RESEARCH PAPER

Cross-Ecosystem Dissemination of Antimicrobial Resistance: A One Health Investigation of Clinical, Hospital, and Livestock Isolates in Jaipur, India

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ABSRACT: This study investigates antibiotic-resistant bacterial isolates from clinical and environmental sources, revealing that resistance is not limited to hospital settings but is now widespread among the general population. The isolation of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* from cattle and poultry suggests that agricultural practices, particularly the use of antibiotics as growth promoters, serve as significant reservoirs for resistance genes. Hospital-derived isolates exhibited notably higher resistance levels, with minimum inhibitory concentrations (MICs) up to 8 µg/mL and multiple antibiotic resistance (MAR) indices as high as 0.55, compared to lower MICs (1–2 µg/mL) and MAR values (0.18–0.22) in cattle isolates. These elevated MAR indices in hospital strains reflect substantial antibiotic pressure in clinical environments. A consistent trend was observed across species: hospital isolates showed higher MICs, MBCs, and MARS indices than those from cattle farms, implying a more intense selective pressure in clinical settings. Notably, most cattle isolates had MAR ≥ 0.2, reinforcing the concept of "One Health," which connects human, animal, and environmental resistance. While some antibiotics (e.g., cefotoxin, ciprofloxacin, and co-trimoxazole) remain effective, rising resistance to fluoroquinolones raises concern about emerging multidrug-resistant strains. The detection of colistin resistance in environmental sources, especially plasmid-mediated mcr-1, underscores the urgency of molecular monitoring and strict antibiotic stewardship in both hospital and agricultural domains.

KEYWORDS: MARI, XRD colistin resistant bacteria, poultry isolates, biofilm, MBC

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INTRODUCTION:

Antibiotics may be defined as antimicrobial substances that have activity against bacteria that may kill or inhibit their growth. Since their discovery, antibiotics have played central role in treatment of wide range of infectious diseases. However, with each new antibiotic introduced, resistance soon followed, underscoring the evolutionary arms race between microbes and medicine[1]. Unfortunately, most of the antibiotics consumed by humans or animals are not completely metabolized in their bodies and thus they release these antibiotics into the environment through excreta and urinal ways[2]. Antibiotic residues may lead to adverse effects in the environment, such as the development of resistant bacteria/resistance genes and may eventually become a threat to human health. Antibiotic resistance is traditionally reported as developing at or above the antibiotic minimum inhibitory concentration (MIC), due to the acquisition of genetic traits by mutation(s) or (HGT) horizontal gene transfer these new traits are passed on to new offspring[3].

The antibiotic-resistant genes (ARGs) present in the antibiotic-resistant bacteria (ARB) are associated with integrons and transposons present in the plasmids which further increases the risk of transmission of antibiotic resistance (AR) through horizontal gene transfer. Meanwhile, animal habit, including exploiting antibiotics unacceptable and not hurling them apart seemly, have possessed a huge influence on how these resistance genes disperse and evolve in the environment[4]. use of a wide spectrum of antibiotics for purposes unlike medical and veterinary vigilance, like praising the maturation of livestock, complicates the dilemma of drug resistance[5]. Antibiotic-resistant bacteria are becoming increasingly problematic for healthcare systems globally. Biocide pollution contributes to the evolution and dissemination of bacterial resistance among humans, animals, and the environment by creating favourable conditions for selecting resistant bacteria and facilitating the transmission of genetic material across ecological boundaries[6]. Drug-resistant pathogenic bacteria are continuously identified in both clinical and community settings whose phenotypic resistance can be attributed to genes acquired from another organism through horizontal gene transfer (HGT) via conjugation, transformation, or transduction. According to the study the use of manure from cattle, chicken, swine, and sewage is the primary source of antibiotic pollution[7]. Several studies before and after 2014 indicate that consumers can still purchase antibiotics without a valid prescription as pharmacists still dispense antibiotics to their customers/clients/patients by attending to their symptoms[8].

Over 35,000 human-kind are picture to passed away to infection by bacteria resistant to anti-microbe annually in the EU and the nations that composed the European Economic Area. Infections in both hospital and community settings invoke towards 874,000 disability-adjusted life years, that sum up into direct and indirect expenditure of over EUR 1.5 billion[9]. By 2050, nevertheless, antibiotic-resistant diseases are anticipated to dismiss up to 10 million human-kind yearly and cost the globally economy across $100 trillion[10]. The development of numerous microbes in Indian rivers which is anti-microbial resistance is a dangerous health hazardous. In addition, the insufficient sewage treatment results in the discharge of inadequately treated waste into the rivers. Multidrug-resistant bacteria in water sources is a recognized health hazard to the community. The rivers of Metropolitan are very prone and serve as a reservoir of antibiotic-resistant bacteria with antibiotic resistance genes.A WHO reported: there should be a effective information of the numerous international associations, handling of antibiotics in consider to the impurities of surface water by antibiotics[11]. Human health is harshly affected by the increase in AMR, basically owing to elevated grade of infections and mortality. It is now more difficult to treat infections that were preliminarily easily treated with general drug, leading to influences the risk of complications and lengthen illnesses[12].

Given the limited preferences for handling patients with Gram-negative infections, antibiotic resistance in these microbes suggest a terribly serious risk. The groups' carbapenem resistance is usually induced by the construction of carbapenem-hydrolyzing β-lactamases, or carbapenemases, which consists of enzymes across molecular classes A and B in most regions[13]. *Bacillus polymyxa*is the origin of the 1947 invention of the polymyxin class of antibiotics, that is one such competitor. More than fifteen varieties of polymyxins have been isolated and characterized, among which the most prominent ones are polymyxin B and polymyxinE (also known as colistin). The polymyxins exhibit significant inhibitory effect against the Gram-negative bacterial pathogens, therefore, it has emerged as an ultimate choice to target such pathogens that have already become resistant to the frontline antibiotics such as β-lactams[14].

The excessive use of polymyxin E (i.e., colistin) and polymyxin B in animal production has led to the emergence of transferable resistance toward these antibiotics, leaving few or no options for treatment of the associated infections. The emergence of mobile colistin-resistance genes (i.e., plasmid-borne MCR) is especially troubling, since it enables rapid spreading of resistance among Gram-negative bacterial species[15]. Colistin is essential in veterinary medicine and is considered irreplaceable for specific intestinal enterotoxemia and septic E. coli infections in pigs and poultry. However, some Enterobacteriaceae are inherently resistant to colistin[16]. Colistin resistance mechanisms in *Enterobacterales* primarily involves the disruption of the upstream PmrAB and PhoPQ two-component system (TCS) control caused by mutations in the regulatory genes[17]. Colistin resistance is prevalent among Gram-negative bacteria in urinary tract infections, with rates varying between 3 and 28% for *P. aeruginosa* and 2.8 and 10.5% for *K. pneumoniae*. antibiotics from different families inhibit bacteria using various mechanisms, and synergy assessment has clarified the interaction of two drugs in combination against bacterial isolates. Therefore, combination therapy could suppress bacterial communities more efficiently in comparison to monotherapy colistin-carbapenems combination therapy to inhibit the biofilm community of Gram-negative bacteria[18]. This study focuses on bacteria that exhibit colistin resistance and explore other therapeutic options that maysuppress or inhibit the growth. Theexperiment involvedcollecting sample from multiple locations, followed by isolation and screening of bacterial strain for colistin resistance and subsequent antibiotic susceptibility test.

METHODOLOGY

2.1.1 Clinical Samples

The sample collection for the study was conducted between January and February 2025 at the Jaipur National University, Jaipur. Bacterial samples described in this study are from cultures obtained by the microbiology and clinical pathology department as part of routine care for hospitalized infected patients. No additional clinical specimens were obtained for purposes of research; therefore, informed consent was not required. Clinical samples were cultured on Blood agar and MacConkey agar[19].

2.1.2 Environmental Samples

Clinical, Poultry and Cattle farm soil and urine samples were collected from JNU hospital and Gopalji farms, Jagatpura, Jaipurduring January-February 2024-2025. The samples were collected in pre-sterilized plastic zip-bags and plastic bottles, respectively, pH and temperature were recorded. The samples were transported under ice cold condition from collection point to the research station at School of life and basic sciences.

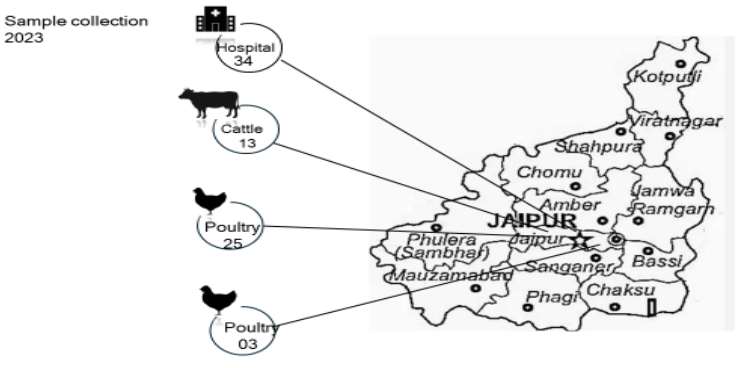


Figure 1: Geographical Distribution of sample collection sites for isolation of colistin resistant bacteria in Jaipur

2.2 Isolation and screening of colistin resistant bacterial isolates

2.2.1 Sample processing and enrichment:

One ml of each sample was added to 100 ml of pre-sterilized colistin enrichment broth medium. Each water sample (200 ml) was separately filtered through 0.25 µm filter paper (Axiva), subsequently used to seed 100 ml enriched broth. All samples were incubated in orbital shaking incubator (Remi C-24 BL) at 37 °C for 24 h at 180 rpm[20].

2.2.2 Preparation of McFarland Standards

McFarland Standards are used to standardize the approximate number of bacteria in a liquid suspension by comparing the turbidity of the test suspension with that of the McFarland Standard**.** A McFarland Standard is a chemical solution of barium chloride and sulfuric acid; the reaction between these two chemicals results in the production of a fine precipitate, barium sulphate. When shaken well, the turbidity of a McFarland Standard is visually comparable to a bacterial suspension of known concentration as indicated below. Mix McFarland Standard on a vortex mixture prior to examination. Ensure that the McFarland Standard is aliquoted into a tube that is the same size and diameter as the tube used to prepare the test suspension, Prepare a test suspension by obtaining a fresh, pure culture of the test organism and inoculating a suitable broth, In the presence of good lighting, visually compare the turbidity of test suspension with that of the McFarland standard by comparing the clarity of the lines, If the test suspension is too light, inoculate with additional organisms or incubate tube until turbidity matches that of the standard. If dilution is necessary, use a sterile pipette and add sufficient broth or saline to obtain a turbidity that matches that of the standard.

2.2.3 Detection of colistin resistant *bacterial isolates:*

The strains of colistin resistant *bacteria* were detected by determination of minimum inhibitory concentration of colistin by agar dilution method. Different concentrations of colistin ranging from 0.0625 to 32 µg/ml were prepared. One millilitre of each of these concentrations was added to 19 ml of Mueller–Hinton agar cooled to around 50 °C and allowed to solidify in sterilized petri plates. Then the concentration of the broth containing test organism was adjusted to 0.5 McFarland standards. Prior to inoculation, further (1:10) dilution of the broth containing test organism was performed. The plates were then inoculated with the test organisms and were incubated aerobically at 35 °C for 24 h, which were then examined for bacterial growth and interpreted according to CLSI guidelines.

2.2.4 The cross-resistance study-

Susceptibility test was carried out over the isolated bacterial strains by using different antibiotics. The wells contained different antibiotics namely Penicillin G(10 units),Cefoxitin (100mcg), Nalidixic acid (15mcg), Norfloxacin (2mcg)Amikacin, and ciprofloxacin (30mcg), Co-Trimoxazole (5mcg) Levofloxacin (30mcg) and many more all against Colistin was used. Bacterial inoculum was preparedas described by Lalitha, 2004 with little modification. Briefly, the bacterial inoculum of clinical isolates was prepared in normal saline with turbidity equivalent to 0.5 McFarland standards. 500µl of inoculums was spread onto the sterile Muller Hinton Agar (MHA) plates, and the antibiotic disc was placed and incubated at 37oC for 24 hours. The appearance of a clear zone was noted down (Lalitha, 2004)

2.2.5 Identification of Colistin Resistant Bacterial isolates

The colistin resistant bacteria isolates exhibiting multi drug resisting properties with pronounced *in vitro* biofilm activity were identified on the basis of morphological, cultural and biochemical characteristics with the help of Bergey's Manual of Systematic Bacteriology (Kreig and Holt, 1984) and Probabilistic Identification of Bacteria (PIB) computer kit (Bryant, 2003)**.**

2.2.6 Antibiotic susceptibility test

The MIC of colistin was determined by agar dilution method following the Clinical and Laboratory Standards Institute (CLSI) guidelines. Briefly, Mueller–Hinton (MH) agar was prepared and the final concentrations of colistin ranging from 0.06 to 128 mg l1 were added to MH agar (Hi-Media) in Petri plates, mixed well and allowed to solidify. Then, bacterial inoculum grown overnight was diluted in saline (10 µl in 4.99 ml) and placed on the surface of the agar (1 µl) within the marked grids placed under the plates. The inoculum was allowed to dry and was then incubated at 37 C for 20 h. The lowest concentration of colistin with the absence of visible bacterial growth was accepted as the MIC. The results were interpreted using the CLSI guidelines. Briefly, MH agar plates were prepared and bacterial inoculation adjusted to 0.5 McFarland turbidity standards were swabbed on the surface of the agar. Plates were dried for 5 min and Ertapenem Ezy MIC strips (Hi-Media) were placed in the centre of the plate and incubated at 37 C for 20 h. E-test strips were labelled from 0.002 to 32 mg l1 and the MIC was determined. The results were interpreted using the CLSI guidelines. For colistin, the MIC was determined using the micro-broth dilution method. Colistin was added at final concentrations ranging from 0.06 to 128 mg. Then, bacterial inoculation of different dilutions (CLSI guidelines) from overnight-grown cells were added to the respective wells and incubated at 37 C for 20 h. The results were interpreted using the CLSI guideline[21].

2.2.7Minimum Inhibitory Concentration (MIC)- Broth Micro dilution (BMD) method

The MIC of colistin was determined by broth micro dilution (BMD) method.Quality control strains (*Klebsiella pneumoniae*and *P. aeruginosa* ATCC 27853) yielded MICs within recommended ranges, confirming assay reliability. Colistin sulphate (analytical grade) was dissolved in sterile water to make a 10 mg/mL stock solution. The stock was filter-sterilized, aliquoted and stored at −20°C until use. Colistin serial dilution were prepared in Muller Hinton broth to yield final concentration ranging from 0.03–128 µg/mL (or limited to the 0.03–16 µg/mL). Bacterial suspension was prepared by suspending three to five well-isolated overnight colonies in sterile 0.9% NaCl and adjusting turbidity equivalent of a 0.5 McFarland standard (≈1–2 × 10^8 CFU/mL). The suspension was diluted to obtain a final inoculum of ≈5 × 10^5 CFU/mL per well. Plates were incubation at 35 ± 1°C for 20 hours. The MIC was recorded as the lowest concentration of colistin producing complete inhibition of visible growth, where an MIC ≤2 µg/mL is typically considered susceptible.

2.2.8 Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentration (MBC) testing was performed after MIC determination y broth micro dilution. The MBC was defined as the lowest antibiotic concentration that reduces the initial bacterial inoculum by ≥99.9% (≥3-log10 reduction) under the same incubation conditions used for MIC determination. After recording the MIC reading (18 h), 10 µL (or 100 µL referencing laboratory protocol) aliquots were taken from wells at and above the MIC concentration (i.e., MIC, 2×MIC, 4×MIC, etc.), that showed no visible growth and were subculture onto antibiotic-free Mueller-Hinton agar for duplicates in all tests. The Plates were incubated at 35°C for 24 h, after which the colonies were counted and compared with the growth control plated at time zero. The MBC was defined as the lowest antibiotic concentration achieving ≥99.9% kill, or ≤0.1% survival of the original inoculum. For spot plating, the MBC was recorded as the concentration that yielded no colonies, or a ≥3-log reduction compared with control.

2.2.9 Multiple antibiotic resistance (MAR) index calculation

The MAR index was calculated to quantify the aggregate antibiotic- resistance burden of each isolate, and were tested by MIC determination according to CLSI and EUCAST guidelines. MAR was calculated using Krumperman's formula: MAR= (number of antibiotics to which the isolate is resistant to) / (total number of antibiotics tested). MAR ≥0.2 was interpreted as indicative of isolates originating from high-risk sources with frequent antibiotic exposure. MAR values were calculated for each isolate and subsequently compared between cattle-farm and hospital-derived isolates to identify resistance patterns.

2.3 Identification of Colistin Resistant Bacterial isolates

The colistin resistant bacteria isolates exhibiting multi drug resisting properties with pronounced *in vitro* biofilm activity were identified on the basis of morphological, cultural and biochemical characteristics with the help of Bergey's Manual of Systematic Bacteriology (Kreig and Holt, 1984) and Probabilistic Identification of Bacteria (PIB) computer kit (Bryant, 2003)**.**

(a) Cattle farm Urine sample collection

(b) Cattle farm soil sample



(c)Poultry farm sampling site

(d) Poultry faecal soil sample

Figure 2: Collection of samples.

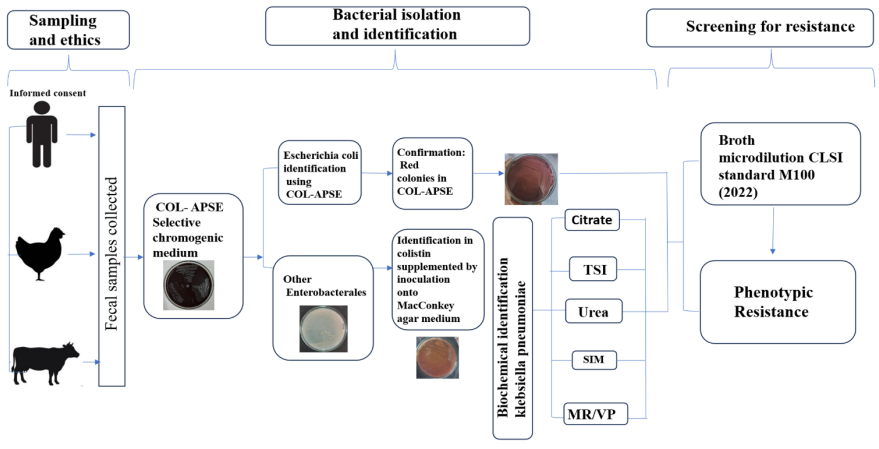


Figure 3:Processing and identification of potent colistin resistant bacteria

2.4 Biofilm Formation

Crystal violet staining method

Crystal violet (CV) was used for the qualitative determination of microtitters of biofilms grown *in vitro* in boiler tubes containing cattle farm, Hospital and poultry farms urine and soil samples.

Different tubes of nutrient broth were prepared. Samples collected from hospitals, cattle farm and poultry farm were (urine and soil sample) were inoculated in the nutrient broth and incubated at37◦C for 24 h. Next day broth was decent without touching attached film. Add 200 µl Crystal violet in tubes. Wash excess crystal violet and air dry the tubes. Observe biofilm in inner walls of tubes.

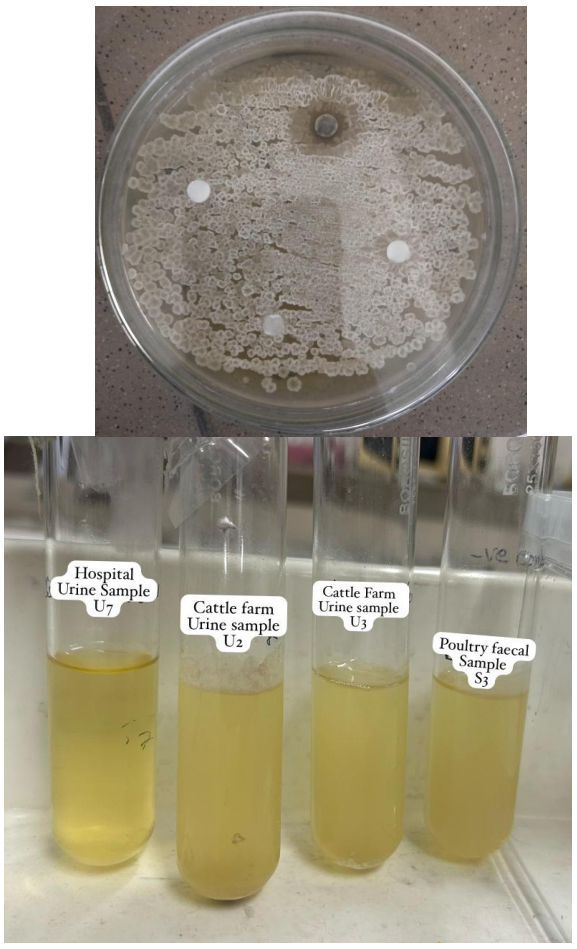


Figure 4: Nutrient Broth inoculated with samples.

2.5 Result and Discussion:

A total of 75consecutive, non-repetitive (resistant and susceptible) bacterial isolates were collected from different sources such as hospital, cattle fields, and poultry farms. Majority of bacterial isolates were isolated from hospitals (42.7%), poultry (33.3%), cattle fields (17.3%). This analysis reveals that colistin resistance is not found in the field of clinical but also appearing in community and agricultural practices. The distribution of susceptible isolates was similar, with the most susceptible isolates being in poultry (29.3%), hospitals (28%), and cattle fields (24%). These suggest that reservoirs of both resistant and susceptible bacteria coexist in different ecosystems.

. Most of the MCR harbouring isolates have been *Enterobacteriaceae,* such as *Escherichia coli* and *Salmonella enterica* which are common to animals and humans. To ensure standardization of bacterial density during antimicrobial susceptibility testing, the 0.5 McFarland turbidity standard was used, which is commercially available. which corresponds to 1.5 × 10^8 CFU/ml. This allowed accurate performance of the Kirby-Bauer disc diffusion method Colistin has been widely used in agricultural production and veterinary medicine for decades, and the recent global dissemination of mobilized colistin resistance (MCR) genes from animals to humans seriously threats the clinical use of colistin. Hospital urine samples frequently yielded colistin-resistant isolates, whereas cattle field samples (both cow urine and soil) and poultry faecal soil showed variable resistance. For instance, resistant strains were recorded in cattle field urine sample (U2), (U3) and poultry faecal sample (S3), and hospital urine sample (U7)and U9 while several other samples remained susceptible. (Table 1)

Table 1: Distribution of colistin-resistant and susceptible bacterial isolates across different sample sources

Subject	Sample	Location	Susceptible/ Resistant
Hospital	Urine (U1)	JNU	Susceptible
Cattle farm1	Cow Urine (U2)	Jagatpura	Resistant
Cattle farm 1	Soil (S1)	Jagatpura	Susceptible
Cattle field 2	Cow Urine(U3)	Jagatpura	Resistant
Cattle farm 2	Soil (S2)	Jagatpura	Susceptible
Poultry	Faecal Soil (S3)	Jagatpura	Resistant
Poultry	Feeding Soil (S4)	Jagatpura	Susceptible
Hospital	Urine (U4)	JNU	Susceptible
Hospital	Urine (U5)	JNU	Susceptible
Hospital	Urine(U6)	JNU	Susceptible
Hospital	Urine(U7)	JNU	Resistant
Hospital	Urine(U8)	JNU	Susceptible
Hospital	Urine (U9)	JNU	Susceptible
Hospital	Urine (U10	JNU	Susceptible

The continued isolation of resistant isolates from clinical and agricultural sources indicates a degree resistance factors sharing between environments. Contaminated growing colonies of colistin resistant bacteria demonstrate in (Figure 5), while inhibition zones receivesusceptibility to colistin, (Figure 6). Visual confirmation of resistance and susceptibility is important for confirming the results of antimicrobial susceptibility testing.

(a)

(b)

Figure 5: (a)Agar plate showing contaminated growing colonies of resistant bacteria, (b) Agar plate showing dense contaminated bacterial colonies with no zone of inhibition

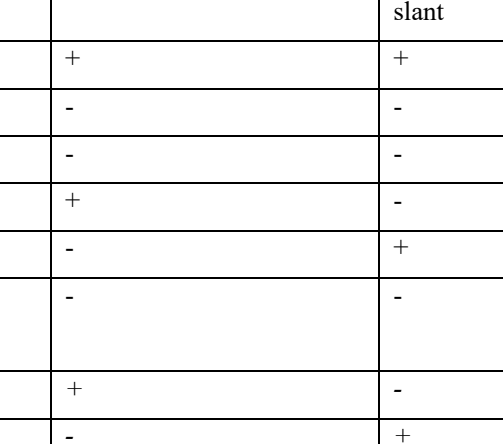


Figure 6: Zone of inhibition produced against colistin - susceptible bacterial isolates.

Bacterial species were identified by biochemical investigation. The types of biochemical reactions each organism undergoes act as a thumb print for its identification. The results confirmed the presence of *Klebsiella pneumonia* and *Pseudomonas aeruginosa*

Table 2: Biochemical characterization of isolated bacterial species

Tests	**Result (1)**	**Result (2)**
Gram staining	Gram negative	Gram negative
Catalase	+	+
Indole	-	-
Citrate	+	+
Urease	+	-
TSI	Acidic butt &Acidic slant	Alkali butt & Alkali slant
Gas	+	+
H_2S	-	-
Methyl Red	-	-
Voges Proskauer	+	-
Motility	-	+
Phenylalanine Deaminase	-	-
Lysine	+	-
Arginine	-	+
Ornithine	-	+
Glucose	+	+
Sucrose	-	-
Lactose	-	-
Mannitol	+	-
Result	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
PIB WIN score	0.978	0.976

+: Positive, -: Negative

Cross-resistance studies revealed alarming patterns. Human pathogen that causes infections primarily in patients in intensive care units (ICUs) is highly antibiotic resistant. Colistin is one of the last-line antibiotics for treating infections; however, colistin-resistant strains are becoming increasingly common. The resistant isolates U1, U2, S2, and U14 demonstrated full resistance to colistin, as expected (Table 3). All of the resistant isolates had the same amount of resistance to ampicillin and nystatin. However, many antibiotics such as ciprofloxacin, Nalidixic acid, Co-trimoxazole,Cedoxitin and streptomycin remained effective, showing distinct zones of inhibition (Figure 8). This means that while it is concerning that colistin resistance is prevalent, other treatment options are still viable. Nonetheless, the resistance of some isolates to fluoroquinolones (ex. Levofloxacin and nalidixic acid) also indicate the pathogens potential for multi-drug resistance.

Table 3: Antibiotic susceptibility profile of colistin-resistant isolates

ANTIBIOTICS	Cattle farm Urine sample (U2)	Cattle farm Urine sample (U3)	Poultry faecal sample (S3)	Hospital Urine sample (U7)
Ampicillin (AMP)	Resistant	Resistant	Resistant	Resistant
Nalidixic acid (NA)	Susceptible	Susceptible	Resistant	Resistant
Norfloxacin (NX)	Susceptible	Susceptible	Susceptible	Susceptible
Ciprofloxacin (CIP)	Susceptible	Susceptible	Resistant	Susceptible
Co-trimoxazole (COT)	Susceptible	Resistant	Susceptible	Resistant
Levofloxacin (LE)	Susceptible	Susceptible	Resistant	Resistant
Nystatin (NS)	Resistant	Resistant	Resistant	Resistant
Cefoxitin (CX)	Susceptible	Susceptible	Resistant	Resistant
Penicillin	Susceptible	Susceptible	Susceptible	Susceptible
Streptomycin	Susceptible	Susceptible	Resistant	Susceptible
Amikacin	Susceptible	Susceptible	Susceptible	Susceptible
Colistin	Resistant	Resistant	Resistant	Resistant

Figure: 7 Cross-resistance assay showing inhibition zone for susceptible antibiotics and absence of zones for resistant antibiotic

As for the MIC/MBC ratio,it was generally ≤4 for most isolates, confirming that colistin was bactericidal against these strains.Most isolates had colistin MICs ranging from 0.25 to 4 µg/mL. Using EUCAST/CLSI interpretive criteria. TheMBC values were typically 1–2 dilutions higher than MIC values, indicating that colistin had bactericidal activity at slightly higher concentration. For the majority of isolates, MBC values were between 0.5– 8 µg/mL.Antibiotic susceptibility testing across various classes is highly heterogeneous regarding resistance. MAR index values of the isolates span from 0.1 to 0.6. Importantly, the MAR index for hospital isolates was 0.38, which was significantly greater than for cattle isolates (0.21). According to Krumperman's criteria, if MAR≥0.2, the isolate is at high risk of prolonged exposure to antibiotics. If the MAR index for hospital isolates is compared to cattle isolates, Z% of hospital isolates exceeds the MAR index cut-off, implicating greater antimicrobial pressure in the clinical setting.The study assessed the Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Multiple Antibiotic Resistance (MAR) indices of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and isolates obtained from cattle field samples and hospital environments. This study provides significant differences in the resistance profiles of environmental (cattle) and clinical (hospital) sources, consistent with previous epidemiological observations that increasingly demonstrate the diffusion of multidrug resistance (MDR) between both veterinary and human domains.

In the case of *Pseudomonas aeruginosa*, isolates from cattle had lower MIC values (1-2 µg/mL) and MAR index (<0.22), while strains from hospitals had higher MIC values (maximum 8 µg/mL) and MAR index (≥0.36). This suggests that there is more selective pressure in a hospital environment, in keeping with studies that showed that *P. aeruginosa* often develops resistance to colistin and other last-line antibiotics in intensive care settings[22,23]. Moreover, MAR index indicate that clinical isolates MAR index (≥0.2) can be considered high-risk sources of contamination, in keeping with Krumperman's (1983) interpretation of a MAR index threshold and with applications in environmental and clinical surveillance[24,25]. Similarly, *Klebsiella pneumoniae* isolates from hospitals showed higher resistance, with in vitro MICs of 4–8 µg/mL and an MAR index of 0.50, compared to the cattle farm isolates (MIC 0.5–2 µg/mL; MAR index 0.20–0.25). The hospital-based isolates expressed a clear multidrug resistance (MDR) phenotype, According to previous work that described the threat of carbapenem- and colistin-resistant *K. pneumoniae* as a threat to global health[26,27,28]. In contrast, the isolates derived from cattle farm exhibited lower resistance levels yet the detection of MAR of >0.2 would suggest the presence of antibiotic pressure in livestock environment[29,30].

Figure: 8 MIC, MBC, and MAR index of *Pseudomonas aeruginosa*

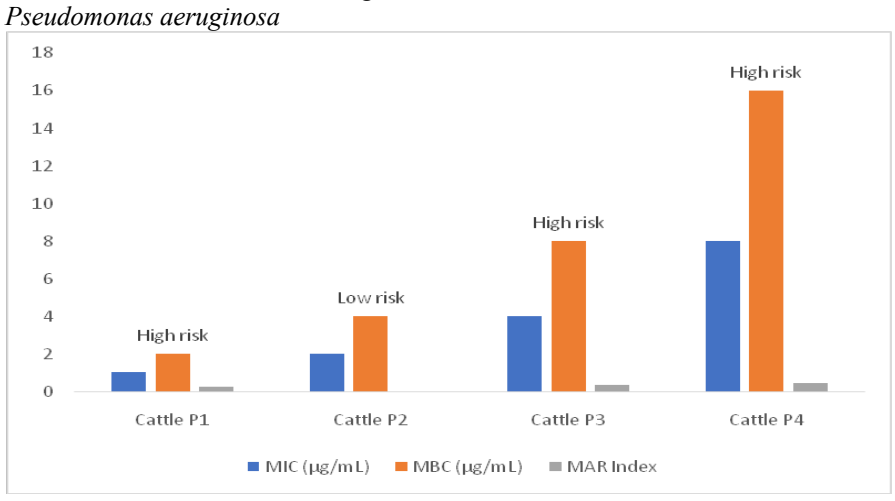
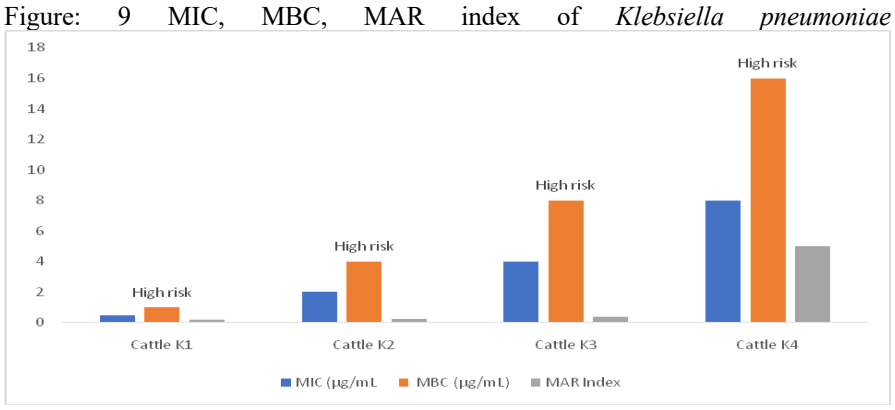
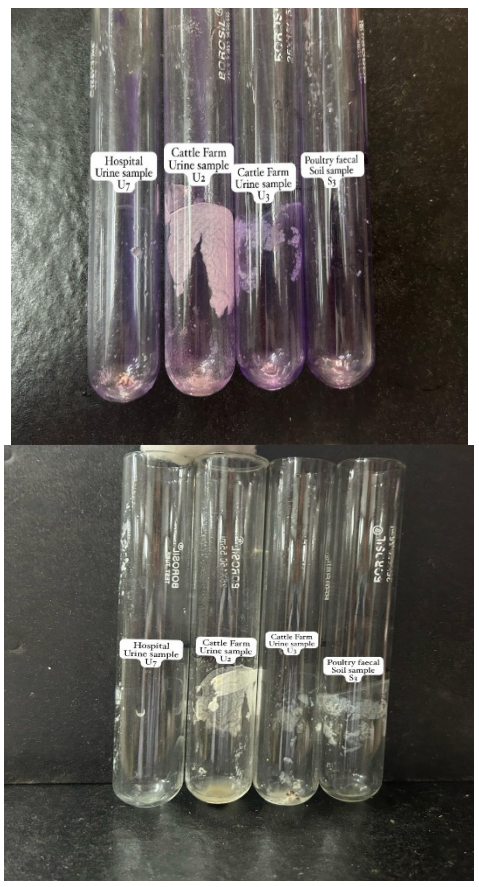


Figure: 9 MIC, MBC, MAR index of *Klebsiella pneumoniae*



During hospital isolates shows remarkable resistance, with MICs up to 8 µg/mL and MAR indices as high as 0.55, cattle isolates showed relatively lower MICs (1–2 µg/mL) and MAR values (0.18–0.22). The significantly higher MAR indices in hospital isolates indicate the pathogen's resilience to high antibacterial stress, supporting surveillance reports from both Europe and Asia. Hospital isolates' considerably upraised MAR indices establish the pathogen's resistance to massive antibiotic pressure, confirming surveillance information from Asia and Europe[31,32]. A coherent trend was followedacross all species:higher MICs, MBC and MAR is showed by hospital isolates as compare to cattle-farm isolates,implying elevated pressure to figure out antibiotics in clinical atmosphere. Meanwhile, the aggregate of cattle isolates have MAR ≥0.2, that amplify wherein livestock antibiotic utilizes offers to the circulation of resistance and unify with the concept of "One Health," that interconnect environmental, animal, and human resistance[33,34]. Based on thisdetection, it is incumbent to watch MAR reporter and colistin resistance in both veterinary and clinical settings. The uncovering of colistin resistance in environmental sources invokes agonizes about the horizontal dispense of resistance genes throughout human and animal contagious disease, intensely mcr-1[4,17]. However, adamant antibiotic stewardship, prohibitions on the use of colistin in agriculture, and molecular monitoring of resistance determinants in hospital and cattle ecosystems should all be segment of the preventive endeavor. Biofilm was observed in resistant colistin samples i.e. both Cattle farm urine sample (U2) and (U3), Hospital Urine sample (U7), and poultry faecal sample (S3). Violet colour staining was observed on inner walls of tubes.



(a)

(b)

Figure 10: Biofilm assessment (a) Before Staining, (b) After staining

Conclusion and Future Prospect

This study reveals the presence of antibiotic-resistant bacterial isolates within clinical and environmental settings, and shows that antibiotic resistance is not confined within hospital and it is now endemic among the general population. The isolation of *Klebsiella pneumoniaeand Pseudomonas aeruginosa*. In cattle and poultry, suggests that environmental sectors, notably the use of antibiotics as growth promoters, could serve as an important reservoir for resistant genes. This finding raises concerns regarding the potential spread of these resistant strains among humans through the means of direct interface, consumption of food and environmental contamination. Our findings also suggest that though holistic resistance prevails, a number of other antibiotics such as cefotoxin (100mcg), Ciprofloxacin(30mcg), and Co-trimoxazole(5mcg) are still effective for the majority of the isolates. This offers specialists alternative remedial agents but also highlights the necessity of maintaining the effectiveness of these medicines. The resistance we observed toward fluoroquinolones (levofloxacin(30mcg), nalidixic acid (15mcg) within some of the isolates is critically problematic, it suggests that the prospective development of multidrug-resistant strain can render it ineffective and if it continues, the number of available effective remedial option may become constrained. These findings highlight the importance of sustained AMR surveillance programs within both clinical and agricultural sectors, the effective delivery of antibiotic usage regulations, and health education campaigns among the public. Future studies ought to focus on molecular characterization of resistance determinants such as MCR genes, plasmid profiling, and complete-genome sequence typing, for better depiction and clarity regarding the genetic basis and modes of transmission of the resistance. Such information shall become invaluable for the formulation of focused intervention for the containment of the spread of holistic resistance. Future research should explore the development of novel, eco-friendly antimicrobials and alternative therapeutic strategies to further minimize the emergence of resistance and protect public health.

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