.72 were classified as weak biofilm-formers. 8 E. coli Isolates having 2 × OD cut < OD ≤ 4 × OD cut that is between 0.72 < OD ≤ 1.44 were classified as moderate biofilm-former. E. coli Isolates having OD >4 × OD cut that is OD > 1.44 were classified as Strong biofilm-former.

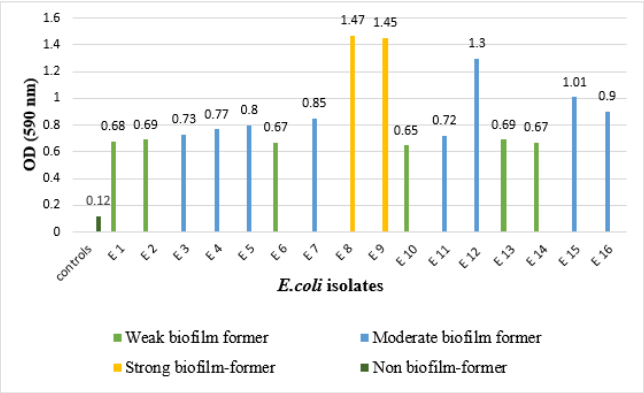

Figure 14: Biofilm formation capacity of isolates of E. coli

Biofilm formation capacity of P. aeruginosa

OD $_{cut}$ = 0.34 (*P. aeruginosa*)

Table 2. Classification of biofilm forming capacity of P. aeruginosa isolates

Criteria for the classification	**Optical density values**
OD ≤ OD $_{cut}$ = non-biofilm-former (NBF)	OD < 0.34
OD $_{cut}$ < OD ≤ 2 × OD $_{cut}$ = Weak biofilm-former (WBF)	0.35 < OD ≤ 0.68
2 × OD $_{cut}$ < OD ≤ 4 × OD $_{cut}$ = Moderate biofilm-former (MBF)	0.68 < OD ≤ 1.36
OD >4 × OD $_{cut}$ = Strong biofilm-former.	OD > 1.36

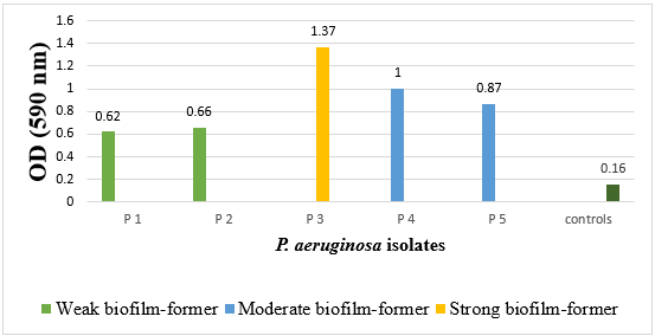

Figure 15. Biofilm formation capacity of isolates of P. Aeruginosa

Antibiogram profiling

The antimicrobial sensitivity patterns of Escherichia coli as shown in Figure 16.A and 16.B, and Pseudomonas aeruginosa isolates as shown in Figure 17.A and 17.B were determined using the disc diffusion method also performed by Iftikhar et al. (2024) [21].

Bacterial suspensions were diluted 1:100 (10µL overnight bacterial culture in 1 mL fresh TSB) to match with the 0.5 McFarland turbidity standard, which equals to 1.5x 108 CFU/m L [22]. Bacterial suspensions were distributed on the Mueller-Hinton agar plates by sterile swabs across the plates. In this study Oxoid antibiotic disks were used for antibiotic susceptibility testing. Antimicrobial impregnated discs (meropenem 10 µg, ciprofloxacin 5 µg, vancomycin 30 µg, erythromycin 10 µg, azithromycin 15 µg, penicillin 1 U, linezolid 30 µg, cefixime 5 µg, enrofloxacin 10 µg and chloramphenicol 30 µg) were placed on the inoculated agar plate on the agar surface at appropriate distances. The test plates were inverted and put in an incubator at 37°C for 24 hours to encourage bacterial growth and diffusion of antibiotics.

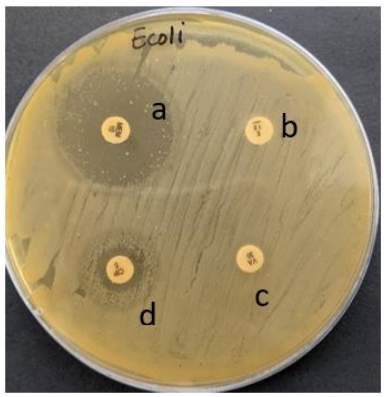

Figure 16.A. Antibiotic susceptibility test of isolated E. coli against a: meropenem, b: erythromycin, c: vancomycin, d: ciprofloxacin.

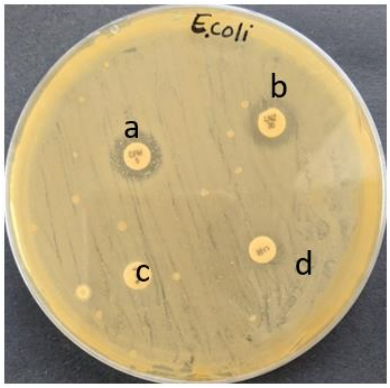

Figure 16.B. Antibiotic susceptibility test of isolated E. coli against a: cefixime, b: linezolid, c: penicillin, d: chloramphenicol.

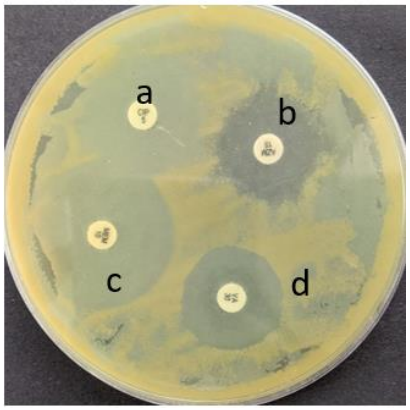

Figure 17.A. Antibiotic susceptibility test of isolated P. aeruginosa against a: ciprofloxacin, b: azithromycin, c: meropenem, d: vancomycin.

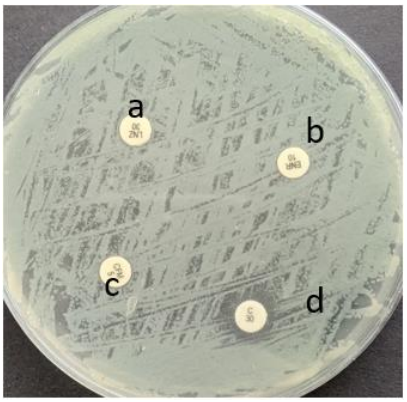

Figure 17.B. Antibiotic susceptibility test of isolated P. aeruginosa against a: linezolid, b: enrofloxacin, c: cefixime, d: chloramphenicol

After incubation, zones of inhibition around each antibiotic disc were accurately measured using millimeter scale. Results were interpreted according to the latest performance standards of Clinical and Laboratory Standards Institute (CLSI) and susceptibility categories (susceptible, intermediate, resistant) were assigned by a comparison to concentration breakpoints. The mean and standard deviation (SD) of the zone of inhibition values were calculated for each antibiotic based on the measurements obtained from all 16 isolates of E. coli and 5 isolates of P. aeruginosa. These statistical values (Mean ± SD) served as a single summarized measure of susceptibility for E. coli and P. aeruginosa as a specie, an approach used by Jabbar et al. (2023) [23] in his antibiogram studies of Mycoplasma Bovis isolated from mastitis This method was used for the construction of a standardized antibiogram of E. coli as shown in Table 3 and antibiogram of P. aeruginosa as shown in Table 4.

Statistical Analysis

Data was analyzed using statistical software SPSS, version 22 [24].

Table 3: Antibiogram of E. coli isolates by disc diffusion assay against commonly used antibiotics

Antibiotic discs	Concentration	Sensitive (S)	Intermediate (I)	Resistant (R)	Zone of Inhibition (mm) Mean ± SD	Antibiotic susceptibility pattern
Meropenem	10µg	≥23	20-22	≤19	25.85 ± 1.16	S (100%)
Ciprofloxacin	5µg	≥21	16-20	≤15	11.52 ± 1.04	R (100%)
Vancomycin	30µg	≥15	10-14	≤9	0	R (100%)
Erythromycin	10µg	≥23	14-22	≤13	0	R (100%)
Penicillin	1 U	≥28	20-27	≤19	0	R (100%)
Linezolid	30µg	≥28	-	≤14	12.25 ± 0.62	R (100%)
Cefixime	5µg	≥19	16-18	≤15	12.48 ± 0.72	R (100%)
Chloramphenicol	30µg	≥18	13-17	≤12	0	R (100%)

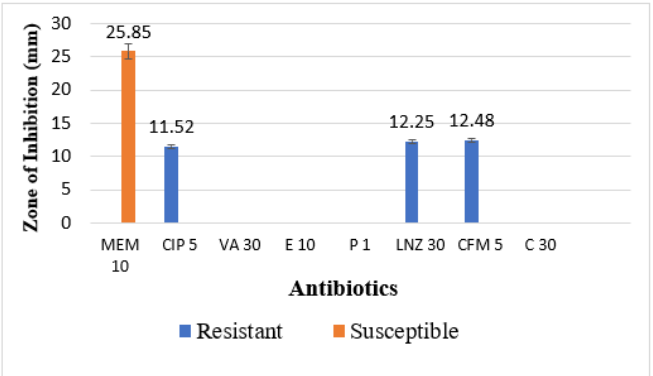

Figure 18. Comparison of mean and standard deviation of zone of inhibition of antibiotic discs against E. coli isolates

Table 4. Antibiogram of P. aeruginosa isolates by disc diffusion assay against commonly used antibiotics

Antibiotic discs	Concentration	Sensitive (S)	Intermediate (I)	Resistant (R)	Zone of Inhibition (mm) Mean ± SD	Antibiotic Susceptibility Pattern
Meropenem	10µg	≥19	16-18	≤15	31.52 ± 1.45	S (100%)
Ciprofloxacin	5µg	≥25	19-24	≤18	24.32 ± 1.15	I (100%)
Vancomycin	30µg	≥12	-	≤9	23.60 ± 0.96	S (100%)
Azithromycin	15µg	≥23	14-22	≤13	24.06 ± 1.26	S (100%)
Enrofloxacin	10µg	≥23	17-22	≤16	0	R (100%)
Linezolid	30µg	≥26	-	≤12	0	R (100%)
Cefixime	5µg	≥19	16-18	≤15	0	R (100%)
Chloramphenicol	30µg	≥21	11-20	≤10	9.54 ± 0.50	R (100%)

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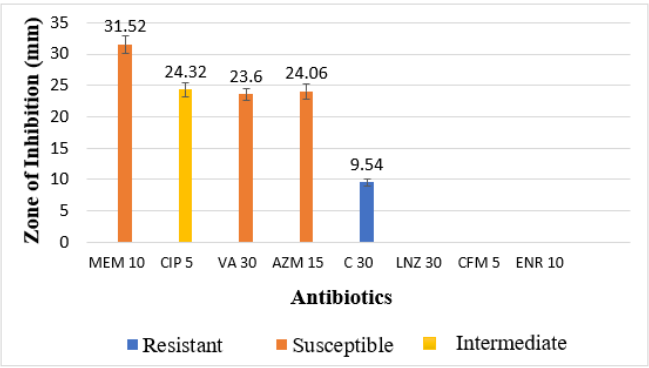

Figure 19: Comparison of mean and standard deviation of zone of inhibition of antibiotic disks against different isolates of P. aeruginosa

Results and Conclusion

Overall, E. coli isolates showed resistance to all the tested antibiotics except meropenem. P. aeruginosa isolates were found resistant to enrofloxacin, linezolid, cefixime and chloramphenicol, and susceptible to meropenem, vancomycin and azithromycin. These results suggest that meropenem could be an effective antibiotic to eliminate mastitis infection at farm caused by E. coli and P. aeruginosa. Moreover, vancomycin and azithromycin could be used to treat mastitis infection caused by P. aeruginosa. E. coli and P. aeruginosa demonstrated varied biofilm formation capabilities, ranging from weak to strong biofilm producers. 38 % of E. coli isolates were classified as weak biofilm formers and 50 % of E. coli isolates were classified as moderate biofilm-former and 12% isolates were classified as strong biofilm formers. P. aeruginosa isolates were classified as weak biofilm formers (40 %) and moderate biofilm-formers (40 %) and strong biofilm-formers (20%). There was 0% non-biofilm former among E. coli and P. aeruginosa isolates as shown in Figure 20.

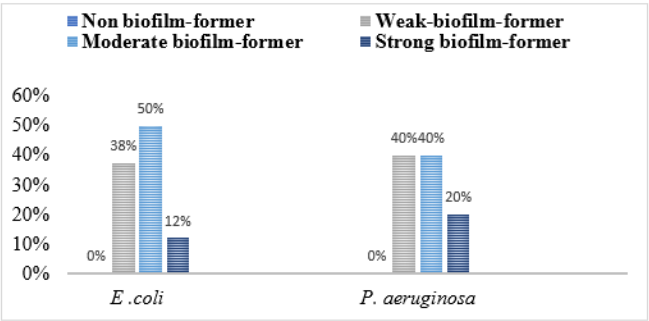

Figure 20. Percentage biofilm formation capacity of E. coli and P. aeruginosa isolates

Discussion

Clinical mastitis in dairy cattle has long been recognized as a significant health and economic challenge in the dairy industry. Historically, mastitis was managed primarily through empirical observation and basic hygiene practices [25]. As dairy farming advanced through the 19th and 20th centuries, particularly with the introduction of commercial antibiotics, the therapeutic landscape of mastitis began to change significantly. However, this advancement brought with it the unintended consequence of antimicrobial resistance, a challenge that now complicates treatment and demands more strategic intervention [11].

This research was carried out to determine the biofilm formation ability and antimicrobial resistance patterns in bacteria isolated from clinical mastitis cases [26]. In this study design, milk samples of fifty lactating cattle showing clinical signs of intramammary infection were obtained. Screening for mastitis was done by use of Surf Field Mastitis Test also performed by Muhammad et al. (2010) [27]. As a result, 32 samples were positive for mastitis. The positive samples were fully microbiologically analyzed using selective culture methods and further confirmed by biochemical identification. Escherichia coli and Pseudomonas aeruginosa have become increasingly problematic due to their ability to resist conventional antibiotics and form protective biofilms, which allow them to survive harsh environmental conditions and evade host immune defenses [28]. Their inherent resistance to many antibiotics is due to capability of bacteria to transfer gene horizontally acquiring novel resistance genes, further complicating treatment strategies [29]. Among the most critical mechanisms contributing to this resistance is the formation of biofilms. Biofilm reduces antibiotic penetration, and the altered metabolic state of bacteria within the biofilm that makes them less susceptible to drugs that typically target active cellular processes [30]. This allows biofilm-associated infections to persist for longer periods and increases the difficulty of achieving complete eradication through conventional therapy [31].

Biofilm formation capacity of E. coli and Pseudomonas aeruginosa was assessed using biofilm assay in 96-well microtiter plate. The optical density (OD) of the crystal violet stained biofilms was measured at 590 nm using a microplate spectrophotometer, following the procedure used by Leoney et al. (2020). The study found that both pathogens exhibited varying degrees of biofilm production. E. coli isolates were classified as strong biofilm-formers (12%), moderate biofilm-formers (50% %) and weak biofilm formers (38 %). Similar results were shown by [32] in his study. 20 % of P. aeruginosa isolates were classified as strong biofilm-formers, 40 % as moderate biofilm-formers and 40 % as weak biofilm-formers. The similar biofilm formation capacity of P. aeruginosa was reported by Huang Y et al. (2024) [33] in his studies. These findings suggest that the ability to form biofilms is common among mastitis-causing isolates and may be linked to their persistence and resistance to antimicrobial treatment [34].

Antibiotic sensitivity testing using disc diffusion assay (Kirby-Bauer method) similar to the method used by Zanichelli et al. [35] revealed significant variation in the effectiveness of commonly used antibiotics For E. coli, meropenem emerged as the most effective antibiotic, Notably, vancomycin, erythromycin, penicillin, and chloramphenicol exhibited no inhibitory effect on E. coli all classified as resistant, suggesting that a high degree of multidrug resistance exists among these isolates. Similarly, Singh et al. [36] also reported high resistance of E. coli isolates, isolated from bovine mastitis cases. In his study the highest sensitivity of E. coli was towards ciprofloxacin unlike our study in which meropenem was

declared as most sensitive which is also demonstrated by Fahim KM et al. [37] in his study carbapenems group of antibiotics (meropenem, imipenem and ertapenem) showed the greatest efficacy against most of the E. coli isolates causing intramammary infections in dairy animals. Similarly, for P. aeruginosa, meropenem found to be the most effective. Other antibiotics such as ciprofloxacin classified as intermediate, azithromycin classified as susceptible, and vancomycin also classified as susceptible. However, P. aeruginosa was resistant against enrofloxacin, linezolid, and cefixime, chloramphenicol indicating a complete lack of sensitivity. These results are in line with the results of research conducted by [33].

These findings clearly indicate a trend of increasing antimicrobial resistance among mastitis pathogens, especially those capable of forming biofilms. The resistance patterns observed in this study underline the importance of conducting routine antibiogram studies before initiating treatment. An antibiogram serves as a valuable tool in identifying the most effective antibiotic for a given infection. This will improve not only treatment outcomes but also help preserve the efficacy of existing antibiotics by reducing unnecessary usage. In the long term, integrating routine antibiotic sensitivity testing into mastitis control programs can contribute to better antimicrobial stewardship and improved overall herd health [38].

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References

[1] Zalewska M, Brzozowska P, Rzewuska M, Kawecka-Grochocka E, Urbańska DM, Sakowski T, Bagnicka E. The quality and technological parameters of milk obtained from dairy cows with subclinical mastitis. J Dairy Sci. 2025;108(2):1285–1300.

[2] Fredebeul-Krein F, Schmenger A, Wente N, Zhang Y, Krömker V. Factors associated with the severity of clinical mastitis. Pathogens. 2022;11(10):1089.

[3] Goulart DB, Mellata M. Escherichia coli mastitis in dairy cattle: etiology, diagnosis, and treatment challenges. Front Microbiol. 2022; 13:928346.

[4] Schukken YH, Chuff M, Moroni P, Gurjar A, Santisteban C, Welcome F, Zadoks R. The "other" gram-negative bacteria in mastitis: Klebsiella, Serratia, and more. Vet Clin North Am Food Anim Pract. 2012;28(2):239–256.

[5] My TT, Manh VD, My BTP, Lan DTM, Binh DX, Duc VM. Antimicrobial resistance and molecular characterization of Escherichia coli isolated from bovine mastitis samples in Nghe an province, Vietnam. Vet World. 2023; 16:743.

[6] Badawy B, Moustafa S, Shata R, Sayed-Ahmed MZ, Alqahtani SS, Ali MS, et al. Prevalence of multidrug-resistant Pseudomonas aeruginosa isolated from dairy cattle, milk, environment, and workers' hands. Microorganisms. 2023;11(11):2775.

[7] Cheng WN, Han SG. Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments. Asian-Australas J Anim Sci. 2020; 33:1699.

[8] Kostakioti M, Hadjifrangiskou M, Hultgren SJ. Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the post-antibiotic era. Cold Spring Harb Perspect Med. 2013; 3:010306.

[9] Pedersen RR, Krömker V, Bjarnsholt T, Dahl-Pedersen K, Buhland R, Jørgensen E. Biofilm research in bovine mastitis. Front Vet Sci. 2021; 8:656810.

[10] Muloi DM, Ibayi EL, Nyotera S, et al. Treatment strategies and antibiotic usage practices in mastitis management in Kenyan smallholder dairy farms. BMC Vet Res. 2025; 21:212.

[11] Sharun K, Dhama K, Tiwari R, Gugjoo MB, Yatoo MI, Patel SK, et al. Advances in therapeutic and managemental approaches of bovine mastitis. Vet Q. 2021; 41:107–136.

[12] Oliver SP, Murinda SE. Antimicrobial resistance of mastitis pathogens. Vet Clin North Am Food Anim Pract. 2012;28(2):165–185.

[13] Karabasanavar N, Manjunatha L, Jeevanand M, Naveenkumar G. Evaluation of Surf Field Mastitis Test (SFMT) for the detection of subclinical mastitis in dairy cows. Asian J Dairy Food Res. 2021; 40:380–383.

[14] Geletu US, Usmael MA, Ibrahim AM. Isolation, identification, and susceptibility profile of E. coli, Salmonella, and Staphylococcus aureus in dairy farms and their public health implication in central Ethiopia. Vet Med Int. 2022; 2022:1887977.

[15] Banerjee S, Batabyal K, Joardar S, Isore D, Dey S, Samanta I, et al. Detection and characterization of pathogenic Pseudomonas aeruginosa from bovine subclinical mastitis in West Bengal, India. Vet World. 2017; 10:738.

[16] Rao KA, Vishwajith S, Sampath S, Lakshminarayana SA. Ventilator-associated pneumonia: an enduring hitch in intensive care units—a study from a tertiary care center. Indian J Microbiol Res. 2019; 6:194–197.

[17] Nguyen HT, O'Donovan LA, Venter H, Russell CC, McCluskey A, Page SW, et al. Comparison of two transmission electron microscopy methods to visualize drug-induced alterations of gram-negative bacterial morphology. Antibiotics (Basel). 2021;10(3):307.

[18] Wolska K, Kot B, Jakubczak A. Phenotypic and genotypic diversity of Pseudomonas aeruginosa strains isolated from hospitals in Siedlce, Poland. Braz J Microbiol. 2012;43(1):274–282.

[19] Thean RK, Ong DX, Heng ZS, Gan SK, Yeo JY. To plate or to simply unfreeze: the question for optimal plasmid extraction. J Biomol Tech. 2021;32(2):57–62.

[20] Leoney A, Karthigeyan S, Asharaf AS, Felix A. Detection and categorization of biofilm-forming bacteria isolated from complete denture patients and visualization using scanning electron microscopy. J Int Soc Prev Community Dent. 2020; 10:627–633.

[21] Iftikhar M, Khan I, Khan SJ, Khan JZ, Rahman SU. Antibiogram and antibiotic resistance patterns in bacterial isolates from Hayatabad Medical Complex, Peshawar. Cureus. 2024;16(10):71934.

[22] Lozano-Guzmán E, Carpio-Montoya JJ, Castro-Ríos ML, Meneses-Mondragón F, Rodríguez-Hernández AM. Low accuracy of the McFarland method for estimation of bacterial populations. Afr J Microbiol Res. 2018;12(31):736–740.

[23] Jabbar A, Ashraf M, Rahman S, Sajid M. Prevalence, molecular characterization and antibiogram of Mycoplasma bovis isolated from milk in Pakistan. Pol J Vet Sci. 2023; 26:461–471.

[24] Aika IN, Enato E. Antibiogram of clinical isolates from primary and secondary healthcare facilities: a step towards antimicrobial stewardship. PLOS Glob Public Health. 2022; 2:0000644.

[25] Ruegg PL. A 100-year review: mastitis detection, management, and prevention. J Dairy Sci. 2017;100(12):10381–10397.

https://doi.org/10.63954/tjh13010

[26] Abebe GM. The role of bacterial biofilm in antibiotic resistance and food contamination. Int J Microbiol. 2020; 2020:1705814.

[27] Muhammad G, Naureen A, Asi MN, Saqib M, Fazal-ur-Rehman. Evaluation of a 3% surf solution (surf field mastitis test) for the diagnosis of subclinical bovine and bubaline mastitis. Trop Anim Health Prod. 2010;42(3):457–464.

[28] Mirghani R, Saba T, Khaliq H, Mitchell J, Do L, Chambi L, et al. Biofilms: formation, drug resistance and alternatives to conventional approaches. AIMS Microbiol. 2022;8(3):239–277.

[29] Michaelis C, Grohmann E. Horizontal gene transfer of antibiotic resistance genes in biofilms. Antibiotics (Basel). 2023;12(2):328.

[30] Schönborn S, Krömker V. Detection of the biofilm component polysaccharide intercellular adhesin in Staphylococcus aureus infected cow udders. Vet Microbiol. 2016; 196:126–128.

[31] Zhao A, Sun J, Liu Y. Understanding bacterial biofilms: from definition to treatment strategies. Front Cell Infect Microbiol. 2023; 13:1137947.

[32] Zuo J, Lv Z, Lian L, Wu Z, Fu S, Zhang H, et al. Difference analysis on virulence genes, biofilms and antimicrobial susceptibility of Escherichia coli from clinical and subclinical bovine mastitis. Vet Sci. 2025;12(2):132.

[33] Huang Y, Chen P, Cao H, Zhou Z, Xu T. Characterization of Pseudomonas aeruginosa isolated from bovine mastitis in Northern Jiangsu Province and correlation to drug resistance and biofilm formability. Animals (Basel). 2024;14(22):3290.

[34] Rudenko P, Sachivkina N, Vatnikov Y, Shabunin S, Engashev S, Kontsevaya S, et al. Role of microorganisms isolated from cows with mastitis in the Moscow region in biofilm formation. Vet World. 2021;14(1):40–48.

[35] Zanichelli V, Monnier AA, Gyssens IC, Adriaenssens N, Versporten A, Pulcini C, et al. Variation in antibiotic use among and within different settings: a systematic review. J Antimicrob Chemother. 2018; 73:17–29.

[36] Singh A, Chhabra D, Sikrodia R, Shukla S, Sharda R, Audarya S. Isolation of E. coli from bovine mastitis and their antibiotic sensitivity pattern. Int J Curr Microbiol App Sci. 2018;7(10):9–15.

[37] Fahim KM, Ismael E, Khalefa HS, Farag HS, Hamza DA. Isolation and characterization of E. coli strains causing intramammary infections from dairy animals and wild birds. Int J Vet Sci Med. 2019;7(1):61–70.

[38] Laborda P, Gil-Gil T, Martínez JL, Hernando-Amado S. Preserving the efficacy of antibiotics to tackle antibiotic resistance. Microb Biotechnol. 2024;17(7):14528.

Declarations:

Authors' Contribution:

- All authors Conceptualization, data collection, interpretation, drafting of the manuscript
- h Intellectual revisions
- The authors agree to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed

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