"ANTIBIOTIC RESISTANT PROFILING OF VETERINARY PATHOGENS"

DISSERTATION SUBMITTED TO ST. TERESA'S (AUTONOMOUS) COLLEGE, ERNAKULAM IN PARTIAL FULFILLMENT OF THE REQUIREMENT

FOR THE AWARD OF

DEGREE OF MASTER OF SCIENCE IN ZOOLOGY

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2022-2024

CERTIFICATE

This is to certify that the dissertation entitled "**Antimicrobial resistant profiling of veterinary pathogens**" is an authentic record of original project work carried out by "**LAKSHMI L**" (**Reg. No.:SM22ZOO009**) during the academic year **2022-2024**, under the external guidance of Dr. Jasmine C, Research Director, Enfys Lifesciences Pvt. Ltd, Kochi and the internal guidance of Dr.Keziya James, Assistant Professor, Department of Zoology, St. Teresa's College, for the partial fulfilment of the requirement of the Degree of Masters of Science in Zoology from St. Teresa's College, Ernakulam.

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DECLARATION

I hereby declare that this dissertation entitled "**Antimicrobial resistant profiling of Veterinary pathogens**" submitted to Mahatma Gandhi University, Kottayam in the partial fulfilment for the award of Master of Science in Zoology, is a record of original project work done by me, and no part thereof has been submitted to any other course. To the best of my knowledge, this project does not include any content that has been previously published or written by someone else, unless proper acknowledgment has been given to the original source.

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ACKNOWLEDGEMENT

Foremost, I am grateful to God almighty for the opportunity to pursue this endeavour, for the guidance and wisdom that I believe will come from a higher power, and for the strength, perseverance, peace of my mind and good health in order to finish this dissertation.

Dr. Keziya James, Assistant Professor, Department of Zoology, St. Teresa's College (Autonomous), Ernakulam and Dr. Jasmine C, Research Director, Enfys Lifesciences were my research mentors. I would like to sincerely thank them for their continuous support and efforts, patience, motivation, enthusiasm and immense knowledge. Their guidance helped a lot in completion of my dissertation.

Dr. Jasmine C, Research Director, Enfys Lifesciences Pvt. Ltd, Kochi have helped me throughout my dissertation work and I would like to express my sincere gratitude towards her for her constant support and guidance.

Beside my mentors, I would like to express my heartfelt gratitude to Dr. Soja Louis, Head of the Department of Zoology, St. Teresa's College (Autonomous), Ernakulum, for her constant support, guidance and inspiration to complete my dissertation.

Also, I would like to thank our principal Dr. Alphonsa Vijaya Joseph for providing an opportunity and support to successfully complete this project work.

I profusely thank Mrs. Gayathri R, Mrs. Indu Vasudevan, Dr. Demaris Benny, Shana Nazrin for their valuable reinforcement and suggestion throughout. Also, I extend my gratitude to my parents for the moral and emotional support.

Last but not the least, my profound thanks to all my friends, non- teaching staffs and family of both St. Teresa's College and Enfys lab for the heartening support to complete my dissertation.

LAKSHMI L

LIST OF ABBREVIATIONS

SL. NO	ABBREVIATION	EXPANSION
1	%	Percentage
2	&	And
3	°C	Degree Celsius
4	Hrs	Hours
5	G	Gram
6	AMR	Antimicrobial resistance
7	WHO	World health organization
8	Mm	Millimetre
9	Ml	Millilitre
10	pH	Potential of Hydrogen
11	Sec	Seconds
12	µM	Micrometre
13	µL	Microlitre
14	Rpm	Revolutions Per Minute
15	Mg ml-1	Milligrams per millilitre
16	Mins	Minutes
17	Bp	Base pair
18	Kbp	Kilo base pair
19	VP	Veterinary pathogen
20	+	Positive
21	-	Negative
22	/	Per
23	Fig	Figure
24	16S rDNA	16 S ribosomal deoxy ribonucleic acid
25	BLAST	Basic local alignment search tool
26	Blastn	Blast Nucleotide
27	Dntp	Deoxy ribonucleotide triphosphate
28	Eg.	Example
29	et al.,	And other

30	Etc.	Exectra
31	i.e.	That is
32	NCBI	National Centre for Biotechnology information
33	PCR	Polymerase chain reaction
34	RNA	Ribonucleic acid
35	SDS	Sodium docedyl sulphate
36	TAE	Trips acetate EDTA
37	Taq	*Thermus aquaticus*
38	TE buffer	Tris EDTA buffer
39	TLC	Thin layer chromatography
40	UV	Ultra Violet

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ABSTRACT

Antimicrobial resistance (AMR) is a pressing global concern threatening both human and animal health. This study aimed to assess the antibiotic resistance profiles of veterinary pathogens collected from the Veterinary Hospital in Ernakulam. A total of 18 veterinary samples namely VP1, VP2, VP3, VP4, VP5, VP6, VP7, VP9, VP10, VP11, VP12, VP13, VP15, VP16, VP17, VP18, VP19 and VP20, were subjected to rigorous analysis using the Kirby-Bauer method to determine antimicrobial susceptibility patterns. Additionally, biochemical tests, such as the Indole, Methyl Red, Voges-Proskauer, and Citrate (IMVIC) test, were conducted to corroborate findings. Molecular biology techniques, including PCR and sequencing, were employed for confirmatory purposes. The results revealed a spectrum of resistance patterns among the pathogens, with some strains exhibiting susceptibility to antibiotics, while others displayed intermediate resistance. However, a concerning portion of the pathogens demonstrated resistance to multiple antimicrobial agents, indicating the presence of multidrug-resistant strains. These findings underscore the critical importance of prudent antibiotic usage in veterinary medicine.

In light of the increasing prevalence of antimicrobial-resistant pathogens, it is imperative to adopt a judicious approach to antimicrobial drug administration. The indiscriminate use of antibiotics contributes to the emergence and spread of resistant strains, diminishing the effectiveness of treatment options. Therefore, the promotion of antimicrobial stewardship practices and the implementation of effective infection control measures are paramount to mitigate the antimicrobial crisis. Although gaps in knowledge about AMR and areas for improvement are obvious, there is not any clearly understood progress to put an end to the persistent trends of antimicrobial resistance.

This study serves as a timely reminder of the urgent need for concerted efforts from all stakeholders to address antimicrobial resistance comprehensively. By fostering collaboration among veterinary professionals, policymakers, and the public, can strive towards preserving the efficacy of antimicrobial agents and safeguarding both animal and human health.

INTRODUCTION

The discovery of antibiotics stands as one of the most important scientific advancements in recent history, having saved millions of lives. However, the rise of antimicrobial resistance (AMR) presents a significant threat to public health, putting this progress at risk. The healthcare system is currently grappling with major challenges caused by antimicrobial resistance. To mitigate the future impact of multidrug resistant organisms, new antimicrobial strategies, revised use of antimicrobial medications, and public health campaigns are essential (Marston et. al., 2016).

AMR is a pressing concern that refers to the ability of microbes to withstand the effects of antimicrobial drugs. Originally, these medications worked well to treat illnesses brought on by fungus, viruses, bacteria, and parasites. However, the rise of AMR has made infections more difficult to treat and has increased the risk of disease spread, severe illness, and even death. The misuse and overuse of antimicrobial drugs in humans, animals, and agriculture are significant factors contributing to the development of AMR. To address this issue, efforts are being made to promote the appropriate use of antimicrobials, enhance infection prevention and control practices, and develop new drugs and treatments. Recognizing the gravity of the situation, organizations like the World Health Organization (WHO) and governments worldwide have identified AMR as a priority. In both human and veterinary medicine, AMR poses a critical global health threat, undermining the effectiveness of antibiotics and presenting challenges in disease management. Particularly in veterinary practice, where antibiotics are crucial in combating infectious diseases, the escalation of AMR raises profound concerns regarding animal health, food safety, and the potential for zoonotic transmission.

The importance of comprehending resistance mechanisms and implementing efficient antimicrobial stewardship practices in veterinary medicine is underscored by the prevalence of antimicrobial-resistant pathogens. Antibiotics are drugs utilized to combat or prevent bacterial infections. Animals that are frequently administered antibiotics are at a heightened risk of developing antibiotic resistance. Antibiotics function by impeding bacterial cell wall synthesis, protein, DNA, and RNA synthesis (Regea, 2018). Inappropriate use of antibiotics in animal management, particularly in pets and livestock, has led to the emergence of resistance in bacteria. Transmission of antibiotic-resistant strains from wild and domestic animals can occur through the food chain, environment, or direct contact with animals. AMR poses a significant challenge to the empirical management of infections, resulting in a shortage of effective antibiotics and escalating healthcare costs. The rapid emergence of resistant bacteria worldwide threatens the effectiveness of antibiotics, which have been instrumental in

revolutionizing medicine and preventing millions of deaths. Bacterial infections are once again becoming a major concern, decades after the introduction of antibiotic treatment. The epidemic of antibiotic resistance is linked to the overuse and misuse of these drugs, as well as the pharmaceutical industry's struggles in developing new medications due to stringent regulations and limited financial incentives. (Varriale L et.a.l, 2020*)*

Common bacterial infections in animals consist of atopic dermatitis, pyoderma, urinary tract infections, pulmonary infections, mastitis, caused by various bacteria such *as Leptospira Spp., Streptococcus Spp., E. coli, Salmonella Spp. etc*. Improper utilization of antibiotics in certain cases can contribute to the emergence of antibiotic-resistant pathogens, creating obstacles to future medical treatment. One of the primary challenges in antimicrobial therapy is the development of multidrug-resistant bacterial strains. Antibiotics undoubtedly play a crucial role in advancing healthcare and medicine, but the rapid emergence of new resistance mechanisms and the declining effectiveness in treating common infectious diseases fail standard treatment. This leads to prolonged illness, increased healthcare costs, and a significant risk of mortality. Bacteria acquire resistance to antibiotics through various means, including possessing a naturally impermeable membrane, utilizing efflux pumps to expel chemicals from the bacterial cell, producing enzymes that can break down antibiotics, and modifying the target of antibiotics. Wild animals, in addition to companion animals, serve as carriers for the dissemination of antibiotic-resistant genes (Allen et. al., 2010). Antibiotic resistance is frequently seen in *E. coli* and *Staphylococcus aureus*. Dogs and cats, as companion animals, play a crucial role as potential reservoirs for transmitting antibiotic resistant pathogens. This is due to their frequent exposure to antibiotics and close interaction with humans.

In addition to the possible spread of antibiotic resistance mechanisms and residues, the overuse of antibiotics in the veterinary industry might have negative effects on the environment and public health. While some bacteria are present in animals' gastrointestinal tracts by nature, if their populations grow to an unacceptably high level, they can develop into diseases that can spread from animals to people.

Companion animals play a role in the spread of methicillin resistant *Staphylococcus aureus,* which poses a risk to their owners. The proximity between pets and humans creates an ideal environment for the transmission of bacteria through direct contact or contamination within the household. Some bacteria found in the gut of animals carry genes that make them resistant to antibiotics, and these genes can be transferred to other bacteria through horizontal gene transfer. As a result, antibiotic resistance is further spread, ultimately affecting humans. These bacteria carry genes that are clinically significant in the field of medicine (Guardabassi et. al., 2004). Due to the increasing resistance of bacteria to

antibiotics, antimicrobial chemotherapeutic treatments have been implemented, including the use of selective drugs to combat these resistant strains. Zoonotic diseases linked to pets occur sporadically, making it difficult to determine their prevalence due to challenges in monitoring and validating disease transmission from pets. Consequently, it is crucial to analyze the presence of infectious agents in pets and focus on the risk factors associated with bacterial transfer to humans. This analysis aims to enhance hygiene practices in veterinary medicine (Lloyd, 2007). The rise of antibiotic resistance necessitates reliance on second and third-line treatments, which can have detrimental effects on patients, including organ failure, prolonged care, and recovery. Additionally, the prescription of broad-spectrum antibiotics often leads to bacterial mutations, further exacerbating antibiotic resistance and causing additional complications. To address this issue, a global initiative was launched in 2007 known as 'One World-One Health'. This initiative aims to raise awareness among farmers, stakeholders, medical professionals, veterinarians, and others about the importance of monitoring and controlling activities related to veterinary practices, particularly concerning zoonotic transmission of antibiotic-resistant pathogens. (Palma et. al., 2020). The antibiotic susceptibility test is considered crucial for verifying the sensitivity of the selected antimicrobial agent and determining the sensitivity to antibiotics of particular bacterial isolates. A thorough grasp of the disc diffusion method helps medical practitioners determine which antibiotics are susceptible to treating a given illness and makes treatment recommendations. Understanding how urgent it is to address this growing situation, the goal of this study is to do a thorough evaluation of the antibiotic resistance profiles of veterinary diseases obtained from the Veterinary Hospital in Ernakulam.

AIM AND OBJECTIVE

AIM

This study aims to understand antimicrobial resistant profiling of veterinary pathogens.

OBJECTIVES

- To collect veterinary pathogens from Veterinary hospitals.
- To assess the antimicrobial susceptibility of Veterinary pathogens using the disc diffusion method
- To identify the pathogens biochemically using the IMVIC test method.
- To identify the pathogens using molecular techniques.

RELEVANCE OF THE WORK

Antimicrobial resistance threatens the efficacy of antimicrobial drugs, which are essential for treating both human and animal infections. By studying resistance in veterinary pathogens, researchers can identify strategies to preserve the effectiveness of these drugs for both veterinary and human medicine. Overall, studying antimicrobial resistance in veterinary pathogens is vital for safeguarding animal health, protecting public health, ensuring food safety, promoting a one health approach, and preserving the efficacy of antimicrobial drugs.

REVIEW OF LITERATURE

According to a different study by Bodey et al. (1983), *Pseudomonas aeruginosa* has grown in importance over the past 20 years as a pathogen. In most hospitals, it is the cause of 10% to 20% of infections. Patients with burn wounds, cystic fibrosis, acute leukaemia, organ transplants, and intravenous drug addiction are more likely to contract this kind of infection. Hospitals are often home to *P. aeruginosa*, which can spread epidemics through a variety of hospital-related objects. Long-term hospital patients are more likely to become colonised by this bacterium, which increases their risk of infection. Meningitis, pneumonia, septicemia, endophthalmitis, endocarditis, and malignant external otitis are among the most serious illnesses brought on by *Pseudomonas*.

The severity of the patient's underlying ailment determines the patient's prognosis following a *Pseudomonas* infection. The advent of penicillins and antipseudomonal aminoglycosides has greatly improved the prognosis for these illnesses. Neutropenic patients have shown remarkable benefit from carbenicillin and ticarcillin. However, for best outcomes, therapy must be started very away. Several novel medications with antipseudomonal activity, including cephalosporins, penicillins, and other β-lactams, have been brought to market recently, potentially providing new avenues for the treatment of these illnesses.

An earlier study conducted by Eugster et., al (2001)., made use of Pulsed field gel electrophoresis which was used to type isolates of *Acinetobacter baumannii, Enterococcus faecalis, Enterococcus faecium, and Staphylococcus intermedius* from infected surgical wounds and various forms of infections in a veterinary teaching hospital. In 1998–1999, a multi-resistant *A. baumannii* strain was found to have caused the first cluster of illnesses in dogs and cats. Following the intensive care unit's washing and disinfection, this strain vanished. A second multi-resistant strain emerged in 2000 and caused a similar cluster of infections. It also infected a patient at the adjacent horse clinic. During this period, no multiresistant *S. intermedius* strain was seen, and there was no indication that this bacterium was being transmitted between patients. During the same time frame, it was also noted that two cats with surgical wound infections had been infected with a multiresistant strain of *E. Faecium*. The current investigation shows that hospital nosocomial resistance issues in veterinary care may resemble those in human medicine. His findings imply that preventive interventions may need to be tailored to the problematic organism and that the epidemiology of nosocomial infections with *A. baumannii* and *E. faecium* may differ from that of *S. intermedius* infections.

Zoonotic diseases are a crucial component of the World Health Organization's global health initiatives, according to a study done by Pal (2005). With more than 250 zoonotic illnesses that can spread from a variety of animal species to people, it is obvious that these illnesses have a big effect on general health. Nowadays, zoonoses account for 80% of all human illnesses, with significant death and

morbidity rates observed in all age groups and genders. Preventing and controlling zoonotic infections has been given top priority by the primary health system. Furthermore, there is a serious risk to human health from the introduction of novel zoonoses. It is alarming that the plague has returned to Gujarat and Maharashtra after a 28-year absence, indicating a breakdown in public health measures. Similarly, concerns over the possible spread of bovine tuberculosis to people are being raised by the disease's reappearance in American cattle, which poses a serious risk to public health. Rapid changes in the environment have made it possible for a variety of zoonotic pathogens to arise and cause diseases in humans. Some zoonotic illnesses seemed difficult to eradicate entirely because of their complex epidemiology. They did, however, emphasise that early diagnosis and chemotherapy, personal hygiene, environmental sanitation, better animal husbandry, improved food hygiene, vaccination, health education, and close collaboration between veterinary and medical departments can all help to lower the prevalence and incidence of these diseases. Furthermore, it is imperative to stress the ongoing participation of public health veterinarians in international health initiatives.

According to Chomal et., al. (2005) when significant outbreaks of infectious diseases started to happen globally in the late 1980s, many scientists who had previously believed that infectious diseases were only found in developing nations or the past, were taken aback by the idea that infectious diseases were on the rise. Numerous studies have pointed to the deterioration of the public health system as one of the causes of newly emerging and reemerging infectious illnesses. Disease onset frequently coincides with ecological changes brought about by human activities like migration, urbanization, forestry, agriculture, or dam construction, as stated by Morse. It's shocking to learn that the majority of these new illnesses are caused by zoonotic bacteria and viruses. Recently, several novel zoonoses have been discovered. Numerous of these illnesses were either unidentified because they could not identify the infectious agent or differentiate them from other clinical syndromes, or they were unintentionally found. Recent discoveries of novel infections have mostly been made possible by advances in molecular biology techniques or epidemiological research. Veterinarians were crucial in the diagnosis, isolation of the causing organisms, and comprehension of the infection's epidemiology for each of these illnesses or infections. As it should be in many other nations, the veterinary profession plays a critical role in public health and is growing once again in the United States. They suggested that the veterinary profession needs to employ surveillance, clinical awareness and curiosity, epidemiology, and laboratory training as key competencies to tackle the challenge of newly emerging zoonoses.

A study by Neilan (2013) pointed out that the Dogs naturally have *Staphylococcus intermedius* on their skin and in their mouths. Since the pathogen is frequently confused with *Staphylococcus aureus*, the

precise rate of infections in humans remains unknown despite the paucity of case reports. There have been documented cases of everything from soft tissue infections to brain abscesses. Dog exposure has been linked to the majority of documented instances in humans. A month after a total elbow replacement, they presented the case of a 73-year-old female patient who developed a surgical site infection due to *S. intermedius*. This is the first recorded instance of *S. intermedius* infection of a human mechanical prosthesis. It was believed that the patient's dog was the main source of the infection. Following the determination of susceptibilities, the patient was treated with vancomycin, cefazolin, and rifampin. According to their case reports, patients usually react favourably to customized antibiotics and recover fully or almost fully. His findings implied that when making a differential diagnosis of an invasive infection in people who have had close contact with dogs, *S. intermedius* should be considered.

The focus of recent research by Mehta, et. al., (2018) has been on Zoonotic influenza viruses, which cross the animal-human barrier, cause disease in humans and have been linked to deadly pandemics. This review discusses their structure, mutation relationship, infection pathogenesis, history, and epidemiology, emphasizing the need for improved diagnostic and treatment methods. The avian influenza A(H7N9) epidemic is currently in its fifth and greatest wave, which we are currently seeing. Numerous other zoonotic influenza viruses are also in circulation; these include the avian influenza viruses A(H5N1) and A(H5N6), the swine influenza viruses A(H1N1)v, A(H1N2)v, and A(H3N2)v. The first documented case of avian influenza A(H7N4) infection in humans occurred most recently.

An examination of the literature on AMR was conducted as part of the Hughes et. al., (2019) study as part of the "one health" concept, with a focus on "antimicrobial resistance" and "One Health." AMR is the global health concern that most embodies the idea of One Health. AMR is a significant global problem that affects people, the environment, and animals. This relates to all three of these areas because of the excessive and negligent use of antibiotics in several businesses (agricultural, livestock, and human medicine). The spread of resistance is aided by improper use of antibiotics, ineffective infection control, agricultural waste, environmental contaminants, and the movement of people and animals with resistant bacterial diseases. Their research sought to examine the many players engaged in One Health by analysing the AMR issue from a health standpoint.

One of the previous studies by Neelam Saba (2021) indicate that Zoonotic illnesses, also referred to as “zoonoses”, pose a problem to all public health-related professions, not only veterinarians. Illnesses that have become more frequent during the last 20 years or that are probably going to become more common soon are referred to as emerging diseases. Emerging and re-emerging illnesses continue to

pose a hazard to human health and are on the rise. The majority of diseases that have been reported to be emerging or re-emerging over the last three decades are zoonotic, especially those that have viral origins. Notable outbreaks of diseases including SARS-CoV2 (COVID-19), Nipah, Avian Influenza (H5N1), Swine Influenza (H1N1), West-Nile Fever, Ebola, Zika, etc. have brought attention to the effects of these illnesses recently. The unexpected introduction of the aforementioned diseases as well as the recurrence of uncommon disease outbreaks such as leptospirosis, brucellosis, rabies, plague, antibiotic resistance, etc., can have a significant impact on national economy as well as the health of humans and animals. Numerous factors are believed to be involved in the emergence of emerging and re-emerging zoonoses, even though the precise causes are unknown. These intricate components can be divided into three categories: ecological, human, and infectious origin factors (bacteria, viruses, and prion).

The research conducted by Siva et. al., (2021) indicates that the restricted treatment options in veterinary medicine are facing challenges due to the growth of methicillin-resistant Staphylococcus pseudintermedius (MRSP) antibiotic resistance and epidemic genetic lineages. In cases of canine pyoderma, MRSP has been identified as a major pathogen.

AMR is a major global public health concern, according to a study found by Guirazza et. al., (2021). The effective and proper use of antibiotics, including those created especially to fight. AMR bacteria, is one of the primary tactics in AMR control. An important development is the availability of new compounds for the treatment of AMR bacteria. Physicians are becoming able to treat antibiotics with precision by understanding resistance patterns and mechanisms of action. It is stressed that maintaining these newly developed antimicrobials for use in the future will be made easier by rigorous adherence to antimicrobial stewardship standards.

According to a study by Nardini et. al., (2022), population expansion and industrialization have led to a race to boost food and supply productivity. This leads to an increase in the number of humans who frequent and reside in forested regions, interacting with wildlife and the parasites and vectors that accompany it, trafficking and consuming wildlife, contaminating water sources, and gathering rubbish. The world's forests, which are essential to preserving the planet's ecological and climatic equilibrium, are being destroyed at a rapid rate as a result of the simultaneous expansion in cattle and agricultural output for human consumption. In addition to bacterial (leptospirosis, tuberculosis, and Lyme borreliosis), fungal, parasitic, and viral pathogens (Ebola virus, hantaviruses, Hendravirus, Nipah virus etc.), this article examines the human activities that contribute to the emergence and resurgence of zoonotic pathogens. These illnesses pose a major threat to the worldwide community. They concluded

by highlighting the urgent need to apply the One Health concept as a coordinated worldwide approach to educate people about the science driving the fight against zoonotic infections and to boost public awareness to lessen the threat to both humans and animals.

According to a survey by Yavonne (2014), exotic animal pet ownership is growing in popularity, particularly among children. Furthermore, it's estimated that 75% of newly discovered infectious diseases are caused by zoonotic infections. The implications of these two phenomena are of concern to the public health community. They looked over research on zoonoses in household animals. This required looking out policies and procedures for zoonoses in household pets across the jurisdictions of Canadian public health organisations. Pets are still predominantly associated with several diseases and outbreaks, both reportable and nonreportable, including cutaneous larval migrans, tularaemia, salmonellosis, and infections caused by the Human Lymphocytic Choriomeningitis Virus. There have been suggestions that certain pet diets and treats could be the source of zoonotic diseases. Children under five years old and individuals with impaired immune systems were recognised as potential high-risk groups. Acute care centres, veterinary clinics, nursery schools, schools, summer camps and private homes were among the places where there was a high risk of zoonotic disease transmission. It has been determined that improper pet handling and inadequate hand hygiene are the two primary risk factors. To lessen the public's exposure to pet zoonoses, the veterinary, public health, and regulatory sectors will need to step in as the pet industry continues to expand. They recommended that the current surveillance systems be improved, that rules be created to fill in the gaps in the pet food industry, that protocols and policies be developed at the federal and provincial levels of government, that the public be made aware of the risks involved in handling pets, and that there be more cooperation between the human and animal health sectors.

According to the research conducted by Devnath et. al., (2022), bats have been identified as a crucial species for mitigating the transmission of zoonotic infectious illnesses, which includes the COVID-19 pandemic and subsequent outbreaks such as SARS and Ebola. Little is known about AMR that is shared by people and bats, despite mounting concerns about rising AMR globally during the present epidemic. They have looked at the evidence of AMR in bats and discussed the planetary health component of AMR in this work to elucidate how the origin, diffusion, and persistence of AMR at the human-animal interface are related. The discovery of clinically significant resistant bacteria in wildlife and bats has important ramifications for disease transmission, treatment strategies, and zoonotic pandemic surveillance. They used PubMed and Google Scholar to search MEDLINE for relevant papers (n = 38) that contained information on resistant bacteria in bats before September 30, 2022. Research evaluating the prevalence of AMR based on bat species, location, and period has produced a

wide range of results. Bats are the primary source of Gram-positive and Gram-negative bacteria that are resistant to commonly administered medicines. The growing number of studies conducted in recent years that have revealed clinically relevant multi-drug resistant bacteria in bat samples, such as Colistin-resistant Enterobacterales and Methicillin-resistant *Staphylococcus aureus* (MRSA), which produce ESBLs, is a concerning development, they pointed out.

METHODOLOGY

SAMPLE COLLECTION

About 18 samples were collected from the pets affected by bacterial infections and were received from the Cochin Pet Hospital, Vidyanagar crossroad, Kadavanthra, Kochi, Kerala, India.

MATERIALS AND METHODS

Materials:

The Luria Bertani Medium, antibiotic discs, Mueller- Hinton agar, used in this study were from HiMedia Laboratories Pvt, Ltd.

Purification and maintenance of bacterial isolates:

About 18 distinct bacterial samples obtained from the Cochin pet hospital underwent purification using the streak plate culture method on a 90 mm sterile petri plate. Subsequently, the plates were placed in an incubator set at 37ºC for 24 hours, and allowed for the growth of the individuals colonies. These colonies were then extracted using a sterile loop. Following this, a slant culture was prepared using the Luria Bertani medium. The cultured slant tubes were then incubated overnight in a 37ºC incubator, and the purity of each bacterial isolate was determined using gram staining method. Under sterile conditions, a smear was prepared by transferring a loopful of samples to drop of water placed on a slide. The mixture was then gently mixed, air-dried and heat fixed over a gentle flame. The smear was then flooded with crystal violet and gently washed off with tap water after a minute. Gram's iodine was used to fix the dye, and allowed it to stand for a minute. Decolorizing agent was added, rinsed off with water within 5 seconds, and finally, the smear was counterstained with safranin for 45 seconds. After drying, the smear was observed under a microscope. The purified bacterial isolates were cryopreserved for storage. These isolates were then inoculated into test tubes containing 5 ml LB broth. A loopful of isolate was taken from the previously prepared slant using a sterilized inoculation loop and transferred into the LB broth through gentle agitation. All the samples were cryopreserved in 30% glycerol for long-term purposes. All serological experiments were strictly conducted in the laminar airflow chamber.

ANTIBIOTIC RESISTANCE PROFILE

The disc diffusion method was used to determine the antibiotic resistance profile of 18 isolates. This method was initially developed in the 1950s and later refined by W. Kirby and A. Bauer. It was

standardized by the World Health Organization in 1961. To perform the test, a bacterial inoculum was applied to the surface of a Mueller-Hinton agar plate by streak plate method. After streaking, commercially prepared paper antibiotic discs with fixed dosages were placed on the agar surface using sterilized forceps (Table.1). The plates were then incubated in an incubator at 37ºC for 16-24 hours before the results were determined (Jorgensen & Ferraro, 2009). The zone of inhibition around each antibiotic disc was measured to the nearest millimeter using an antibiotic zone scale. The interpretation of the zone diameter for each drug was done according to the criteria published by the Clinical and Laboratory Standards Institute (CLSI).

Table 1: Different antibiotics used in the study, based on generation

Sl. No	Antibiotics	Abbreviation	Generation	Dosage
1	Amikacin	AK	3rd	30mcg
2	Ampicilin	AMP	3rd	10mcg
3	Azithromycin	AZM	2nd	15mcg
4	Clindamycin	CD	1st	2mcg
5	Cefpodoxime	CPD	2nd	10mcg
6	Trimethoprim	COT	3rd	25mcg
7	Cefalexin	CN	1st	30mcg
8	Cefixime	CFM	3rd	5mcg
9	Cefuroxime	CXM	2nd	30mcg
10	Ceftriaxone	CTR	3rd	30mcg
11	Cefpirome	CFP	4th	30mcg
12	Ciprofloxacin	CIP	2nd	5mcg
13	Doxycyclin hydrochloride	DO	2nd	30mcg
14	Erythromycin	E	1st	15mcg
15	Enrofloxacin	EX	3rd	10mcg
16	Faropenem	FAR	3rd	5mcg
17	Gentamicin	GEN	3rd	10mcg
18	Imipenem	IC	3rd	10mcg
19	Levofloxacin	LE	3rd	5mcg
20	Linezolid	LZ	4th	30mcg
21	Metranidazole	MT	1st	4mcg
22	Nalidixic acid	NA	1st	30mcg
23	Oflaxacin	OF	2nd	5mcg
24	Tetracyclin	TE	1st	30mcg
25	Vancomycin	VA	3rd	30mcg

BIOCHEMICAL TESTS

IMViC REACTIONS

The present study used four IMViC reactions which includes the Methyl red test, the Voges Proskauer test, the Indole test, and the Citrate Utilization test for the identification of bacteria belonging to Enterobacteriaceae family.

Indole test:

Principle: Using the enzyme typtophanase, certain bacteria may convert the amino acid tryptophan into indole. Using either Kovac's or Ehrlich's reagent, indole production is identified. The reagent turns red when indole combines with the aldehyde in it. The red colours are concentrated in a ring at the top by an alcohol layer..

Procedure: The test bacterium is cultured in tryptophan-containing peptone water and incubated at 37 °C overnight. A few drops of Kovac's reagent (Para-dimethyl aminobenzaldehyde, isoamyl alcohol, and condensed hydrochloric acid) was added after incubation. Ehrlich's reagent has a higher sensitivity for identifying the synthesis of indole in non-fermenters and anaerobes. Positive results are observed when a red or pink coloured ring forms at the top.

Example: *Escherichia coli*: Positive; *Klebsiella pneumoniae*: Negative.

Methyl red (MR) test:

Principle: This is done to assess an organism's capacity to ferment glucose into stable acid end products. Certain bacteria surpass the system's buffering capacity by producing so much acid during the fermentation of glucose. A pH indicator called methyl red maintains its red hue at pH values of 4.4 or lower.

Procedure: A phosphate buffer and glucose are combined in glucose phosphate broth, which is infected with the test bacteria and incubated for 48 hours at 37 °C. To stay acidic over the 48-hours, the organism that produces mixed acid needs to generate enough acid to surpass the phosphate buffer. A five-drop of MR reagent was added is used to measure the medium's pH. A red colour development will indicate a positive and a yellow colour as MR negative organisms.

Example: *Eschericihia coli*: Positive; *Klebsiella pneumoniae*: Negative

Voges Proskauer (VP) test:

Principle: The VP test finds butylene glycol makers while the MR test helps identify mixed acid manufacturers. Butylene glycol is produced using acetoin, also known as acetyl-methyl carbinol, as an intermediary. In this experiment, the test broth is incubated, and then two reagents—40% KOH and alpha-naphthol—are added and exposed to ambient oxygen. If acetoin is present, it is oxidized to diacetyl in the presence of air and KOH. Next, in the presence of alpha-naphthol, diethyl combines with the guanidine components of peptone to give a red colour. Alpha-naphthol serves as both a colour intensifier and a catalyst.

Procedure: After being added to glucose phosphate broth, the test bacteria was allowed to incubate for a minimum of 48 hours. The test broth was mixed with 0.6 cc of alpha-naphthol and shaken. The soup was then agitated after 0.2 ml of 40% KOH was added. The tube were let to stand for fifteen minutes. When the colour red appears, the test is considered positive. Given that the reagents develop their colour to their fullest within an hour, the negative tubes must be kept for one hour. Examples: *Escherichia coli*: Negative*; Klebsiella pneumoniae*: Positive

Citrate utilization test:

Principle: This test determines whether an organism can use citrate as its only source of energy and carbon. On a medium that contains sodium citrate and the pH indicator bromothymol blue, bacteria are injected. Inorganic ammonium salts are also present in the medium and serve as the only source of nitrogen. The enzyme citritase is used to break down citrate into oxaloacetate and acetate. Pyruvate and CO2 are the byproducts of further oxidative acetate breakdown. An alkaline pH is produced when sodium citrate and ammonium salt are used, respectively, to produce Na2CO3 and NH3. This causes the medium's colour to shift from green to blue.

Procedure: After being removed from a straight wire, bacterial colonies were injected onto Simmon's citrate agar slope and left to incubate overnight at 37 ℃.

Examples: *Escherichia coli:* Negative; *Klebsiella pneumoniae*: Positive.

Determination of MAR index:

MAR Index or multiple antibiotic resistance index is calculated as the number of antibiotics to which an isolate is resistant (a) divided by the total number of antibiotics used in the study against which the isolates are tested (b).

MAR INDEX = a/b

Gram staining

The gram-staining procedure was carried out to classify the bacterial isolates into gram-positive and gram-negative bacteria.

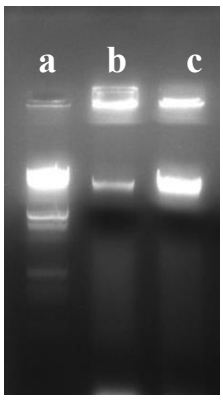
The slide smear was prepared by transferring a small amount of suspended culture onto a microscope slide using an inoculation loop. The smear was spread uniformly (15mm) over the slide using the inoculation loop and kept for drying. The air-dried samples were then treated using crystal violet stai n for about 1 min and washed using distilled water. Subsequently, the smear is coated with iodine solution for 1 min and the slide is rinsed with distilled water. This was followed by the addition of decolourizer, typically a blend of ethanol and acetone for 5 seconds. Lastly, the smear is counterstained with a basic fuchsin solution for 1 min. The fuchsin solution is then rinsed off with water, and water remained was soaked up using bibulous paper. Ultimately, the slide was examined under oil immersion microscope. VP1, VP2, VP3, VP6, VP7, VP9, VP10, VP12, VP13, VP15, VP16, VP17 and VP20 isolates appeared to be purple colour which implied that they were gram-positive strains.

VP4, VP5, VP11, VP18, VP19 appeared to be pink coloured and implied that they were gram-negative strains. When the gram-stained isolates were observed under the microscope, it displayed the shape of the bacterial colony. VP6, VP7, VP9, VP10, VP12, VP13, VP15, VP16, VP17 and VP20 exhibited coccus shape whereas VP1, VP2, VP3, VP4, VP5 and VP11
exhibited rod-shaped bacterial colonies.

DNA isolation

DNA was isolated from a loopful of well-grown bacteria using the standard phenol-chloroform method as described by Sambrook and Russel in 2001. Initially, the culture was grown at 37 ℃ in Lysis buffer (NaCl 400 mM, Sucrose 750 mM, EDTA 20 mM, and TrisHCl 50 mM) with 1 mg ml−1 lysozyme. Following this, SDS (1%) and proteinase K (100 µg ml−1) were introduced to the mixture, and the incubation was carried out for 2 hours at 55 ℃. The crude DNA was then extracted twice using chloroform: isoamyl alcohol (700 µl of 24:1 mixture), and the DNA-containing aqueous phase was collected by centrifugation at 10000 rpm for 10 minutes. Subsequently, isopropanol (0.6 volume) was added, allowing the DNA to precipitate at −20 °C for 60 minutes. After pelleting the DNA, it was washed twice with 70% ethanol and dried at room temperature for 20-30 minutes. Finally, the DNA was dissolved in TE buffer or Milli Q water (~30 µl), and its quality was verified through Agarose Gel Electrophoresis. (Figure.1)

Figure 1 : GEL image of DNA extraction : (a)



λ DNA /Hind III Digest : (b)

DNA of sample VP17 and (c)

DNA of sample VP19

PCR

The amplification of the 16S rRNA genes of bacterial DNA was conducted in a reaction volume of 20 µl. The reaction mixture comprised of 1 µl DNA (10–50 ng), 1 µl each of Forward and Reverse primers (10 picomoles µl-1), and 10 µl Emerald Amp GT PCR master mix (Takara). The cycling conditions employed were as follows: initial denaturation at 95 ◦C for 2 min, followed by cycle denaturation at 95 ◦C for 40 s, annealing at 55 ◦C for 40 s, and extension at 72 ◦C for 1.5 min. This cycle was repeated for a total of 30 cycles, followed by a final extension for 10 min at 72 ◦C. To verify the success of the PCR reaction, 5µl of the PCR product was electrophoresed on a 1% agarose gel containing ethidium

bromide at 120 V for approximately 45 minutes in 1X TAE Buffer. The resulting gel image was captured using the UV gel documentation system for future reference (Figure 2). Furthermore, a 100bp DNA ladder from Thermo was loaded alongside the PCR products as a size marker.

Table 2: Details of primers used for bacterial 16s rRNA amplification

Sl. No.	Primer name	Primer Sequence (5'-3')	Annealing temperature	Reference
1	27F	AGAGTTTGATC(AC)TGGCTCAG	55^0C	(Lane, 1991)
2	1492R	GGTTACCTTGTTACGACTT		

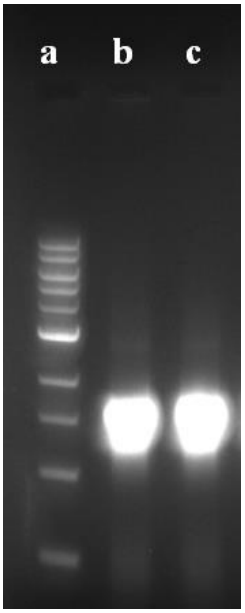


Figure 2: (a) 1kb ladder (b) VP17 (c) VP19

PCR product clean up

The PCR products were further treated with ExoSAP-IT PCR Product Clean-up Reagent and were used as a template for sequencing PCR

Sanger sequencing PCR

Sequencing PCR was done with ABI PRISM Big Dye terminator ready reaction mix (Life Technologies, USA).

Purification

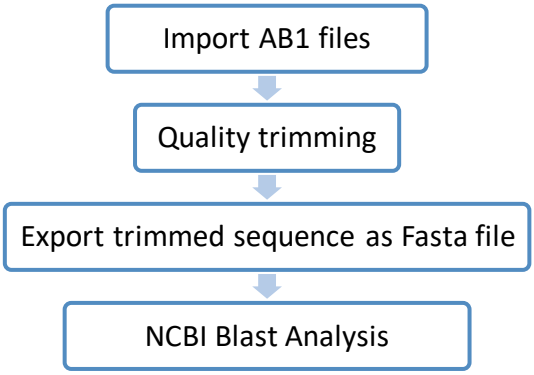
The cycle extension products were purified following ethanol/EDTA/ sodium acetate precipitation.

Machine

Applied Biosystems ABI 3730xl DNA

Bioinformatics Analysis

Workflow:



The sequences were quality-checked and trimmed using the software Sequencher V4.10.1 (Gene Codes Corporation, Ann Arbor, MI USA)

Trimmed Sequences were searched in NCBI using BLASTn tool and identity of the sample was confirmed based on percentage similarity and query coverage of the nearest neighbours

RESULTS

Isolation and Purification of samples

The study purified 18 bacterial samples from Cochin pet hospital using streak plate culture method.

Antibiotic resistant profiling

The PCR revealed that the samples taken from the veterinary pet hospital in Cochin, Ernakulam, had a significant incidence of *Pseudomonas aeruginosa* and *Staphylococcus pseudointermedius* pathogens. According to the graph showing complete zone of inhibition for each isolate, it is found that antibiotics such as Metronidazole, cefixime, nalidixic acid and vancomycin show complete zone for each isolate resistant.

Gram staining:

The gram-staining showed that the VP1, VP2 , VP3, VP6, VP7, VP9, VP10, VP12, VP13, VP15, VP16, VP17 and VP20 isolates appeared to be purple coloured and indicated as gram-positive strains. VP4, VP5, VP11, VP18, VP19 appeared to be pink coloured and indicated as gram-negative strains. When the gram-stained isolates were observed under the microscope, it displayed VP6, VP7, VP9, VP10, VP12, VP13, VP15, VP16, VP17 and VP20 as coccus shape whereas VP1, VP2, VP3, VP4, VP5 and VP11 as rod-shaped bacterial colonies (Table.3 and Figure.3)

Table 3: showing the result of gram staining

ISOLATES	GRAM POSITIVE/ NEGATIVE	SHAPE
VP1	Gram positive	Rod shaped
VP2	Gram positive	Rod shaped
VP3	Gram positive	Rod shaped
VP4	Gram negative	Rod shaped
VP5	Gram negative	Rod shaped
VP6	Gram positive	Cocci shaped
VP7	Gram positive	Cocci shaped
VP9	Gram positive	Cocci shaped
VP10	Gram positive	Cocci shaped
VP11	Gram negative	Rod shaped
VP12	Gram positive	Cocci shaped
VP13	Gram positive	Cocci shaped

VP15	Gram positive	Cocci shaped
VP16	Gram positive	Cocci shaped
VP17	Gram positive	Cocci shaped
VP18	Gram negative	Cocci shaped
VP19	Gram negative	Cocci shaped
VP20	Gram positive	Cocci shaped

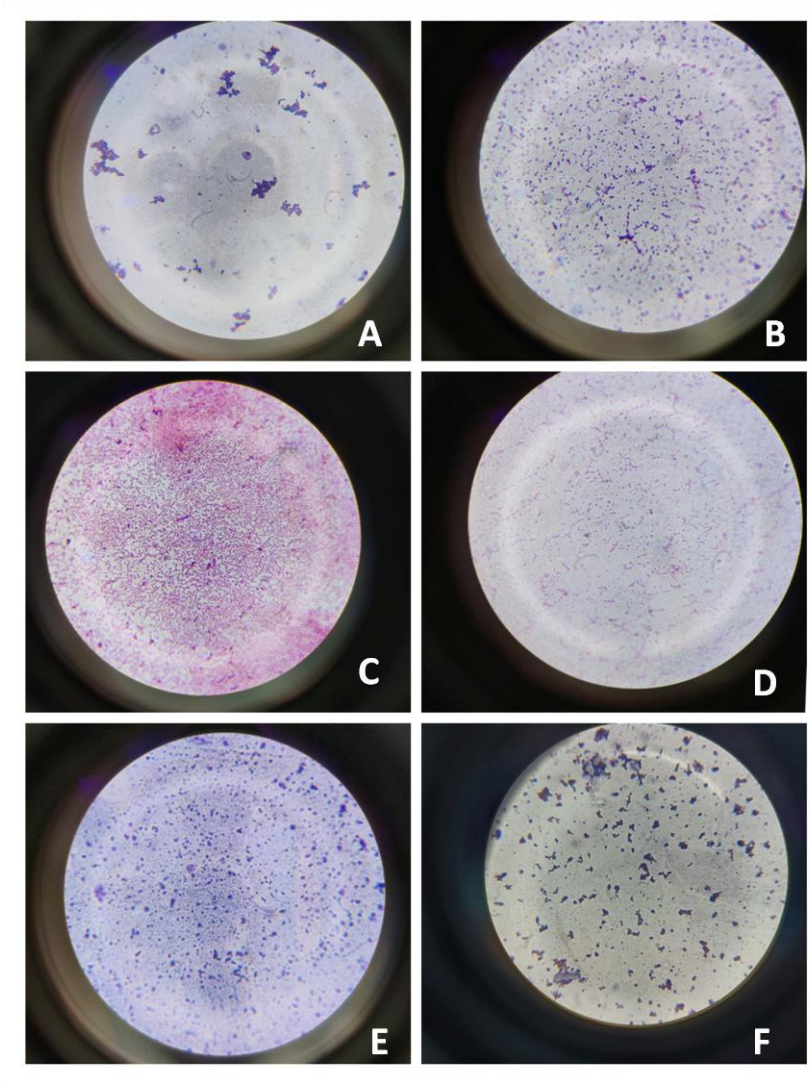


Fig 3 : showing the result of gram staining of some isolates

(A) , (B), (E), (F) : Microscopic image of gram positive bacteria
(C), (D) : image showing the gram negative bacteria

IMVIC TEST

Table 4: showing the result of IMVIC test

SL. NO.	SAMPLE	INDOLE TEST	METHYL RED TEST	VOGES PROSAUER	CITRATE TEST
1	VP1	-	-	+	+
2	VP2	-	-	-	+
3	VP3	-	-	-	+
4	VP4	-	-	-	+

5	VP5	-	-	-	+
6	VP6	+	-	+	-
7	VP7	-	-	-	+
8	VP9	-	-	+	-
9	VP10	+	+	+	-
10	VP11	-	-	-	+
11	VP12	+	+	+	-
12	VP13	-	-	+	-
13	VP15	-	-	+	-
14	VP16	-	-	-	-
15	VP17	+	+	+	+
16	VP18	-	-	-	+
17	VP19	+	-	+	+
18	VP20	+	-	+	-

From the indole test these following samples VP6, VP10, VP12, VP17, VP19, VP20 are positive (*E coli*) and VP1, VP2, VP3, VP4, VP5, VP7, VP9, VP11, VP13, VP15, VP16, VP18 are negative (*Klebsiella*).

From the Methyl red test, VP10, VP12, VP17 are positive (*E coli*) and VP1, VP2, VP3, VP4, VP5, VP6, VP7, VP9, VP11, VP13, VP15, VP16, VP18, VP19, VP20 (*Klebsiella*).

From the Voges Proskauer test, VP1, VP6, VP9, VP10, VP12, VP13, VP15, VP17, VP19, VP20 are positive (*Klebsiella*) and VP2, VP3, VP4, VP5, VP7, VP11, VP16, VP18 are negative (*E coli*).

From the Citrate utilization test, VP1, VP2, VP3, VP4, VP5, VP7, VP11, VP17,VP18, VP19 (*Klebsiella*) are positive and VP6, VP9, VP10, VP12, VP13, VP15, VP16, VP20 are negative (*E coli*) (Table.4 and figure 4-8).

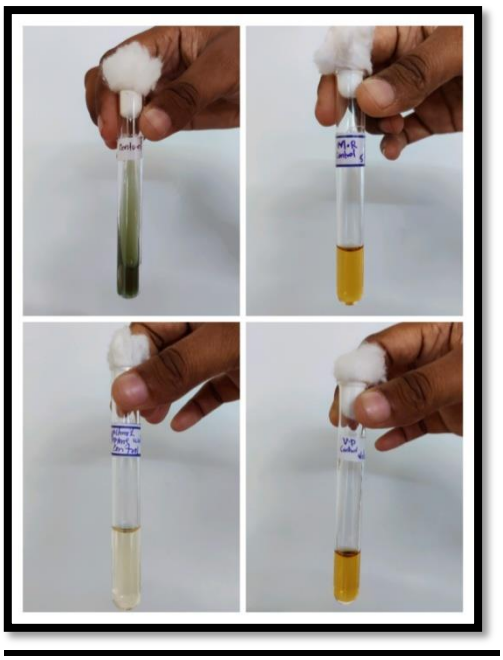


Fig 4: showing the control of IMVIC test

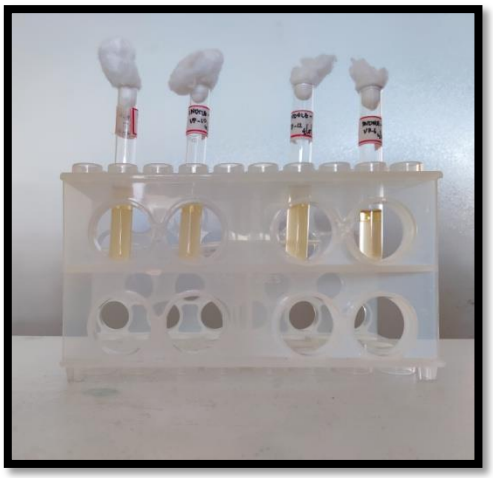


Fig 5 : Result of Indole test

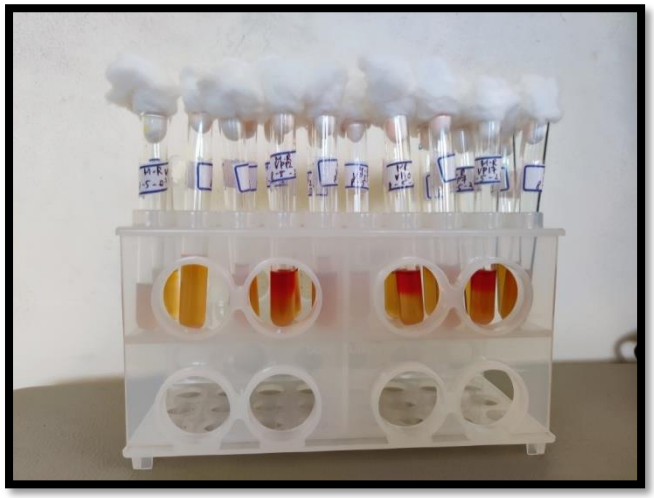


Fig 6: showing the result of Methyl red test

Fig 7: showing the result of Voges Proskauer

Fig 8 : showing the result of Citrate utilization test

Table 5: MAR index of each isolate

SL.NO	SAMPLE	MAR INDEX
1	VP1	0.16
2	VP2	0.52

3	VP3	0.48
4	VP4	0.4
5	VP5	0.52
6	VP6	0.16
7	VP7	0.12
8	VP9	0.48
9	VP10	0.08
10	VP11	0.6
11	VP12	0.4
12	VP13	0.48
13	VP15	0.12
14	VP16	0.44
15	VP17	0.24
16	VP18	0.6
17	VP19	0.6
18	VP20	0.24

Result of MAR indicates, the resistance was identified to be higher in VP11 and lower in VP10 (Table.5 and figure 9)

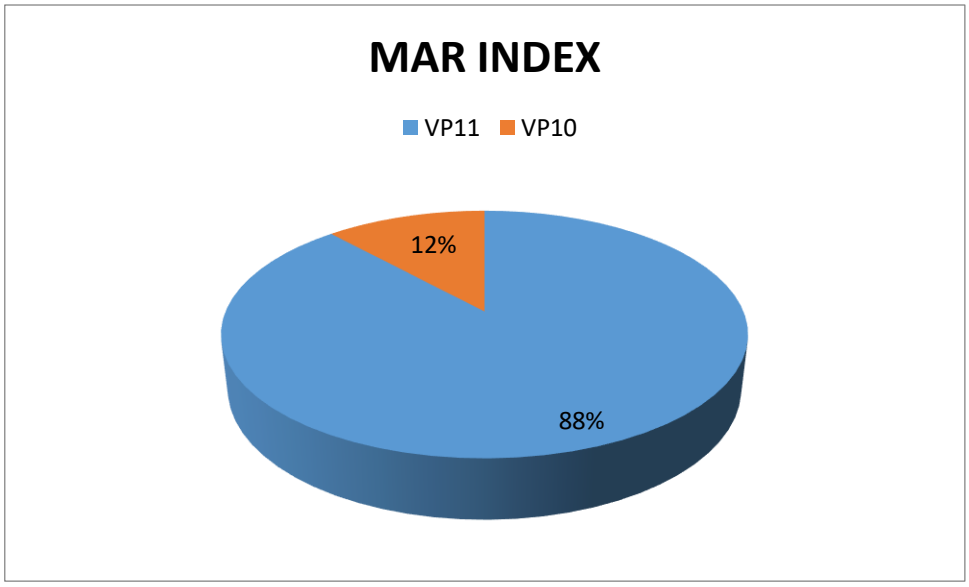


Fig 9 : Pie-chart representing the MAR index of VP10 and VP11

ANTIBIOTIC RESISTANCE PROFILE

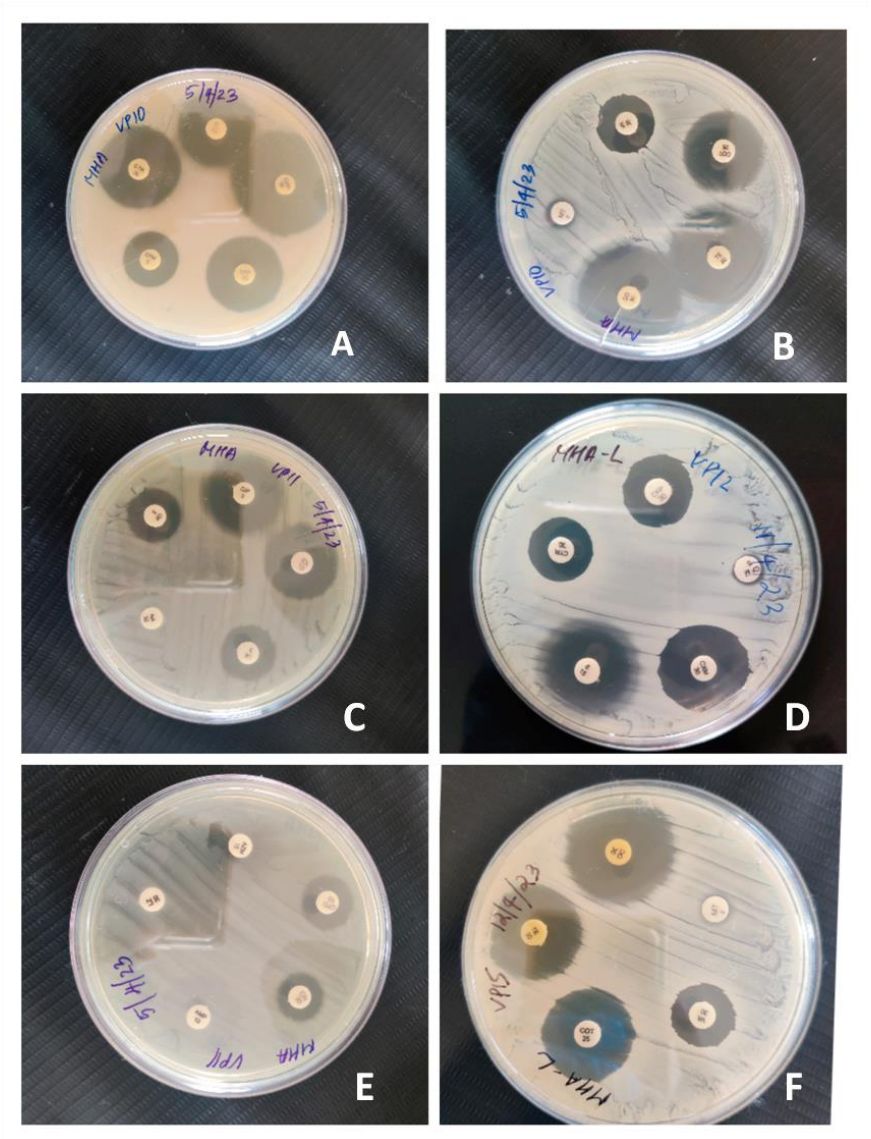


Fig 10: Representative images of clearing zones of different diameters found in the study against antibiotics tested.

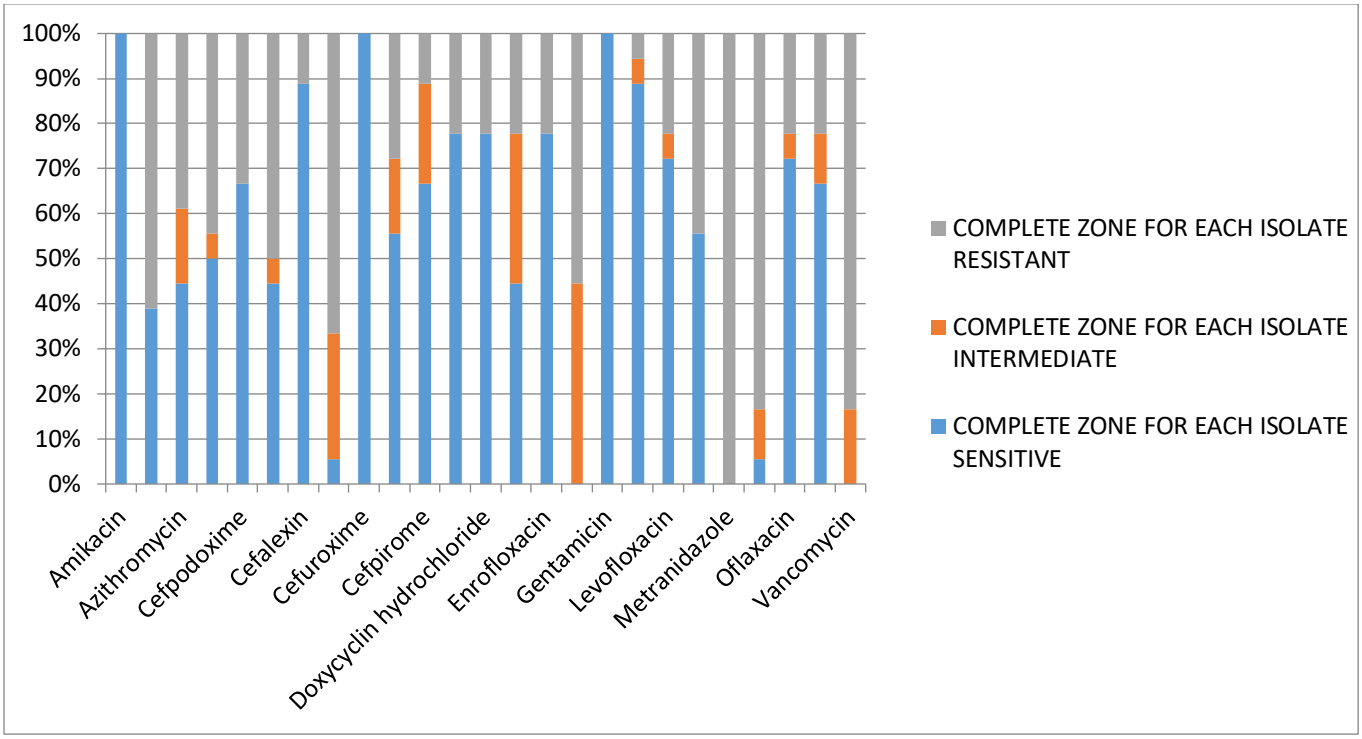
A - all pathogens showing zone of clearance

B, C, D, E, F - most pathogens are resistant

Table 6: showing the complete zone for each isolate

ANTIBIOTICS	COMPLETE ZONE FOR EACH ISOLATE		
	SENSITIVE	INTERMEDIATE	RESISTANT
Amikacin	18	-	-
Ampicilin	7	-	11
Azithromycin	8	3	7
Clindamycin	9	1	8
Cefpodoxime	12	-	6
Trimethoprim	8	1	9
Cefalexin	16	-	2
Cefixime	1	5	12
Cefuroxime	11	-	-
Ceftriaxone	10	3	5
Cefpirome	12	4	2
Ciprofloxacin	14	-	4
Doxycyclin hydrochloride	14	-	4
Erythromycin	8	6	4
Enrofloxacin	14	-	4
Faropenem	-	8	10
Gentamicin	18	-	-
Imipenem	16	1	1
Levofloxacin	13	1	4
Linezolid	10	-	8
Metranidazole	-	-	18
Nalidixic acid	1	2	15
Oflaxacin	13	1	4
Tetracyclin	12	2	4
Vancomycin	-	3	15

Fig 11: Graphical representation of the response of bacteria to the tested antibiotics. The response was classified into Susceptible (blue), Intermediate (orange) and Complete Resistance (grey).



SANGER SEQUENCING

>VP19 *Pseudomonas aeruginosa*

TGCAGCACCTGTGTCTGAGTTCCCGAAGGCACCAATCCATCTCTGGAAAGTTCTCAGCA
TGTCAAGGCCAGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTT
GTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTC
GACTTATCGCGTTAGCTGCGCCACTAAGATCTCAAGGATCCCAACGGCTAGTCGACATC
GTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTC
AGTGTCAGTATCAGTCCAGGTGGTCGCCTTCGCCACTGGTGTTCCTTCCTATATCTACGC
ATTTCACCGCTACACAGGAAATTCCACCACCCTCTACCGTACTCTAGCTCAGTAGTTTTG

GATGCAGTTCCCAGGTTGAGCCCGGGGATGGGACATCCAACTTGCTGAACCACCTACGC
GCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTTCGTATTACCGCGGCTGCTG
GCACGAAGTTAGCCGGTGCTTATTCTGTTGGTAACGTCAAAACAGCAAGGTATTAACTT
ACTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGG
CATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGA
GTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCG
TCGCCTTGGTAGGCCTTTACCCCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGC
GTGAGGTCCGAAGATCCCCCACTTTCTCCCTCA

>VP17 *Staphylococcus pseudintermedius*

GCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAGCTTGATGTA
GGGAAGAACAAATGTGTAAGTAACTGTGCACATCCTGACGAGTACCTAACCAGAAAGC
CACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGA
ATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCACGGC
TCAACCGTGGAGGGTCATTGGAAACTGGAAAACTTGAGTGCAGAAGAGGAAAGTGGAA
TTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGG
CTTTCTGGTCTGCAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGTT
ACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTT
AGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAA
CTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGC
AACGCGAAGAACCTTACCAAATCTTGACATCCTTTGACCGCTCTAGAGATAGAGTTTTC
CTCTTCGGAGGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGAT
GTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGAACTTAGTTGCCATCATTCAGTTGG
GCACTCTAAGTTGACTGCCGGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATC
ATCATGCCCCTTATGATTTGGGGCTACA

Table 7 - NCBI BLAST ANALYSIS

Sl. No.	Sample Name	Sequence Length (bp)	Nearest Neighbour	E value	% Identity	Query coverage
1	VP17	847bp	*Staphylococcus pseudintermedius*	0.0	99.06	100

2	VP19	869bp	*Pseudomonas aeruginosa*	0.0	99.65	100

The number of isolates that are susceptible, intermediate and completely resistant as per the zone interpretation chart provided by CLSI and EUCAST.

Table 8: Effect of antibiotics for the bacterial sample VP1

Sl. No	Antibiotics	Bacterial isolate	Diameter (mm)	Sensitive	Intermediate	Resistant
1	Amikacin	VP1	32			
2	Ampicilin		22			
3	Azithromycin		31			
4	Clindamycin		-			
5	Cefpodoxime		31	✓		
6	Trimethoprim		21	✓		
7	Cefalexin		28	✓		
8	Cefixime		30	✓		
9	Cefuroxime		23	✓		
10	Ceftriaxone		39	✓		
11	Cefpirome		30	✓		
12	Ciprofloxacin		29	✓		
13	Doxycyclin hydrochloride		26	✓		
14	Erythromycin		34	✓		
15	Enrofloxacin		39	✓		
16	Faropenem		31		✓	
17	Gentamicin		32	✓		
18	Imipenem		34	✓		
19	Levofloxacin		30	✓		
20	Linezolid		-			✓
21	Metronidazole		-			✓
22	Nalidixic acid		28	✓		
23	Oflaxacin		27	✓		

24	Tetracyclin		20	✓		
25	Vancomycin		-			✓

VP1 is resistant to Linezoid, Metronidazole and Vancomycin, intermediate to Faropenem, and susceptible to the rest of the antibiotics tested.

Table 9: Effect of antibiotics for the bacterial sample VP2

Sl. No	Antibiotics	Bacterial isolate	Diameter (mm)	Sensitive	Intermediate	Resistant
1	Amikacin	VP2	22	✓		
2	Ampicilin		-			✓
3	Azithromycin		-			✓
4	Clindamycin		-			✓
5	Cefpodoxime		24	✓		
6	Trimethoprim		10			✓
7	Cefalexin		18	✓		
8	Cefixime		-			✓
9	Cefuroxime		-			✓
10	Ceftriaxone		18			✓
11	Cefpirome		26	✓		
12	Ciprofloxacin		29	✓		
13	Doxycyclin hydrochloride		17	✓		
14	Erythromycin		11			✓
15	Enrofloxacin		24	✓		
16	Faropenem		-			✓
17	Gentamicin		23	✓		
18	Imipenem		21		✓	
19	Levofloxacin		22	✓		
20	Linezolid		-			✓
21	Metronidazole		-			✓
22	Nalidixic acid		10			✓

23	Oflaxacin		21	✓		
24	Tetracyclin		18	✓		
25	Vancomycin		-			✓

The sample VP2 is resistant to Ampicillin, Azithromycin, Clindamycin, Trimethoprim, Cefixime, cefuroxime, ceftriaxone, erythromycin, faropenem, linezoid, metronidazole, nalidixic acid and vancomycin and intermediate to imipenem, and sensitive to the rest of antibiotics used.

Table 10: Effect of antibiotics for the bacterial sample VP3

Sl.No	Antibiotics	Bacterial isolate	Diameter (mm)	Sensitive	Intermediate	Resistant
1	Amikacin	VP3	28	✓		
2	Ampicilin		-			✓
3	Azithromycin		18	✓		
4	Clindamycin		-			✓
5	Cefpodoxime		22	✓		
6	Trimethoprim		11			✓
7	Cefalexin		20	✓		
8	Cefixime		-			✓
9	Cefuroxime		-			✓
10	Ceftriaxone		17		✓	
11	Cefpirome		25	✓		
12	Ciprofloxacin		25	✓		
13	Doxycyclin hydrochloride		10			✓
14	Erythromycin		12			✓
15	Enrofloxacin		25	✓		
16	Faropenem		-			✓
17	Gentamicin		27	✓		
18	Imipenem		23	✓		
19	Levofloxacin		21	✓		

20	Linezolid		-			✓
21	Metronidazole		-			✓
22	Nalidixic acid		-			✓
23	Oflaxacin		21	✓		
24	Tetracyclin		12		✓	
25	Vancomycin		-			✓

The sample VP3 is resistant to ampicillin, clindamycin, trimethoprim, cefixime, cefuroxime, doxycyclin, erythromycin, faropenem, linezolid, metronidazole, nalidixic acid, and intermediate to tetracycline and susceptible to the rest of antibiotics used.

Table 11: Effect of antibiotics for the bacterial sample VP4

Sl.No	**Antibiotics**	**Bacterial isolate**	**Diameter (mm)**	**Sensitive**	**Intermediate**	**Resistant**
1	Amikacin	**VP4**	25	✓		
2	Ampicilin		-			✓
3	Azithromycin		17		✓	
4	Clindamycin		-			✓
5	Cefpodoxime		22	✓		
6	Trimethoprim		-			✓
7	Cefalexin		19	✓		
8	Cefixime		-			✓
9	Cefuroxime		-			✓
10	Ceftriaxone		19	✓		
11	Cefpirome		26	✓		
12	Ciprofloxacin		27	✓		
13	Doxycyclin hydrochloride		17	✓		
14	Erythromycin		18		✓	
15	Enrofloxacin		23	✓		
16	Faropenem		-			✓

17	Gentamicin		22	✓		
18	Imipenem		23	✓		
19	Levofloxacin		21	✓		
20	Linezolid		-			✓
21	Metronidazole		-			✓
22	Nalidixic acid		10			✓
23	Oflaxacin		21	✓		
24	Tetracyclin		13		✓	
25	Vancomycin		-			✓

The sample 4 is resistant to ampicillin, clindamycin, trimethoprim, cefixime, cefuroxime, faropenem, linezoild, metronidazole, nalidixic acid and vancomycin and intermediate to azithromycin, erythromycin and tetracycline , and sensitive to the rest of antibiotics used.

Table 12: Effect of antibiotics for the bacterial sample VP5

Sl.No	**Antibiotics**	**Bacterial isolate**	**Diameter (mm)**	**Sensitive**	**Intermediate**	**Resistant**
1	Amikacin	**VP5**	27	✓		
2	Ampicilin		-			✓
3	Azithromycin		13			✓
4	Clindamycin		-			✓
5	Cefpodoxime		21	✓		
6	Trimethoprim		10			✓
7	Cefalexin		15	✓		
8	Cefixime		-			✓
9	Cefuroxime		-			✓
10	Ceftriaxone		18			✓
11	Cefpirome		22	✓		
12	Ciprofloxacin		27	✓		
13	Doxycyclin hydrochloride		14	✓		

14	Erythromycin		14			✓
15	Enrofloxacin		24	✓		
16	Faropenem		-			✓
17	Gentamicin		25	✓		
18	Imipenem		21	✓		
19	Levofloxacin		19	✓		
20	Linezolid		-			✓
21	Metronidazole		-			✓
22	Nalidixic acid		-			✓
23	Oflaxacin		19	✓		
24	Tetracyclin		18	✓		
25	Vancomycin		-	✓		

The sample VP5 is resistant to ampicillin, azithromycin, clindamycin, trimethoprim, cefixime, cefuroxime, ceftriaxone, erythromycin, faropenem, linezolid, metronidazole, nalidixic acid and others are sensitive to the rest of antibiotics used.

Table 13: Effect of antibiotics for the bacterial sample VP6

Sl.No	**Antibiotics**	**Bacterial isolate**	**Diameter (mm)**	**Sensitive**	**Intermediate**	**Resistant**
1	Amikacin		27	✓		
2	Ampicilin		22	✓		
3	Azithromycin		12			✓
4	Clindamycin		19	✓		
5	Cefpodoxime		26	✓		
6	Trimethoprim		28	✓		
7	Cefalexin		31	✓		
8	Cefixime		-			✓
9	Cefuroxime		27	✓		
10	Ceftriaxone		14		✓	
11	Cefpirome	**VP6**	25	✓		

12	Ciprofloxacin		30	✓		
13	Doxycyclin hydrochloride		30	✓		
14	Erythromycin		16	✓		
15	Enrofloxacin		33	✓		
16	Faropenem		29		✓	
17	Gentamicin		27	✓		
18	Imipenem		34	✓		
19	Levofloxacin		25	✓		
20	Linezolid		30	✓		
21	Metronidazole		-			✓
22	Nalidixic acid		-			✓
23	Oflaxacin		25	✓		
24	Tetracyclin		28	✓		
25	Vancomycin		19	✓		

The sample VP6 is resistant to azithromyin, cefixime, metronidazole and nalidixic acid, and intermediate to ceftriaxone and faropenem and sensitive to the rest of antibiotics used.

Table 14: Effect of antibiotics for the bacterial sample VP7

Sl.No	**Antibiotics**	**Bacterial isolate**	**Diameter (mm)**	**Sensitive**	**Intermediate**	**Resistant**
1	Amikacin		23	✓		
2	Ampicilin		17			✓
3	Azithromycin		20	✓		
4	Clindamycin		27	✓		
5	Cefpodoxime		23	✓		
6	Trimethoprim		25	✓		
7	Cefalexin		29	✓		
8	Cefixime		18		✓	
9	Cefuroxime		30	✓		

10	Ceftriaxone	**VP7**	28	✓		
11	Cefpirome		26	✓		
12	Ciprofloxacin		27	✓		
13	Doxycyclin hydrochloride		30	✓		
14	Erythromycin		28	✓		
15	Enrofloxacin		29	✓		
16	Faropenem		32		✓	
17	Gentamicin		25	✓		
18	Imipenem		36	✓		
19	Levofloxacin		23	✓		
20	Linezolid		29	✓		
21	Metronidazole		-			✓
22	Nalidixic acid		-			✓
23	Oflaxacin		23	✓		
24	Tetracyclin		28	✓		
25	Vancomycin		16	✓		

The sample VP7 is resistant to ampicillin, metronidazole and nalidixic acid and intermediate to cefixime and faropenem and sensitive to the rest of the antibiotics used in the study.

Table 15: Effect of antibiotics for the bacterial sample VP9

Sl.No	**Antibiotics**	**Bacterial isolate**	**Diameter (mm)**	**Sensitive**	**Intermediate**	**Resistant**
1	Amikacin		18	✓		
2	Ampicilin		9			✓
3	Azithromycin		14		✓	
4	Clindamycin		20	✓		
5	Cefpodoxime		-			✓
6	Trimethoprim		16	✓		
7	Cefalexin		20	✓		

8	Cefixime	**VP9**	-			✓
9	Cefuroxime		19	✓		
10	Ceftriaxone		13			✓
11	Cefpirome		15		✓	
12	Ciprofloxacin		-			✓
13	Doxycyclin hydrochloride		24	✓		
14	Erythromycin		20		✓	
15	Enrofloxacin		14			✓
16	Faropenem		22			✓
17	Gentamicin		20	✓		
18	Imipenem		26	✓		
19	Levofloxacin		-			✓
20	Linezolid		21	✓		
21	Metronidazole		11			✓
22	Nalidixic acid		-			✓
23	Oflaxacin		-			✓
24	Tetracyclin		23	✓		
25	Vancomycin		14			✓

The sample VP9 is resistant to ampicillin, cefpodoxime, cefixime, ceftriaxone, ciprofloxacin, enrofloxacin, faropenem, levofloxacin, metronidazole, nalidixic acid, oflaxacine and vancomycin and intermediate to azithromycin, cefpirome and erythromycin and sensitive to the rest of the antibiotics used.

Table 16: Effect of antibiotics for the bacterial sample VP10

Sl.No	**Antibiotics**	**Bacterial isolate**	**Diameter (mm)**	**Sensitive**	**Intermediate**	**Resistant**
1	Amikacin		23	✓		
2	Ampicilin		18	✓		
3	Azithromycin		20	✓		

4	Clindamycin	**VP10**	25	✓		
5	Cefpodoxime		26	✓		
6	Trimethoprim		25	✓		
7	Cefalexin		36	✓		
8	Cefixime		18		✓	
9	Cefuroxime		33	✓		
10	Ceftriaxone		25	✓		
11	Cefpirome		25	✓		
12	Ciprofloxacin		30	✓		
13	Doxycyclin hydrochloride		30	✓		
14	Erythromycin		25	✓		
15	Enrofloxacin		31	✓		
16	Faropenem		31		✓	
17	Gentamicin		22	✓		
18	Imipenem		36	✓		
19	Levofloxacin		26	✓		
20	Linezolid		26	✓		
21	Metronidazole		10			✓
22	Nalidixic acid		10			✓
23	Oflaxacin		24	✓		
24	Tetracyclin		26	✓		
25	Vancomycin		17	✓		

The sample VP10 is resistant to the following antibiotics, metronidazole and nalidixic acid, and intermediate to cefixime and faropenem and sensitive to the rest of the antibiotics used.

Table 17: Effect of antibiotics for the bacterial sample VP11

Sl.No	**Anibiotics**	**Bacterial isolate**	**Diameter (mm)**	**Sensitive**	**Intermediate**	**Resistant**
1	Amikacin		23	✓		
2	Ampicilin		-			✓

3	Azithromycin	**VP11**	-			✓
4	Clindamycin		-			✓
5	Cefpodoxime		15			✓
6	Trimethoprim		-			✓
7	Cefalexin		12	✓		
8	Cefixime		-			✓
9	Cefuroxime		-			✓
10	Ceftriaxone		23	✓		
11	Cefpirome		17			✓
12	Ciprofloxacin		25	✓		
13	Doxycyclin hydrochloride		16	✓		
14	Erythromycin		-			✓
15	Enrofloxacin		22	✓		
16	Faropenem		-			✓
17	Gentamicin		20	✓		
18	Imipenem		24	✓		
19	Levofloxacin		17		✓	
20	Linezolid		-			✓
21	Metronidazole		-			✓
22	Nalidixic acid		-			✓
23	Oflaxacin		15		✓	
24	Tetracyclin		10			✓
25	Vancomycin		-			✓

The sample VP11 is resistant to ampicillin, azithromycin, clindamycin, cefpodoxime, trimethoprim, cefixime, cefuroxime, cefpirome, erythromycin, faropenem, linezolid, metronidazole, nalidixic acid, tetracyclin and vancomycin and intermediate to levofloxacin and oflaxacin and the rest are sensitive to the antibiotics used.

Table 18: Effect of antibiotics for the bacterial sample VP12

Sl.No	Antibiotics	Bacterial isolate	Diameter (mm)	Sensitive	Intermediate	Resistant
1	Amikacin	VP12	23	✓		
2	Ampicilin		25	✓		
3	Azithromycin		25	✓		
4	Clindamycin		26	✓		
5	Cefpodoxime		10			✓
6	Trimethoprim		25	✓		
7	Cefalexin		21	✓		
8	Cefixime		-			✓
9	Cefuroxime		23	✓		
10	Ceftriaxone		11	✓		
11	Cefpirome		18		✓	
12	Ciprofloxacin		11			✓
13	Doxycyclin hydrochloride		22	✓		
14	Erythromycin		22	✓		
15	Enrofloxacin		16			✓
16	Faropenem		23			✓
17	Gentamicin		27	✓		
18	Imipenem		30	✓		
19	Levofloxacin		10			✓
20	Linezolid		26	✓		
21	Metronidazole		-			✓
22	Nalidixic acid		-			✓
23	Oflaxacin		-			✓
24	Tetracyclin		27	✓		
25	Vancomycin		15			✓

The sample VP12 is resistant to cefpodoxime, cefixime, ciprofloxacin, enrofloxacin, faropenem, levofloxacin, metronidazole, nalidixic acid, oflaxacin and vancomycin and intermediate only to cefpirome and sensitive to the rest of the antibiotics used.

Table 19: Effect of antibiotics for the bacterial sample VP13

Sl.No	Antibiotics	Bacterial isolate	Diameter (mm)	Sensitive	Intermediate	Resistant
1	Amikacin	VP13	21	✓		
2	Ampicilin		15			✓
3	Azithromycin		13			✓
4	Clindamycin		17		✓	
5	Cefpodoxime		14			✓
6	Trimethoprim		-			✓
7	Cefalexin		32	✓		
8	Cefixime		18		✓	
9	Cefuroxime		29	✓		
10	Ceftriaxone		22	✓		
11	Cefpirome		25	✓		
12	Ciprofloxacin		-			✓
13	Doxycyclin hydrochloride		17	✓		
14	Erythromycin		20		✓	
15	Enrofloxacin		15			✓
16	Faropenem		30		✓	
17	Gentamicin		22	✓		
18	Imipenem		34	✓		
19	Levofloxacin		10			✓
20	Linezolid		23	✓		
21	Metronidazole		-			✓
22	Nalidixic acid		11			✓
23	Oflaxacin		-			✓
24	Tetracyclin		10			✓
25	Vancomycin		14			✓

The sample VP13 is resistant ampicillin, azithromycin, cefpodoxime, trimethoprim, ciprofloxacin, enrofloxacin, levofloxacin, metronidazole, nalidixic acid, oflaxacin, tetracycline and vancomycin and intermediate to clindamycin, cefixime, erythromycin and faropenem and sensitive to the rest of antibiotics used.

Table 20: Effect of antibiotics for the bacterial sample VP15

Sl. No	Antibiotics	Bacterial isolate	Diameter (mm)	Sensitive	Intermediate	Resistant
1	Amikacin	VP15	22	✓		
2	Ampicilin		29	✓		
3	Azithromycin		23	✓		
4	Clindamycin		23	✓		
5	Cefpodoxime		22	✓		
6	Trimethoprim		22	✓		
7	Cefalexin		24	✓		
8	Cefixime		16		✓	
9	Cefuroxime		28	✓		
10	Ceftriaxone		22	✓		
11	Cefpirome		24	✓		
12	Ciprofloxacin		22	✓		
13	Doxycyclin hydrochloride		26	✓		
14	Erythromycin		23	✓		
15	Enrofloxacin		24	✓		
16	Faropenem		28		✓	
17	Gentamicin		23	✓		
18	Imipenem		35	✓		
19	Levofloxacin		20	✓		
20	Linezolid		28	✓		
21	Metronidazole		-			✓
22	Nalidixic acid		-			✓

23	Oflaxacin		19	✓		
24	Tetracyclin		24	✓		
25	Vancomycin		15			✓

The sample VP15 is resistant to metronidazole, nalidixic acid and vancomycin, and intermediate to cefixime and faropenem and sensitive to the rest of the antibiotics used.

Table 21: Effect of antibiotics for the bacterial sample VP16

Sl.No	Antibiotics	Bacterial isolate	Diameter (mm)	Sensitive	Intermediate	Resistant
1	Amikacin	VP16	23	✓		
2	Ampicilin		26			✓
3	Azithromycin		25	✓		
4	Clindamycin		24	✓		
5	Cefpodoxime		10			✓
6	Trimethoprim		25	✓		
7	Cefalexin		15	✓		
8	Cefixime		-			✓
9	Cefuroxime		20	✓		
10	Ceftriaxone		15		✓	
11	Cefpirome		16			✓
12	Ciprofloxacin		-			✓
13	Doxycyclin hydrochloride		22	✓		
14	Erythromycin		25	✓		
15	Enrofloxacin		15			✓
16	Faropenem		21			✓
17	Gentamicin		25	✓		
18	Imipenem		30	✓		
19	Levofloxacin		-			✓

20	Linezolid		28	✓		
21	Metronidazole		-			✓
22	Nalidixic acid		16		✓	
23	Oflaxacin		-			✓
24	Tetracyclin		24	✓		
25	Vancomycin		15			✓

The sample VP16 is resistant to ampicillin, cefpodoxime, cefixime, cefpirome, ciprofloxacin, enrofloxacin, faropenem, levofloxacin, metronidazole, oflaxacin, and vancomycin and intermediate to cefriaxone and nalidixic acid and sensitive to the rest of the antibiotics used.

Table 22: Effect of antibiotics for the bacterial sample VP17

Sl.No	**Antibiotics**	**Bacterial isolate**	**Diameter (mm)**	**Sensitive**	**Intermediate**	**Resistant**
1	Amikacin	**VP17**	26	✓		
2	Ampicilin		32	✓		
3	Azithromycin		20	✓		
4	Clindamycin		30	✓		
5	Cefpodoxime		24	✓		
6	Trimethoprim		-			✓
7	Cefalexin		25	✓		
8	Cefixime		16		✓	
9	Cefuroxime		30	✓		
10	Ceftriaxone		23	✓		
11	Cefpirome		29	✓		
12	Ciprofloxacin		25	✓		
13	Doxycyclin hydrochloride		-			✓
14	Erythromycin		22	✓		
15	Enrofloxacin		27	✓		
16	Faropenem		30		✓	

17	Gentamicin		25	✓		
18	Imipenem		35	✓		
19	Levofloxacin		22	✓		
20	Linezolid		28	✓		
21	Metronidazole		-			✓
22	Nalidixic acid		-			✓
23	Oflaxacin		22	✓		
24	Tetracyclin		-			✓
25	Vancomycin		-			✓

The sample VP17 is resistant to trimethoprim, doxycyclin, metronidazole, nalidixic acid, tetracycline, and vancomycin and intermediate to cefixime and faropenem and sensitive to the rest of the antibiotics used.

Table 23: Effect of antibiotics for the bacterial sample VP18

Sl.No	**Antibiotics**	**Bacterial isolate**	**Diameter (mm)**	**Sensitive**	**Intermediate**	**Resistant**
1	Amikacin	**VP18**	22	✓		
2	Ampicilin		-			✓
3	Azithromycin		-			✓
4	Clindamycin		-			✓
5	Cefpodoxime		2			✓
6	Trimethoprim		12		✓	
7	Cefalexin		-			✓
8	Cefixime		-			✓
9	Cefuroxime		-			✓
10	Ceftriaxone		18			✓
11	Cefpirome		20		✓	
12	Ciprofloxacin		30	✓		
13	Doxycyclin hydrochloride		15	✓		

14	Erythromycin		-			✓
15	Enrofloxacin		25	✓		
16	Faropenem		-			✓
17	Gentamicin		18	✓		
18	Imipenem		2			✓
19	Levofloxacin		22	✓		
20	Linezolid		-			✓
21	Metronidazole		-			✓
22	Nalidixic acid		11			✓
23	Oflaxacin		20	✓		
24	Tetracyclin		19	✓		
25	Vancomycin		-			✓

The sample VP18 is resistant to ampicillin, azithromycin, clindamycin, cefpodoxime, cefalexin, cefixime, cefuroxime, ceftriaxone, erythromycin, faropenem, imipenem, linezolid, metronidazole, nalidixic acid and vancomycin and intermediate to trimethoprim and cefpirome and sensitive to the rest of te antibiotics used.

Table 24: Effect of antibiotics for the bacterial sample VP19

Sl.No	**Antibiotics**	**Bacterial isolate**	**Diameter (mm)**	**Sensitive**	**Intermediate**	**Resistant**
1	Amikacin		20	✓		
2	Ampicilin		-	✓		
3	Azithromycin		-	✓		
4	Clindamycin		-			✓
5	Cefpodoxime		20		✓	
6	Trimethoprim		-			✓
7	Cefalexin		-			✓
8	Cefixime		-			✓
9	Cefuroxime		-			✓
10	Ceftriaxone		19			✓

11	Cefpirome	**VP19**	20		✓	
12	Ciprofloxacin		24	✓		
13	Doxycyclin hydrochloride		10			✓
14	Erythromycin		-			✓
15	Enrofloxacin		21	✓		
16	Faropenem		-			✓
17	Gentamicin		22	✓		
18	Imipenem		22	✓		
19	Levofloxacin		21	✓		
20	Linezolid		-			✓
21	Metronidazole		-			✓
22	Nalidixic acid		10			✓
23	Oflaxacin		19	✓		
24	Tetracyclin		15	✓		
25	Vancomycin		-			✓

The sample VP19 is resistant to clindamycin, trimethoprim, cefelexin, cefixime, cefuroxime, ceftriaxone, doxycyclin, erythromycin, faropenem, linezolid, metronidazole, nalidixic acid and vancomycin and intermediate to cefpodoxime and cefpirome and sensitive to the rest of the antibiotics used.

Table 25: Effect of antibiotics for the bacterial sample VP20

Sl. No	Antibiotics	Bacterial isolate	Diameter (mm)	Sensitive	Intermediate	Resistant
1	Amikacin		22	✓		
2	Ampicilin		30	✓		
3	Azithromycin		16		✓	
4	Clindamycin		25	✓		
5	Cefpodoxime		22	✓		
6	Trimethoprim		-			✓

7	Cefalexin	**VP20**	22	✓		
8	Cefixime		15			✓
9	Cefuroxime		28	✓		
10	Ceftriaxone		22	✓		
11	Cefpirome		26	✓		
12	Ciprofloxacin		25	✓		
13	Doxycyclin hydrochloride		-			✓
14	Erythromycin		22		✓	
15	Enrofloxacin		27	✓		
16	Faropenem		30		✓	
17	Gentamicin		24	✓		
18	Imipenem		35	✓		
19	Levofloxacin		22	✓		
20	Linezolid		24	✓		
21	Metronidazole		-			✓
22	Nalidixic acid		17		✓	
23	Oflaxacin		22	✓		
24	Tetracyclin		-			✓
25	Vancomycin		-			✓

The sample VP20 is resistant to trimethoprim, cefixime, doxycyclin, metronidazole, tetracycline and vancomycin and intermediate to azithromycin, erythromycin, faropenem and nalidixic acid and sensitive to the rest of the antibiotics used.

DISCUSSION

The study focuses on the pathogens collected from the Veterinary hospitals and their Antimicrobial resistance profiling were determined. Around 18 samples including the skin scrapping, ear swabs, urinary tract infection, otitis, etc of dogs, cats and birds were collected. From this study we got *Pseudomonas* and *Staphylococcus* species to be resistant.

In this study, the gram positive isolates were VP1, VP2 , VP3, VP6, VP7, VP9, VP10, VP12, VP13, VP15, VP16, VP17 and VP20 whereas VP4, VP5, VP11, VP18, VP19 appeared to be pink coloured. Therefore, these isolates belonged to gram-negative strains. When the gram-stained isolates were observed under the microscope, VP6, VP7, VP9, VP10, VP12, VP13, VP15, VP16, VP17 and VP20 exhibited coccus shape whereas VP1, VP2, VP3, VP4, VP5 and VP11 exhibited rod-shaped bacterial colony.

The number of recorded Gram-positive strains remained relatively stable throughout the study period, showing no significant variation from year to year. Moreover, the number of Gram-positive strains was consistently higher compared to Gram-negative bacterial isolations, with a statistically significant difference (p-value = 0.0007). Among dogs, *Staphylococcus pseudintermedius* was the most commonly identified Gram-positive bacterium, accounting for 65% of the isolations. On the other hand, *Pseudomonas aeruginosa* was the predominant Gram-negative bacterium, representing 36% of the isolated strains (Nocera 2021). One notable advantage of the modified Gram stain is its ability to effectively highlight collagen, enabling its application to various collagen-containing tissues. However, when using this technique on different tissue samples, it may be necessary to optimize the staining times based on the collagen content of each specific sample. For instance, cutaneous tissue, which is abundant in dense collagen fibers, may require shorter staining times compared to tissues with lower collagen abundance or specific locations, such as the lamina propria. Our study has successfully demonstrated the clinical relevance of this modified Gram stain by visualizing bacteria in burn wound samples obtained from both the laboratory and the operating room. Going forward, this

technique can be readily implemented in clinical settings, allowing for rapid examination of infection status in tissue samples (Becerra et. al., 2016). The findings of previous studies align with our own, further supporting the significance of gram-positive strains in our research.

The VP11 (*Pseudomonas*) exhibits a higher MAR index, while VP10 (*Staphylococcus*) demonstrates a lower MAR index, as derived from our study. Our findings align with previous studies, as the Mar index confirms the prevalence of antimicrobial resistance among the pathogens we collected. This is consistent with similar cases reported in the literature, highlighting the persistence and spread of resistant strains in veterinary settings. The Mar index proves to be an invaluable tool in evaluating the extent of resistance and aiding in antimicrobial therapy decisions (Sindeldecker, 2021).

MAR index values exceeding 0.2 suggest a significant risk of contamination from sources where antibiotics are commonly utilized (Osundiya et. al., 2013). The calculation of MAR index for each isolate revealed that the majority of isolates originated from high-risk sources, highlighting the emergence of antibiotic resistance to commonly prescribed antibiotics. The research demonstrated the evolutionary changes in bacteria over time, resulting in the heightened resistance of newly identified strains to antibiotics. These antibiotics specifically target virulence factors. However, a major challenge faced by pharmaceutical companies is the rapid mutation events in bacteria, leading to the acquisition of multi-resistance by each strain. Many bacterial infections today do not respond to first-line treatment, as the treatment protocols heavily rely on broad-spectrum antibiotics, which in turn promote resistance across a wide array of bacterial strains. Prioritizing the treatment of pets is essential, as they can serve as potential disease carriers to humans and facilitate the transfer of antibiotic resistance through horizontal gene transfer, thereby enabling transmission from pets to humans.

The high levels of resistance seen in commonly prescribed antibiotics, including Metronidazole, Cefixime, Nalidixic acid, and Vancomycin, underscore the critical importance of responsible antimicrobial usage and surveillance within veterinary medicine. The data presented in the chart illustrating bacterial reactions to these antibiotics underscores the necessity of monitoring resistance trends and implementing targeted strategies to address antimicrobial resistance. Given the close relationship between pets and humans, there is a potential risk of zoonotic transmission. Research conducted in recent years has shown that pet animals are a significant source of antibiotic resistance (Gómez, 2016). Antibiotic resistance is particularly prevalent in cases of urinary tract infections, skin infections, and wound infections. Watanabe's identification of horizontal transfer of R factors (plasmids containing resistance genes) was a key discovery in understanding the persistence of multi-resistant bacteria despite antibiotic treatment (Creager, 2007).

MRSP, a methicillin-resistant bacterium, was initially detected in dogs during the 2000s, with its prevalence being influenced by geographical and clinical factors. Companion animals, particularly dogs, are key in the spread of MRSP, as staphylococci can easily transfer to humans through close contact. Consequently, humans may temporarily carry MRSP after contact with their colonized dogs, potentially resulting in serious disease outbreaks. The management of MRSP infections poses a significant challenge in veterinary medicine, with cases in humans often being misdiagnosed as S. aureus, leading to underreporting. A high percentage (62%) of isolates exhibited multi-drug resistance. The use of antimicrobials can contribute to the co-selection and emergence of resistant strains like MRSP by acquiring mobile genetic elements and mutations (Srednik et. al., 2023). This bacterium has developed resistance to various classes of antibiotics, including β-lactams (e.g., penicillins and cephalosporins), macrolides, and fluoroquinolones. Resistance is primarily acquired through the acquisition of resistance genes like mecA, which encodes methicillin resistance, and different efflux pumps (Carcamo-Tzic et. al., 2022). In this research, the pathogens displayed high resistance to antibiotics such as Metronidazole, Cefixime, Nalidixic acid, and Vancomycin.

The presence of the mecA gene, as identified, is responsible for conferring resistance to methicillin and other β-lactam antibiotics (Perreten et. al., 2010). This discovery underscores the role of horizontal gene transfer in the dissemination of antimicrobial resistance among staphylococcal species. The research demonstrated elevated levels of resistance to β-lactams, macrolides, and tetracyclines, with a significant portion of isolates displaying multidrug resistance. These findings stress the significance of prudent antibiotic use and the monitoring of resistance patterns in veterinary practice (Loeffler et. al., 2007).

Pseudomonas aeruginosa, a Gram-negative bacterium known for its motility and opportunistic nature, is considered a sexually transmitted pathogen and is recognized as the primary cause of endometritis in animals. Due to its natural production of AmpC B-lactamase, P. aeruginosa exhibits resistance to various antibiotic combinations and remains unaffected by lactam inhibitors such as clavulanic acid, sulbactam, and tazobactam currently available in the market. This bacterium is capable of causing a wide array of infections, especially in individuals with compromised immune systems and those suffering from cystic fibrosis. It is linked to numerous healthcare-associated infections, including pneumonia, urinary tract infections, and bloodstream infections, presenting a significant challenge in clinical settings due to its resistance mechanisms.

A study on fluoroquinolone resistance mechanisms in clinical isolates of *Pseudomonas aeruginosa* from cystic fibrosis patients revealed mutations in the gyrA and parC genes, responsible for encoding

DNA gyrase and topoisomerase IV subunits, respectively. These mutations result in decreased susceptibility to fluoroquinolones, underscoring the importance of target site alterations in conferring resistance in *Pseudomonas aeruginosa*. Both *Staphylococcus pseudintermedius* and *Pseudomonas aeruginosa* represent significant challenges in terms of antimicrobial resistance, with implications for both veterinary and human healthcare. Responsible antimicrobial use, surveillance of resistance patterns, and development of alternative treatment strategies are essential for addressing the threat of antimicrobial resistance posed by these pathogens.

Moreover, it highlights the necessity for interdisciplinary cooperation and One Health strategies to effectively address AMR across human, animal, and environmental sectors. Progressing, endeavors to combat AMR should concentrate on formulating alternative treatment approaches, enhancing antimicrobial utilization, and advocating for responsible antibiotic prescribing habits. By giving importance to antimicrobial stewardship and surveillance endeavors, we can ensure the protection of both animal welfare and public health against the escalating challenge of antimicrobial resistance.

CONCLUSION

The research emphasizes the widespread presence of antimicrobial resistance (AMR) in pathogens obtained from veterinary hospitals, with both Gram-positive (*Staphylococcus pseudintermedius*) and Gram-negative (*Pseudomonas aeruginosa*) bacteria showing resistance, including multi-drug resistance. The findings indicated a range of resistance profiles among the pathogens, with some strains being susceptible to antibiotics, while others displayed intermediate resistance. Nevertheless, a troubling proportion of the pathogens exhibited resistance to multiple antimicrobial agents, suggesting the existence of multidrug-resistant strains. The MAR index serves as a useful tool for evaluating resistance levels, underscoring significant risks linked to antibiotic use. Specifically, among the veterinary pathogens collected, VP11 (*Pseudomonas*) stands out with a notably higher MAR index compared to other isolates, indicating an elevated level of antimicrobial resistance. The resistance of *Pseudomonas* to various antibiotic combinations and its ability to cause a wide range of infections pose significant challenges in clinical settings. *Pseudomonas aeruginosa*, in particular, is known for causing severe infections in individuals with compromised immune systems and those with conditions like cystic fibrosis. Due to its resistance mechanisms, such as the production of AmpC β-lactamase and mutations in key genes, treating *Pseudomonas* infections can be challenging, resulting in prolonged illness, increased healthcare costs, and higher mortality rates. These findings highlight the crucial role of judicious antibiotic use in veterinary medicine. Additionally, the close interaction between pets and humans requires attention to the potential for zoonotic transmission of resistant bacteria. The challenges in managing antimicrobial-resistant strains, like methicillin-resistant *Staphylococcus pseudintermedius* (MRSP), emphasize the importance of interdisciplinary collaboration and One Health strategies. Efforts moving forward should prioritize the promotion of responsible antimicrobial use, surveillance, and the development of alternative treatment strategies to address the growing threat of AMR in veterinary medicine. This is crucial to safeguard both animal welfare and public health. To effectively combat the global challenge of antimicrobial resistance (AMR) that impacts individuals, animals, and the environment, the adoption of a One Health approach is essential. This collaborative strategy involves coordination among veterinarians, physicians, public health experts, and epidemiologists. In conclusion, the increasing resistance of bacteria to antibiotics

must be recognized as a significant global health issue. Therefore, it is imperative to explore alternative treatment strategies to enhance quality of life.

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