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Isolation of Streptococcus and Antimicrobial Susceptibility Test From Clinical Equipment and Clinical Case Animals at Veterinary Clinic in Bishoftu Town, Ethiopia

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Abstract

Cross-contamination of pathogenic Streptococcus among infected host to their surrounding environment such as areas in which it's handled for diagnosis and treatment Is a serious concern in the field of veterinary and human medicine. The aim of this study was to isolate multi-antibiotics-resistant streptococcus from animals and clinical equipment in the veterinary teaching hospital, CVMA in Bishoftu town from April 2024 to June 2024. A total of 60 samples were collected, from which 33 were collected purposively from clinical equipment and 27 samples were collected randomly from clinical case animals. Isolation of streptococci was conducted by using morphological and colony characterizing on nutrient agar, Edward media, Gram staining, hemolytic properties on Blood agar, Biochemical tests, and also antimicrobial tests against twenty isolates. In the present study, the prevalence of Multi-drug resistant streptococci was 33.3% with no statistical difference in the prevalence of streptococci between case animal and clinical equipment (P-value > 0.05). An antimicrobial test of ten antibiotic disks against 20 isolates indicated 100% susceptibility to Gentamycin and Streptomycin. However, they were 100% resistant to penicillin, ceftazidime and Methicillin. Therefore, clinical staff should follow strict safety and hygienic practices, and antimicrobial susceptibility tests of antibiotics should be done before administering antibiotics for wound and upper respiratory infections.

Keywords: streptococcus, Isolate, antimicrobial susceptibility test, clinical equipment.

Introduction

Bacterial contamination of hospital equipment is one of the most probable causes of nosocomial infections. These infections are developed within a hospital or other type of clinical care facility and are acquired by patients while they are using the same facility **[18]**. An opportunistic and pathogenic Streptococcus Is one of the bacterial infections accompanying respiratory infections and suppurative infectious diseases of horses, cows, pigs, sheep, monkeys, guinea pigs, mink, and gerbils **[1,2]**. Streptococcus pneumoniae is usually inhibited commensals as it is reserved on the mucosal surface of the upper airways which enables it to transmit through nasal droplets and invade essentially sterile sites, such as the middle ear spaces, lungs, bloodstream, and meninges **[15]**. The fact that the available equipment is shared among cases greatly increases the risk of spread of nosocomial infections or hospital-acquired infections between health workers and patients in health facilities in low-resource settings. This occurs due to resource limitations manifested through practices such as reusing and sharing of single-use medical devices or consumables as well as poor implementation of risk management policies **[12]**. Transmission among infected and healthy humans or animals, colonization of infected animal tissue, and discursions depend on the remarkable ability of Streptococcus. pneumonia to evade or take advantage of the host's inflammatory and immune responses **[15]**.

Antimicrobial resistance of bacterial infections including StreptococcusIs, is currently an important concern of human and veterinary medicine that needs monitoring to obtain information about its resistance levels and effects of intervention measures. **[9]** The emergence of multi-drug resistant (MDR) strains in a hospital environment; particularly in developing countries, is an increasing problem that is an obstacle to the management of HCAIs in Ethiopia, studies reported a high prevalence of HCAIs mainly due to MDR pathogens including the country's largest tertiary referral Hospitals which warrants the critical need for a reassessment of the role played by inanimate environment in the transmission of nosocomial infections **[14]**. On the other hand, antimicrobial therapy is the common anti-infection treatment. However, with the changes in S. pneumoniae serotype and antibiotic resistance over time, the current treatment options are constantly being adjusted as well **[19]**. Emerging antimicrobial resistance in clinical, community and veterinary environments has become a threat to public health worldwide. **[10]**

To identify the appropriate measures in case of a high-case frequency at a farm, it is important to understand whether this pathogen is transmitted between animals or from the environment to the animals. (16). In Ethiopia there is a scarcity of equipment for the diagnosis and treatments of animals attending the clinic for the different cases including surgical treatments and postmortem diagnosis and also there is no frequent cleaning and disinfecting of equipment. Due to this the animals were treated and diagnosed with the same equipment without frequent cleaning and disinfecting of clinical

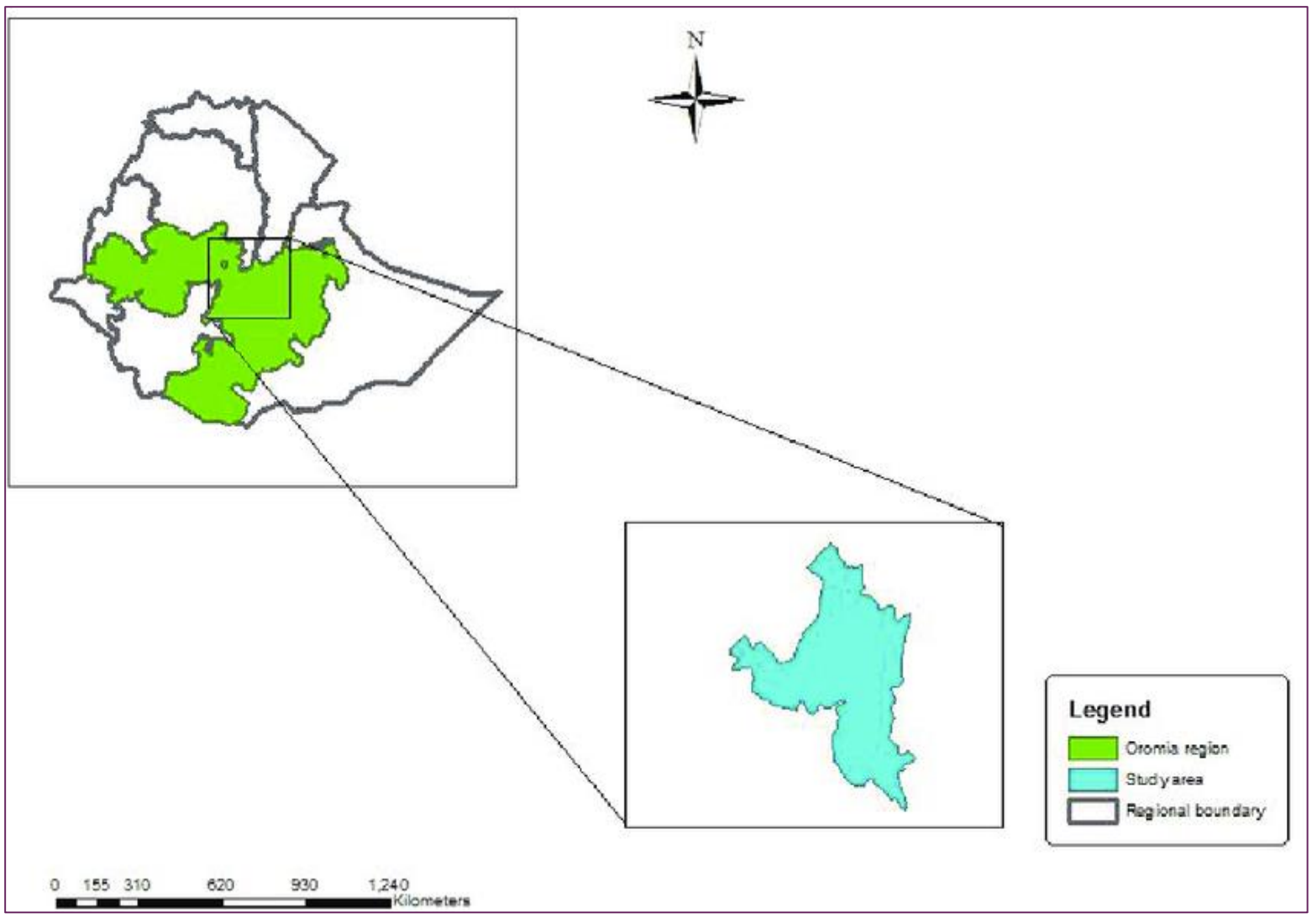
equipment. **Therefore;** the main objective of this research is to isolate multi-antibiotics-resistant streptococcus infection of animals and the role of clinical equipment for the transmission of streptococcus bacterial infection.

Material And Methods

Study Area

The study was conducted from March 2024 to May 2024 on streptococcus isolation in clinical equipment and clinical case animals attending VTH of CVMA in Bishoftu town. the town is located in Oromia regional State laying at a distance of 47.9 kilometers southeast of Addis Ababa (capital city of Ethiopia). Geographically stretched between 8 degrees 43' north -8 degrees 48' north latitude and 38 degrees 00' east - 38 degrees 48' east longitude. according to spatial analysis in this study, the town currently covers a total area of about 14,878 hectares which was 4,520 hectares in the year 2005 **[3]**. Moreover, the town is suited in a tepid to cool sub-moist mid-highland at an average altitude of about 1920 meters above mean sea level with moderate weather conditions.

Figure 1: Geographical Location of Bishoftu



Source

Study Animal and Sample Sources

This study was conducted on the isolation of streptococcus and antimicrobial susceptibility tests on the clinical equipment and clinical case animals attending at veterinary teaching hospital (VTH) at the College of Veterinary Medicine and Agriculture from March 2024 to May 2024. Accordingly, nasal swabs, surface swabs, and wound samples were collected.

Sample Collection and Transportation

A total of 60 samples were collected from the veterinary teaching hospital (VTH) at the College of Veterinary Medicine and Agriculture from which 33 were collected purposively from clinical equipment and 27 samples were collected randomly from clinical case animals. Aseptically, the samples of surface, nasal swab, and wound were collected by using a sterilized cotton swab through rubbing and directly inserted into a 10 ml containing test tube filled with 9 ml of tryptone soya broth as a transport media. During the study period, each sample was labeled with permanent markers handled in the ice box, and transported to the Veterinary Public Health (VPH) Laboratory, CVMA as soon as possible. Then, after arrival at the laboratory, the samples were directly incubated at 37℃ for 24 hours for bacterial enrichment.

Sample Processing and Analysis

Culturing and Colony characterization on Nutrient Agar and Edward medium.

For the purpose of streptococcus isolation, after 24 hours of enrichment, a loopful of the sample taken from tryptone soya broth was inoculated on the nutrient agar to get the bacteria by quadrant Streaking method in order to obtain pure colonies, then incubated at 37ºC for 24 hours. After 24 hrs of incubation, the samples were observed for colony growth and colony morphological characterization. Finally, small, round, smooth, and opaque (cloudy) colonies were observed. According to microbiological procedures, made subculture on a selective media which was called Edward medium in order to get a pure streptococcus colony. The medium is enriched by the addition of 6 % Sheep Blood and it was a modified medium supplemented with collision sulfate and oxolinic acid allowing the growth of all streptococci organisms while inhibiting the growth of the staphylococci and gram-negative strains. The grown bacteria appeared as small, white, and round colonies.

Gram's Staining and Microscopic Examination

According to **[9]**, the clean glass slide was marked with a sample identification number by using a permanent marker, now by using the sterile wire loop, the colony was transferred onto the slide and emulsified in distilled water. Then the emulsified colony was spread over the slide and allowed to dry by passing on the flame of the Bunsen burner three times. the prepared

smear was put on a staining rack over the sink and Primary stain (Crystal violet) was added to the smear and waited for 1 minute. After 1 minute each specimen slide was washed under gentle tap water and gram's Iodine was added and waited for 1 minute. After 1 minute again slide of the specimen was washed under gentle tap water and decolorized with 95% ethanol just for less than 10 seconds. Again, the sample slide was washed under gentle tap water and stained with Carbonyl Fuchsin for 1 minute. Finally, the sample slide was washed under gentle tap water and allowed to air dry, the back of the sample size was cleared with tissue paper, the drop of microscopic oil was dropped and examined under an Oil Immersion lens (100 X) of the compound microscope was conducted. **[9]**

Hemolysis Detection on Blood Agar

hemolysis is a peculiar process caused by hemolysin, a group of proteins produced by certain microorganisms, causing the lysis of the red blood cell membrane in the growth substrate **[13]**. Streptococcus species appeared in three main types of hemolysis patterns seen on blood agar plates. α-hemolytic reaction occurs when the hemoglobin in the red blood cells is reduced to methemoglobin, causing a greenish discoloration on the agar surrounding the colonies and resulting in a partial breakdown of red blood cells. β-hemolysis is characterized by complete lysis of red blood cells, resulting in a clear zone around the bacterial colonies and γ-hemolysis refers to the absence of hemolysis on blood agar plates **[12]**.

Biochemical Tests (Oxidation and Catalase Tests)

Streptococcus species are generally considered to be oxidase-negative, meaning they do not produce the oxidase enzyme. A small amount of the bacterial culture is transferred to a filter paper disk impregnated with an oxidase reagent (tetra methyl-p-phenylenediamine dihydrochloride). When the bacteria produce cytochrome c oxidase, the oxidase reagent turns purple within a few seconds. If there was no color change, the bacteria were considered oxidase-negative. Streptococcus species are generally catalase-negative, meaning they do not produce the catalase enzyme.3% H_2O_2 was added to a bacterial colony on a slide. If the bacteria produced catalase, bubbles of oxygen gas were observed as the hydrogen peroxide was broken down. This was indicated as a positive catalase test result. If no bubbles were observed, the bacteria were considered as catalase-negative.

Antimicrobial Susceptibility Testing

The isolates' antimicrobial susceptibility tests were carried out using the Kirby Bauer disk diffusion method following the Clinical and Laboratory Standards Institute of the United States of America (CLSI, 2019) and the Kirby Bauer Disk Diffusion Susceptibility Test Protocol on Muller Hinton agar medium. A sterile loop was used to transfer a loop full of well-grown colonies on nutrient agar from each confirmed isolate into sterile tubes containing 5 ml of normal saline solution. The inoculated colonies were mixed with saline solution by vortex and smooth suspension was formed. Saline solution was added to the suspension until the turbidity met with the 0.5 McFarland turbidity standards. The bacteria were spread uniformly over the entire surface of the Muller Hilton Agar plate using a sterile cotton swab dipped in the suspension and allowed to dry the plates by keeping them at room temperature for 3 minutes in a biosafety cabinet (CLSI, 2019). Ten antimicrobial disks with the known concentration of antimicrobial were placed on the Muller Hinton Agar plate and the plates were incubated for 24 hours at 37 °C. Using a caliper, the diameters of the clear zones of inhibition produced by diffused antimicrobial on lawn inoculated bacterial colonies were measured to the nearest mm. According to the published interpretive chart, all ten zones of inhibition against ten antimicrobial agents for each isolate were recorded, compared with standards, and finally classified as resistant, intermediate, or sensitive (CLSI, 2019)

Data Management and Statistical Analysis

The collected data were entered into MS Excel (MS Excel, 2019) and analyzed using statistical software. Before being subjected to statistical analysis, the data was screened for errors and properly coded. P-values were employed to assess the presence of association. In all cases, $p < 0.05$ was considered for statistically significant difference whereas p-value >0.05 was considered non-significant.

Results

Isolation of Streptococcus

Out of sixty (60) collected samples which were from animals and clinical equipment used for the treatment of animals, twenty 20/80) of the samples were positive for streptococcus. The bacteria were isolated from animal cases and clinical equipment with a prevalence of 8/27 (29.6%) and 12/33 (36.3%) respectively. **(Table 1)**. Statistically, there was no difference in the prevalence of streptococci between animal cases and clinical equipment (P-value > 0.05) **(Table 1)**.

Table 1: Distribution of Streptococcus Among clinical Materials and Clinical Case Animals

Sample type	No. Observation	No. Positive	Prevalence (%)	X^2	P-Value
Animal case	27	8	29.6	0.8	0.4092
Clinical Equipment	33	12	36.3		
Total	60	20	33.3		

From animal case samples, Streptococcus was isolated from nasal swabs and wounds. Accordingly, the prevalence of streptococcus in nasal swabs was 3/13 (23.1%), while that in wound infection was 5/14 (35.7%) **(Table 2)**. This result indicated that there was no statistical difference in the abundance of streptococcus among the wound and nasal swabs (P-value = 0.1) **(Table 2)**.

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Table 2: Distribution of Streptococcus Among Different Samples Collected From Animals

Sample Source	No of observation	No of positive	Prevalence (%)	X^2	P- value
Nasal Swab	13	3	23.10%	2.7	0.1003
Wound	14	5	35.70%		
Total	27	8	29.60%		

Among the twelve (12) isolates of streptococcus isolated from clinical equipment, 8/23 (34.8%) were isolated from Clinical Environment, while 4/10 (40%) were isolated from the surgery room **(Table 3)**. The frequency of streptococcus isolation was not statistically different between the clinic environment and the Surgery room (P-value > 0.05) **(Table 3)**.

Table 3: Prevalence of Streptococcus in Clinic

Sample Source	No of Observation	No of Positive	Prevalence (%)	X^2	P-value
Clinic Environment	23	8	34.8	0.3	0.5
Surgery room	10	4	40		
total	33	12	36.3		

Antimicrobial Susceptibility Test for Streptococcus Isolates

The antibiotic susceptibility patterns of *Streptococcus* isolates are shown In **Table 4**. The isolates were resistant to the following antibiotics: Methicillin (Met_5), Penicillin (Pen_{10}) and ceftazidime (CAZ_{30}) were 100% resistant to Streptococcus, Oxaciline (OxC_1) (95%), cotrimoxazole (Cot_{25}) (90%), Ampicillin (Amp_{10}) (70%), Vancomycin (Van_{30}) (15%) Oxytetracycline (OT_{30}) (5%) and gentamycin (Gen10) and streptomycin (S_{25}) were (0%) resistance.

Table 4: Susceptibility Pattern of All Isolates from Different Samples to Each Antibiotic Disk Used

Sample Code	Antimicrobials										Frequency of Antibiotics Resisted (%)
	COT_{25}	Van_{30}	Met_5	Gen_{10}	Pen_{10}	Oxc_1	Amp_{10}	CAZ_{30}	S_{25}	OT_{30}	
01	R	S	R	S	R	R	R	R	S	S	6(60%)
02	R	S	R	S	R	R	R	R	S	S	6(60%)
O6	R	S	R	S	R	R	R	R	S	S	6 (60%)
10	R	S	R	S	R	R	R	R	S	S	6(60%)
11	R	S	R	S	R	R	R	R	S	S	6 (60%)
18	R	S	R	S	R	R	I	R	I	S	5 (50%)
19	R	S	R	S	R	R	R	R	S	S	6 (60%)
23	R	S	R	S	R	R	S	R	S	S	5 (50%)
24	R	R	R	S	R	R	R	R	S	S	7 (70%)
25	S	S	R	S	R	R	I	R	S	S	4 (40%)
27	R	S	R	S	R	R	I	R	S	S	5 (50%)
30	I	R	R	S	R	R	R	R	S	S	6 (60%)
32	R	S	R	S	R	R	I	R	S	S	5(50%)
33	R	S	R	S	R	R	R	R	S	I	6 (60%)
34	R	R	R	S	R	R	R	R	I	R	8 (80%)
35	R	S	R	S	R	R	R	R	S	S	6 (60%)
37	R	S	R	S	R	R	I	R	S	S	5 (50%)

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45	R	S	R	S	R	R	R	R	I	S	6(60%)
56	R	S	R	S	R	I	R	R	S	S	5 (50%)
58	R	S	R	S	R	R	R	R	I	S	6 (60%)
Resistant sample (%)	90%	15%	100%	0%	100%	95%	70%	100%	0%	5%	

COT= Cotrimoxazole, Van= Vancomycin, Met= Methicillin, Gen= Gentamycin, Pen10= Penicillin, Oxc = Oxacillin, Amp= Ampicillin, CAZ= ceftazidime, S= streptomycin and OT= Oxytetracycline

Multidrug Resistance of Streptococcus Isolates

The multi-drug-resistant features of streptococcus isolates are shown in Table 5. Out of all 20 Streptococcus isolates tested for Antibiotic susceptibility, all isolates were resistant to three (3) or more antimicrobials. Multi-drug-resistant streptococcus isolates showed 7 different resistant patterns for ten Antibiotic agents tested. Out of 20 multidrug-resistant isolates, 1 (5%) Isolates exhibited resistance to four Antibiotics, 6 (30%) Isolates were resistant to five antibiotics, 11 (55%) of Isolates were resistant to six antibiotics, 1 (5%) of Isolates were resistant to seven antibiotics and 1 (5%) of Isolate were resistant to eight antibiotics **(Table 5)**.

Table 5: Multi-Drug Resistance Pattern of Isolated Streptococcus

No. of Antibiotic	**Resistant Pattern and Number of Isolate**	**Total Numbers of Resistant Isolates (%)**
4	Met_5, Pen_{10}, OXc_1, CAZ_{30} (1)	1 (5%)
5	Cot_{25}, Met_5, Pen_5, OXc_1, CAZ_{30} (5)	6 (30%)
	Cot_{25}, Met_5, Pen_{10}, Amp_{10}, CEFT (1)	
6	Cot_{25}, Met_5, Pen_{10}, OXc_1, Amp_{10}, CAZ_{30} (10)	11(55%)
	Van_{30}, Met_5, Pen_{10}, OXc_1, Amp_{10}, CAZ_{30} (1)	
7	Cot_{25}, Van_{30}, Met_5, Pen_{10}, OXc_1, Amp_{10}, CAZ_{30}(1)	1 (5%)
8	Cot_{25}, Van_{30}, Met_5, Pen_{10}, OXc_1, Amp_{10}, CAZ_{30}, OT_{30} (1)	1 (5%)

COT= Cotrimoxazole, Van= Vancomycin, Met= Methicillin, Gen= Gentamycin, Pen10= Penicillin, Oxc = Oxacillin, Amp= Ampicillin, CAZ= ceftazidime, S= streptomycin and OT= Oxytetracycline

Discussion, Conclusion, And Recommendation

Bacterial cross-contamination plays an important role in healthcare-associated infections (HCAIs) and resistant strain dissemination. The majority of HCAIs are believed to be 68 transmitted directly from patient to patient, but increasing evidence demonstrates that also the medical personnel, as well as the clinical environment (i.e., surfaces and equipment), often are a source of infections. Hospital design and hygienic practices have been largely directed at controlling nosocomial pathogens and resistant strains contaminating air, hands, equipment, and surfaces. A better understanding of how bacterial cross-contamination occurs can provide the basis for the development of evidence-based preventive measures **[14]**. With high numbers of admissions and long patient delays, the risk of transferring infections across patients and health workers is expected to be high. The burden of nosocomial infections is estimated to be up to twenty times higher in low-middle-income countries (LMICs) than the high-income countries **[13]**.

The current study indicated a 33% prevalence of Streptococci isolated from clinical case animals and clinical materials used in the diagnosis and treatment of animals attending the VTH of the College of Veterinary Medicine and Agriculture Addis Ababa University. However, there was no general prevalence of bacteria, the prevalence of streptococci in the current study was agreed with the overall 32.9% contamination occurrence in environment and equipment reported by **[4]** The incidence of Streptococci occurrence obtained in this study was higher than that reported at 16% frequency of Streptococci by**[8]** from pregnant women as he indicated in his A systemic review and meta-analysis study. We also noted high variability in the choice of disinfectants for similar equipment even in the same health facility. This was reportedly due to variations in procurement options although it was not clear why these procurement decisions were made. There was generally a lack of uniform infection control protocols across departments and health facilities sampled which could also contribute to the high bacterial colonization rates. Previous studies have reported that decontamination of medical equipment with 70% alcohol can reduce the infection rates on medical equipment surfaces by more than 80% **[13]** The bacteria had a lower frequency of streptococcus isolation from animal cases (29.6%) than clinical equipment (36.3%), despite there being no statistical difference (P-value > 0.05) **(Table 1)**. The lower contamination frequency of streptococcus in animals amazingly indicates the assumption environmental surface of crash, floor, table, and other surgical equipment would be suitable for bacterial colonization and transmission unless it was frequently disinfected at the end of each examination and treatment of the cases **[4]**.

The antibiotic susceptibility patterns of streptococcus isolates are shown In **Table 4**. The isolates were resistant to the following antibiotics: Methicillin, Penicillin, and ceftazidime were 100% resistant to Streptococcus, Oxaciline (95%); Cotrimoxazole (90%), Ampicillin (70%), Vancomycin (15%); Oxytetracycline (5%); and Gentamycin and streptomycin were 0% resistant. However, the resistance to vancomycin varied from that reported by **[13]**, and the resistance to penicillin was agreed upon by him. Also, the resistance to penicillin varied from the report of Lemma *et al.*,**[8]**. Additionally, the resistance and susceptibility of the left antimicrobials varied in different studies. This drug resistance was the result of using drugs in common and the adaptability of antibiotics by streptococci Isolates. The antimicrobial resistance pattern and the occurrence of multi-drug resistant streptococci may be due to unsuitable use of antibiotics and unrecommended doses of antibiotics In the treatment of infected animals and humans **[10]**.

In conclusion, bacteria of the Streptococcus genus inhabit the skin, throat, and upper respiratory tract. Streptococcus Infections In animals can be a significant concern due to the potential for transmission to humans and other animals. The contamination of equipment can also contribute to the spread of these infections.

In the present study, most streptococcal Isolates were susceptible to gentamycin and streptomycin. Penicillin has been used as a drug of choice for treating streptococcal infection in veterinary teaching hospitals, CVMA. However, antimicrobial resistance including multidrug resistance was observed in several commonly used antibiotics Including penicillin, ceftazidime, Methicillin (100%) and Oxaciline (95%), cotrimoxazole (90%), Ampicillin (70%), Vancomycin (15%), and Oxytetracycline (5%) were resistance. Hence, it is important to periodically monitor the antibiotic resistance patterns to choose empirical treatments for better management of streptococcus Infection. Based on this study: Implementing strict biosecurity measures, such as proper sanitation of equipment and facilities, Regularly monitoring animals for signs of Infection and seeking veterinary care promptly If any Issues arise, and Educating staff and handlers on proper hygiene practices to minimize the risk of transmission, regularly disinfecting equipment and high-touch surfaces to reduce the risk of contamination and developing an action plan for the promotion of rational use of antimicrobials should recommended to all animal health hospitals.

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