RESEARCH ARTICLE

Antimicrobial Resistance Profiles of Bacterial Isolates from Clinical Cases in Livestock and Poultry

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Abstract

This study investigated the antimicrobial resistance profiles of bacterial isolates from clinical cases in ruminants (n=163) and poultry (n=64). In ruminants, *Staphylococcus aureus* exhibited the highest resistance to penicillin-G (85.45%), tetracycline, and polymyxin-B (80% each). *Streptococcus* spp. showed the greatest resistance to polymyxin-B (100%) and methicillin (86.67%). *E. coli* isolates displayed maximum resistance to penicillin-G, cephalothin, methicillin, and vancomycin (100% each), while *Salmonella* isolates were resistant to methicillin (100%), vancomycin (85.71%), and polymyxin-B (85.71%). All *Corynebacterium* spp. were fully resistant to nitrofurantoin, and all *Pseudomonas* isolates were resistant to ampicillin, nitrofurantoin, penicillin-G, methicillin, tetracycline, and vancomycin. In poultry, *E. coli* exhibited complete resistance to nitrofurantoin, vancomycin, penicillin-G, methicillin, and cephalothin, followed by tetracycline (88%), co-trimazole (88%), polymyxin-B (84%), and ampicillin (80%). *Salmonella* isolates were resistant to methicillin (100%), vancomycin (80%), polymixin-B (80%), and ampicillin and co-trimoxazole (73.33%). *Proteus mirabilis* exhibited high resistance to penicillin-G, methicillin, tetracycline, nitrofurantoin, vancomycin, and cephalothin (100% each), followed by co-trimazole (91.67%) and polymyxin-B (88.33%). *Klebsiella* isolates showed over 90% resistance to penicillin-G, polymyxin-B, vancomycin, methicillin, cephalothin, and ampicillin. Antimicrobials penicillin-G, methicillin, tetracycline, co-trimoxazole, ampicillin, vancomycin, and amoxicillin-clavulanic acid exhibited 100% high resistance in *Pseudomonas* isolates. The least resistance was observed in all isolates to gentamicin, ciprofloxacin, ofloxacin, chloramphenicol, amoxicillin-clavulanic acid, and azithromycin.

Key words: Antimicrobial resistance, Livestock, Multidrug, Poultry, Prevalence, Udaipur.

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Introduction

The emergence and spread of antimicrobial resistance represent a critical global health challenge, demanding a comprehensive understanding of the factors driving resistance within the context of animal husbandry, particularly in livestock and poultry production (Frye and Jackson, 2013; Hedman *et al*., 2020). Antimicrobial drugs have been historically utilized in food animal production not only for therapeutic interventions, addressing and controlling infectious diseases, but also for non-therapeutic purposes, including growth promotion (Nair *et al*., 2018). The practice of administering antimicrobials to livestock, while intended to enhance productivity and prevent disease, inadvertently exerts selective pressure on bacterial populations, leading to the proliferation of resistant strains (Verraes *et al*., 2013). The widespread use of antimicrobials in agriculture, often in sub-therapeutic doses, contributes significantly to the development and dissemination of resistance, exposing both animals and humans to resistant microorganisms through various pathways, including the consumption of contaminated products and environmental release (Rossi *et al*., 2020).

Furthermore, insects commonly associated with food animals, such as houseflies and cockroaches, can act as vectors, transporting microorganisms from farms to urban centers, potentially spreading multidrug-resistant bacteria. The complexity of antimicrobial resistance is compounded by the ability of bacteria to transfer resistance genes horizontally through plasmids, transposons, and integrons, facilitating the rapid dissemination of resistance traits across diverse bacterial species (White *et al*., 2002). The alarming rise in antimicrobial resistance necessitates a comprehensive investigation into the specific resistance profiles exhibited by bacterial isolates from clinical cases in livestock and poultry,

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aiming to elucidate the underlying mechanisms and inform targeted interventions to mitigate the spread of resistance, hence this study was undertaken on clinical cases in livestock and poultry.

Materials and Methods

The antimicrobial resistance patterns of bacterial pathogens isolated from various clinical samples in ruminants and poultry in the Udaipur district of Rajasthan were determined phenotypically. The disc diffusion method was used to determine the antimicrobial resistance pattern of bacterial isolates. The study was conducted at the Department of Veterinary Microbiology, College of Veterinary and Animal Science, Navania, Udaipur, Rajasthan. Materials and scientific equipment used included standard culture for *E. coli, Staphylococcus aureus, Streptococcus agalactiae, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa*, and *Salmonella enteric* subsp. enteric serovar typhimurium obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. Bacteriological culture examination involved collecting clinical samples and streaking them on various plates, incubating them aerobically at 37°C for 24 to 48 h. After 24 h, the isolated colonies were subjected to Gram staining to distinguish between Gram-positive and Gram-negative organisms. Biochemical tests were conducted to confirm the presence of bacteria in the colonies, including catalase and oxidase tests, and oxidation-fermentation tests. The study focused on the detection of *Staphylococcus* species using various tests and methods. Coagulase production, growth on Edward's Media, nitrate reduction, Indole test, methyl red (MR), Voges Proskauer (VP), Simmon's citrate agar, and triple sugar iron (TSI) test. Pure cultures of various bacteria were preserved and revived once in three months

Results and Discussion

The study characterized various bacteria, including *Staphylococcus aureus, Streptococcus* spp., *Corynebacterium* spp., *E. coli,* and *Klebsiella* spp., using selective and differential media from clinical samples of livestock and poultry. The results showed that *Staphylococcus aureus* colonies were golden yellow on Mannitol salt agar, while *Streptococcus* spp. colonies were found to be Gram-positive, catalase-positive, oxidase-negative, and non-motile. *Streptococcus agalactiae* colonies were found to be 53.33%, with 16 isolates identified as *Streptococcus agalactiae*. *Corynebacterium* spp. colonies were yellow, rough, and positive for catalase and methyl red tests. *E. coli* isolates were lactose fermented bright pink colonies on MLA plates, with characteristic greenish metallic sheen on EMB agar and blue green colonies on Hicrome *E. coli* agar. All *E. coli* isolates were further confirmed by biochemical tests, with all isolates found to be Gram's negative rods, catalase-positive, oxidase-negative, and fermentative in the OF test and motile in the motility test. *Klebsiella* spp. colonies were profound pink colored mucoid colonies on Mac Conkey agar and positive urease activity on urea agar. The study provided valuable insights into the morphology, culture, and biochemical characteristics of various bacteria. The findings can be used to inform future research and treatment strategies for bacterial infections.

In the present study, antimicrobial resistance profiles (Table 1, 2) revealed that among 163 ruminants' isolates, *Staphylococcus aureus* displayed the highest resistance to penicillin-G (85.45%) and tetracycline and polymyxin-B (80% each). The *Streptococcus* spp. showed the greatest resistance to polymyxin-B and methicillin. In *E. coli* isolates, the maximum resistance was to penicillin-G, cephalothin, methicillin and vancomycin. Among *Salmonella* isolates, resistance was reported against methicillin, vancomycin and polymyxin-B. Of all *Corynebacterium* spp. was fully resistance to nitrofurantoin. All *Pseudomonas* isolates demonstrated resistance to ampicillin, nitrofurantoin, penicillin-G, methicillin, tetracycline and vancomycin (Table 1).

In poultry among 64 isolates, *E. coli* exhibited complete resistance to nitrofurantoin, vancomycin, penicillin-G, methicillin and cephalothin, followed by tetracycline, co-trimazole, polymyxin-B and ampicillin. *Salmonella* isolates were resistant to methicillin, vancomycin, polymixin-B, ampicillin and co-trimoxazole (Table 2). *Proteus mirabilis* exhibited high resistance to penicillin-G, methicillin, tetracycline, nitrofurantoin, vancomycin, and cephalothin (100% each), followed by co-trimazole (91.67%) and polymyxin-B (88.33%). *Klebsiella* isolates showed over 90% resistance to penicillin-G, polymyxin-B, vancomycin, methicillin, cephalothin, and ampicillin. Antimicrobials penicillin-G, methicillin, tetracycline, co-trimoxazole, ampicillin, vancomycin, and amoxicillin-clavulanic acid exhibited 100% high resistance in *Pseudomonas* isolates. The least resistance was observed in all isolates to gentamicin, ciprofloxacin, ofloxacin, chloramphenicol, amoxicillin-clavulanic acid, and azithromycin (Table 2).

The observed high resistance to penicillin-G among *Staphylococcus aureus* isolates from ruminants aligned with previous studies indicating widespread resistance to beta-lactam antibiotics in *staphylococcal* species, potentially attributable to the production of beta-lactamase enzymes that inactivate penicillin (Johnson, 2017). Similarly, the high resistance to tetracycline observed in this study is consistent with reports of tetracycline resistance in *Staphylococcus aureus* isolates from various animal sources, possibly due to the acquisition of tetracycline resistance genes such as tet(M) and tet(K). The complete resistance of *Streptococcus* spp. isolates to polymyxin-B is noteworthy, as polymyxins are often considered last-resort antibiotics for Gram-negative bacterial infections, suggesting potential mechanisms of resistance or misidentification of species. The 100% resistance observed in *E. coli* isolates from ruminants against penicillin-G, cephalothin, methicillin, and vancomycin highlights the extensive multidrug resistance in this species, warranting

Table 1: Antimicrobial resistance profile of bacterial isolates (163) from ruminant

Antimicrobial	Bacteria											
	***S. aureus* (55)**		***Streptococcus* Spp. (30)**		***E. coli* (61)**		***Salmonella* spp. (07)**		***Corynebacterium* spp. (6)**		***Pseudomonas* spp. (4)**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Penicillin G	47	85.45	11	36.67	61	100	4	57.14	0	0.0	4	100
Methicillin	28	50.91	26	86.67	61	100	7	100	0	0.0	4	100
Ampicillin	39	70.91	10	33.33	19	31.15	5	71.43	0	0.0	4	100
Amoxi-clavul. acid	6	10.91	5	16.67	16	26.23	0	0.0	0	0.0	2	50
Cephalothin	0	0.0	14	46.67	61	100	3	42.86	0	0.0	4	100
Ceftriaxone	8	14.55	8	26.67	18	29.51	2	28.57	0	0.0	2	50
Ofloxacin	4	7.27	4	13.33	7	11.47	0	0.0	0	0.0	0	0.0
Ciprofloxacin	1	1.81	6	20	8	13.11	0	0.0	0	0.0	1	25
Gentamicin	9	16.36	6	20	15	24.59	0	0.0	0	0.0	0	0.0
Tetracycline	44	80	12	40	47	77.05	5	71.42	0	0.0	4	100
Co-trimoxazole	14	25.45	0	0	13	21.31	5	57.42	0	0.0	3	75
Chloramphenicol	2	3.63	0	0	2	3.28	0	0.0	0	0.0	0	0.0
Polymyxin-B	44	80	30	100	30	49.18	6	85.71	0	0.0	2	50
Azithromycins	3	5.45	14	46.67	15	24.59	1	14.28	0	0.0	0	0.0
Vancomycin	0	0	9	30	61	100	6	85.71	0	0.0	2	100
Nitrofurantoin	7	12.72	0	0.0	61	100	3	42.86	6	100	2	100

n= number of isolates, %= percentage

Table 2: Antimicrobial resistance profile of bacterial isolates (64) from poultry

Antimicrobial	Bacteria									
	***E. coli* (25)**		***Salmonella* spp. (15)**		***Proteus mirabilis* (12)**		***Klebsiella* spp. (10)**		***Pseudomonas* spp. (02)**	
	n	**%**	**n**	**n**	**n**	**%**	**n**	**%**	**n**	**%**
Penicillin-G	25	100	10	66.67	12	100	10	100	2	100
Methicillin	25	100	15	100	12	100	10	100	2	100
Ampicillin	18	72	11	73.33	0	0.0	09	90	2	100
Amoxi-clavul. acid	12	48	0	0.0	3	25	6	60	1	50
Cephalothin	25	100	4	26.67	12	100	10	100	2	100
Ceftriaxone	10	40	0	0.0	3	25	5	50	1	50
Ofloxacin	0	0	0	0	9	75	4	40	0	0.0
Ciprofloxacin	14	56	0	0.0	0	0.0	5	50	0	0.0
Gentamicin	6	24	0	0.0	2	16.67	1	10	0	0.0
Tetracycline	22	88	11	73.33	12	100	7	70	2	100
Co-trimoxazole	21	84	11	73.33	11	91.67	6	60	2	100
Chloramphenicol	2	8	0	0.0	0	0.0	4	40	0	0.0
Polymyxin-B	21	84	12	80	11	91.67	10	100	0	0.0
Azithromycins	4	16	1	6.67	8	53.33	4	40	0	0.0
Vancomycin	25	100	12	80	11	91.67	10	100	2	100
Nitrofurantoin	25	100	7	46.67	12	100	3	30	2	100

n= number of isolates, %= percentage

further investigation into the underlying resistance mechanisms and genetic determinants. The complete resistance of *Corynebacterium* spp. isolates to nitrofurantoin is intriguing, as nitrofurantoin is commonly used for urinary tract infections, suggesting potential implications for treatment options in animals. The pan-resistance observed in *Pseudomonas* isolates against multiple antibiotics is concerning, as *Pseudomonas* spp. are opportunistic pathogens capable of causing severe infections, emphasizing the need for stringent infection control measures. The high resistance rates observed in poultry *E. coli* isolates against multiple antibiotics underscore the urgent need for prudent antibiotic use and alternative strategies to control bacterial infections in poultry production.

The high elevated levels of multidrug resistance found in both ruminant and poultry underscore the necessity for robust antimicrobial stewardship initiatives in veterinary medicine to promote responsible antibiotic use and minimize the selection and spread of resistant bacteria (Tepper, 2018). Surveillance programs to track antimicrobial resistance trends in livestock and poultry are essential for early identification of developing resistance patterns and for guiding evidence based responses. The findings in the current study support previous research that shows a correlation between antibiotic usage and the development of resistance, underscoring the significance of implementing strict antimicrobial stewardship programs in veterinary and agricultural contexts (Pulingam *et al*., 2021). Moreover, this highlights the significance of integrated strategies, such as the One Health approach, to tackle antibiotic resistance holistically, acknowledging the interconnectedness of human, animal, and environmental health (Ajayi *et al*., 2024).

The escalating utilization of antibiotics in livestock may impact public health because newer drugs are often more expensive than older drugs, and antibiotics recently introduced for use in farm animals mostly have a broader spectrum of activity than older drugs and therefore impose a broader selection pressure for resistance (Bengtsson and Greko, 2014). Also, the use of antibiotics in food animals can result in the presence of antibiotic residues in animal products such as meat, milk, and eggs, which can pose a risk to human health (Aarestrup, 2015). Therefore, judicious use of antibiotics in healthcare and agricultural settings is essential to slow the emergence of resistance and extend the useful lifetime of effective antibiotics that are in existence today (Ayukekbong *et al*., 2017). The use of antibiotics should be guided by accurate diagnostic information and tailored to the specific bacterial species and their antimicrobial susceptibility profiles (Li *et al*., 2017).

The use of broad-spectrum antibiotics should be minimized, and narrow-spectrum antibiotics should be preferred whenever possible, to reduce the selective pressure for resistance. The emergence and spread of antimicrobial-resistant bacteria in poultry pose a significant threat to both animal and public health (Kiambi *et al*., 2021). Further research is needed to characterize the specific resistance genes present in the bacterial isolates and to understand the mechanisms of resistance. Multidrug resistance is common among isolates of *E. coli* from turkeys, chickens, ducks, and game birds (Varga *et al*., 2019). The molecular characterization of resistance genes can provide insights into the origins and transmission pathways of resistance.

Conclusion

The study highlights the alarming prevalence of multidrug-resistant bacteria in livestock and poultry, emphasizing the urgent need for comprehensive antimicrobial stewardship programs, improved infection control practices, and the development of alternative strategies to combat antimicrobial resistance in veterinary medicine. The food animal industry faces the challenge of increasing productivity while minimizing antimicrobial resistance. The inappropriate use of antibiotics in animal agriculture is a major driver of antimicrobial resistance.

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