

Bacteriological Identification among Patients Attending at Jimma University Veterinary Clinic

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Abstract

Findings of conducted study showed that the predominant bacteria in Jimma University Veterinary Clinic. Among predominant bacteria identified includes; *Escherichia coli* 46 (34.3%), *Klebsiella* 23(17.2%), followed by *Staphylococcus aureus* 40(29.9%) and *Streptococcus spp* 25(18.7%). Among 4 species of bacteria isolated, 2 were gram negative and 2 of them was gram positive bacteria. Among the antibiotic susceptibility test, *Staphylococcus aureus* showed the total resistant on penicillin while tetracycline showed to have the highest sensitivity. *Escherichia coli* showed the total resistant on gentamicin (100%). Veterinary Clinic is at risk of acquiring bacterial infections due to the presence of mentioned bacteria during this study. The big issue is that these infections resist to antibiotics used in treatment according to results of the study. Health care workers in Veterinary Clinic should improve hygiene to control clinic acquired infections and much attention should be done during diagnosis to improve treatment of patients.

1. Introduction

Veterinary clinics acquired infections are known to be common cause of morbidity and mortality among animal patients. Antibiotic-resistant bacteria as the etiology of infection have been expanding at an alarming rate. These infections are due to Multi-Drug Resistant (MDR) bacteria and seriously lead to health care problem, and a daily challenge among Veterinarians dealing with diagnosis and treatment of patients. Cross-contamination of pathogens among patients of inanimate surfaces around patients in Veterinary clinics has been clearly identified in outbreaks; contamination can take place either by microorganisms transfer that contaminated health workers' hands or direct patient shedding of microorganisms while performing their task related to patient care and treatment. Infections crossing from patient to another as well as from Veterinary clinic workers to patients constructed huge hazards in health care especially in developing country. These infections are mostly known to be causes of morbidity and mortality of animals on the worldwide. Contamination due to bacteria in clinics are related directly or indirectly to the use of antibiotics in improper way by number of owners. Haphazard use of antibiotics and absence of knowledge are the most imperative variables for the rise, selection, and spread of antibiotic-resistant organisms in the environment. This improper use of Antibiotics creates opportunities of new strains of bacteria appearance that resist to current used antibiotics. Many of the antimicrobial agents are utilized in feed of animal production for controlling diseases and mostly used as growth promoter that is continuously disseminating in human food chain leads serious health problem in human and animals. Cattle that comes to veterinary clinics were a potential source for the contamination of the environment and farm products by antibiotic-resistant of *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus spp*. present in cow milk, dung/cow urine. The way of how Veterinary clinics environment is in terms of hygiene and other activities related to patient care build the way of spreading various antimicrobial-resistance pathogens. Both gram negative and gram positive bacteria resist to antibiotics and most of them are Staphylococci wide variety of Enterobacteriaceae, *Pseudomonas* species, *Acinetobacter* species or *Candida* species. These pathogens are zoonotic in nature and can

transmit to human from animals through contaminated milk, meat, water, and direct contact with animals or their environmental equipment. *Salmonella* spp. is the most ubiquitous organisms in nature and major food-borne zoonotic pathogen, it is also one of the pathogens listed in the WHO priority pathogen list. Livestock manure contains microbial constituents, which make it a potential source of pathogenic microorganisms for animals and human.

Despite all effort done by veterinarian care setting to reduce the burden of infections in Veterinary care unit, the rate of these infections continue to be a burden, however, patients in Veterinary clinics are at high risk of various infections due to their health state. However, these Veterinary clinics patients are often at risk of infection due to impairing effects of their diseases and therapies on the immune system as well as some operative treatment consequences. Infection categorization is a very big issue in various infection surveillance, control and management, especially in Veterinary clinics as most of patient in this service are risky to get second infection. Health setting acquired infection especially in Veterinary clinics based on time spent at health setting enough to distinguish bacterial infection from community infections. Veterinarians have taken this inconsideration and confirmed that infection that can occur after pre-treatment clinic is not considered to be nosocomial or Veterinary clinics acquired infection.

2. Materials And Methods

2.1 Study design and sample collection

This study was conducted from January to July 2020 to December 2020 and 134 animal patients comes to Jimma University Veterinary Clinics were targeted. A total of 134 samples were collected from the clinic where each of the animals contributed 134 samples consists of 36 cow dung (faecal), 35 milk, 19 pus, swab 30 and 14 urine. Inclusion criteria were all patients who diagnosed clinically have bacterial infection. The study was carried out using cross section method to collect the different samples from the patients in aseptic condition and transferred to laboratory on bacteriology service and using Ice box. Specimen was collected from Veterinary clinics and tested on Microbiology and Bacteriology unit of laboratory. All specimens were registered when collected.

2.2 Bacterial identification techniques

Different techniques were used for bacterial identification. Bacterial identification techniques used during the study including gram staining for gram positive and gram negative bacterial differentiation, catalase test to differentiate staphylococci and streptococcus, coagulase test to differentiate staphylococcus aureus (positive) from coagulase negative staphylococci, Mac-Conkey agar was used to identify enterobacteriaceae, however all samples were incubated in Incubators to maintain the growth of bacteria.

2.3 Antibiotic susceptibility tests

Antibiotic susceptibility test of bacteria was conducted regarding criteria of clinical and performed laboratory standard institute, using the Kirby-Bauer disc diffusion on Mueller Hinton agar. Isolates were grown in peptone water at 37⁰C turbidity was matched with 0.5 McFarland standards. The placement of

various types of antibiotic on culture was taken place on Mueller Hinton agar plate and incubated for 24hr on 37⁰C both the sensitivity and resistance were confirmed by means of measuring the diameter of inhibition zone.

3. Results

3.1 Demographic characteristics of the participants

Table below shows demographic characteristics of target population based on age, sex and body condition scores of patients in Veterinary clinics

Table 1
demographic characteristics of the participant regarding to Age group:

Age	Frequency	Percent
Adult	35	26.1%
Old	39	29.1%
Young	60	44.8%
Total	134	100%

Table 2
demographic characteristics of the participant regarding to Gender group:

Gender	Frequency	Percent
Female	72	53.7%
Male	62	46.3%
Total	134	100%

Table 3
demographic characteristics of the participant regarding to Body condition score group:

Body condition score	Frequency	Percent
good	25	18.7%
medium	52	38.8%
poor	57	42.5%
Total	134	100%

Table 4
Sample type collected and diagnostic results in Veterinary clinics

Samles	Frequency	Percent
Feacal	36	26.9%
Milk	35	26.1%
Pus	19	14.2%
Swab	30	22.4%
Urine	14	10.4%
Total	134	100%

3.2 Microorganisms identification

The below figure 1 shows the percentage of isolated bacteria in Veterinary clinic. Isolated bacteria are as follows: *Escherichia coli* 46 (34.3%), *Klebsiella* 23(17.2%), followed by *S.aureus* 40(29.9%) and *Streptococcus spp* 25(18.7%). Among 4 species of bacteria isolated, 2 were gram negative and 2 of them was gram positive bacteria.

Table 5
Microorganisms identified from diagnostic results in Veterinary clinics

Organisms	Frequency	Percent
<i>E.coli</i>	46	34.3%
<i>Klebsiella</i>	23	17.2%
<i>S.aureus</i>	40	29.9%
<i>Streptococcus spp</i>	25	18.7%
Total	134	100%

3.3 Antibiotic susceptibility test of isolated bacteria

Frequently isolates of *Staphylococcus aureus* were 40(29.9%) as presented on the Table 5 above. The antibiotics susceptibility test isolated on *Staphylococcus aureus*, is that it was totally resistant to Penicillin 36(90%), it also showed the highest sensitivity to Gentamycin 40 (100%) and Vancomycin 38 (92%)while Tetracycline 26 (65%) and moderate Sensitivity Ciprofloxacin 24 (60%). In treatment of infection due to *Staphylococcus aureus*, the results of the study show that Penicillin is not the correct drug to treat *Staphylococcus aureus* related infection at Jimma University Veterinary clinics, however, resistant factors can be later studied in other researches.

According to Figure 5 above, Streptococcus is also among isolated bacteria, and their frequency was 25(18.7%). In this study the antibiotic susceptibility test for isolate on Streptococcus, showed the total resistance on amoxicillin 25(100%) and moderately resist on ciprofloxacin 15(60%) and cefotaxime 13(52%) while imipenem showed sensitivity on 17 (68%). The same study was conducted in Nigeria where Results showed the general resistance to ceftriaxone, cefuroxime, amoxicillin, ampicillin, and ciprofloxacin and augmentin which are the drugs of choice to treat thyroid fever in the area of study. Although, sensitivity was seen to ofloxacin and chloremphenicol, despite stopping these drugs in treatment of thyroid fever in the area. The antibiotic susceptibility test on E.Coli isolated whose frequency were 46 showed the total resistance on gentamicin 17(100%) moderately resist on ceftazidime 20(23%) and chloremphenicol 9(47%). The sensitivity was showed on imipenem 16(94.1%). The study conducted in Ethiopia where the highest isolation rate was obtained from urine samples 203(45.5%). High resistance rate to erythromycin (89.4%), amoxicillin (86.0%) and tetracycline (72.6%) were seen. However, high sensitivity was seen on nitrofurantoin (96.4%), norflaxocin (90.6%), gentamicin (79.6%) as well as ciprofloxacin. Different multiple antimicrobial resistances of 74.6% and the increase of resistance rates to all antimicrobials except ciprofloxacin were kept. Antibiotic resistance was found to be greater than sensitivity in all cases of test conducted during this study. It is understandable; treating second infection can be very difficult and increase resistance of bacteria to existing antibiotics. The resistance increased basing on factors that favor the bacteria to infect the patient. S.aureus is most resistant bacteria and is known to be predominant to cause nosocomial infections especially in skin and soft tissue due to its location in human body. In Veterinary Clinic while treating some infections, thinking on second infections may be thought due to exposure of patients.

3.4 Antibiotics susceptibility tests of Staphylococcus aureus

Both sensitivity and resistant rate of staphylococcus aureus were shown using various antibiotics. Penicillin, Gentamycin, Vancomycin, Tetracycline and Oxacillin are antibiotics used for antibiotics susceptibility test of Staphylococcus aureus. The antibiotics susceptibility test isolated on Staphylococcus aureus, is showed that it was totally resistant to Penicillin 36 (90%), it also showed the highest sensitivity to Gentamycin 40 (100%) and Vancomycin 38 (92%) while Tetracycline 26 (65%) and moderate Sensitivity Oxacillin 24 (60%). The level of sensitivity and resistance shows the right antibiotics to kill Staphylococcus aureus. In treatment of infection caused by Staphylococcus aureus Gentamycin and Vancomycin can be used as the first antibiotic for disease treatment.

3.5 Antibiotics susceptibility test of Streptococcus

The antibiotics susceptibility test used for isolate Streptococcus spp including Imipenem, ciprofloxacin, cefotaxime and amoxicillin. It has been shown that Salmonella isolate totally resist to amoxicillin 25 (100%) and moderately resist to ciprofloxacin 15 (60%) and cefotaxime 13(52%) while Imipenem 17 (68%) showed sensitive. By strict analysis more other drugs are needed to treat infections related to Salmonella typhi level of resistance to be high. However, Imipenem can be used considering the result of this study.

3.6 Antibiotics susceptibility test of *Escherichia coli*

Gentamicin, Imipenem, chloramphenicol and ceftazidime are antibiotics susceptibility test isolated on of *E. coli*, where the rate of sensitivity and resistant were assessed. Gentamicin, imipenem, chloramphenicol and ceftazidime used for antibiotics test of *Escherichia coli*. *Escherichia coli* isolated showed totality of resisting on on Gentamicin 44 (95.65%) moderate on ceftazidime 24(52.2%) and chloramphenicol 22(47.8%) and sensitivity high on imipenem 45(97.8%). The very low percentage of the two microorganisms; *Klebsiella* and *Streptococcus* spp were also isolated, however, *Streptococcus* spp was seen in pus and *Klebsiella* was seen in cow dung and urine specimen. *Klebsiella* was seen to be resistant to gentamicin and tetracycline; and high sensitivity to penicillin. *Streptococcus* spp was resistant to Imipenem but sensitive to cefotaxime.

4. Discussion

The study was carried out for identifying common bacteria in affected animals that were comes to Veterinary Clinics at Jimma University. As shown on the Table 4, samples including urine 14 (10.4%), swab 30(22.4%), pus 19(14.2%), milk 35(26.1%), and faecal 36(26.9%) were taken for different techniques of bacterial identification. The results of this study, the total of 134 patients were tested, 62(46.3%) were male and 72(53.7%) were female. Female occupied a great number of patients comparing to male. Another study conducted in India showed the high prevalence of bacterial infection in females (73.57%) comparing to males whose prevalence was (35.14%) and this study is correlated with other studies that show the high prevalence of infection in veterinary clinic for females comparing to males. The main sample corrected in veterinary clinic was swab sample, but the results showed that animals with swab samples are low risked compared to others considering percentages of positive results. Patients with pus and faecal samples in veterinary clinic are at high risk of infections compared to others. This due to kinds of diseases that favours second infections. Pus is mostly taken for animals with wound infections and these infections easily infect the patient. Around 59% of all diagnosed patient with pus sample were infected which show high risk of infections among these group of patients. Patients with faecal sample also showed high risk of infections, and it usually known that Gastrointestine are good medium for microorganism growth. It was seen that 60% of patients diagnosed with faecal sample were infected and this a proof showing that feces harbors simply microorganisms and grow. Predominant bacteria in this study were identified in veterinary clinic, both gram negative and gram positive were shown with their percentages on Figure1. The entire prevalence of isolated gram negatives was 69(51.49%) while prevalence for gram positives was 65(48.50%). Among gram positives isolated including *Staphylococcus aureus* with 40(29.9%) while isolated gram negative bacteria were *Escherichia coli* 46 (34.3%), *Streptococcus* 25 (18.7%) and *Klebsiella pneumoniae* 23 (17.2%). The similar study was conducted in Iraq by Nasser et al., (2013), where 68 of pure colony isolated were identified including 24 (35.29%) gram positive bacterial isolates and 44 (64.71%) of gram negative bacterial isolate. Another study carried out in Iran by Hassanzadeh & Hadi (2009) the gram negative 37(4.6%) bacteria were significantly more involved infections compared to gram positive bacteria 9 (10.9%). Looking at number

of conducted studies in Veterinary clinic, gram negatives are more prevalent than gram positives, researchers and difference scientists should wonder on causes. A part from deep analysis and identification of predominant bacteria, Escherichia Coli, was found to be highest prevalent compared to others. This bacterium lives in environment, and in lower intestines of warm blooded animals. Actually this bacterium leaves move from the host' s fecal flora to the urogenital tract and cause UTI. This is a justification of the the high presence of E. Coli in feecal and urine samples according to the results of this studies. It can be a cause of other infections but mostly known to cause UTI. The second bacteria dominated is Staphylococcus aureus. These bacteria are known to be among animals/human microbiota and mostly reside on skin and upper respiratory tract. The results of this study shows that Staphylococcus aureus was mostly found in pus samples and swab samples. The reason of positive test in these sample should be understood, Staphylococcus aureus as microbiota, is known to be opportunistic pathogen in case of immune suppression. Thus serious of follow up should be done during patients staying in veterinary clinics with the risk of being infected with Staphylococcus aureus. Staphylococcus aureus is known to cause nosocomial infection and the cause was explained above. Taking care of patients with much hygiene, and avoiding simple contacts of them will reduce the spread of these kind of infection in veterinary clinic.

4.1 Limitation

- The study was conducted at Jimma University Veterinary Clinics to identify common bacteria in Veterinary clinics; however, a little sample has taken due to limitation of budget to work with all Veterinary clinics in and around Jimma area.
- The second limitation is that only Jimma University Veterinary clinics has clinical microbiology laboratory with techniques of bacterial identification like bacterial culture and others in and around Jimma area Veterinary Clinics.

5. Conclusion And Recommendations

The patients comes to Veterinary clinics are more susceptible to bacterial infections. They are frequently resistant to various used antibiotics; the predominant infecting bacteria in Veterinary clinics were staphylococcus aureus and Streptococcus which are gram positive bacteria, Klebsiella and Escherichia coli which is gram negative bacteria and showed a high resistance to number of antibiotics used during this study. The high increase in resistance of microbes to previous and recent antibiotics is a factor for health facilities to control the spread of infections in terms of improving treatments system as well as providing hygiene to health settings environments and infrastructures.

Based on the above conclusion the following recommendations are forwarded:

- The ministry of Livestock and Fisheries in collaboration with researchers should plan researches related to antibiotic susceptibility to know if used antibiotics are effective to treat infections in veterinary Clinics.

- Animal health education should be provided to the public in terms of using given antibiotics for treatment in a correct way.
- Farms and Veterinary clinics should improve hygiene and use effective disinfectants and antibiotics.

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