بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Antimicrobial Resistance in Dairy Farms in Khartoum State, Sudan

مقاومة المضادات الميكروبية فى مزارع الألبان بولاية الخرطوم -السودان

A Thesis Submitted in Fulfillment of the Partial Requirements of Master Degree in Preventive Medicine

By

Sabah Ebead Mohammed Omer

B.V.M. College of Veterinary Medicine, Sudan University of Science and Technology (2009)

Supervisor: Dr. Sumaia Awad Elkariem Ali Mohammed

November, 2021

Dedication

To my mother

My sister

My colleagues

Sabah

Acknowledgement

Primarily, I would like to thank God Almighty for giving me the ability and strength to complete this project successfully.

I am grateful to my supervisor, Dr. Sumaia Awad Elkariem Ali, for supervising this research, whose suggestions and instructions were the main contributor to the success and completion of this project.

I am also grateful to Prof. Mohahamed Tagaldeen for helpful recommendations and data analysis, as well as Dr. Naglaa Abdoelhakeem Abbas, for her informative remarks and contributions to this project.

thanks to Sudan University of Science and Technology's College of Veterinary Medicine's Department of Microbiology, for hosting this study and supplying all of the essential necessities for sample culture and preservation. thanks especially to technician Najwa Abdullah for her continuous help.

Many thanks to the head of the Department of Bacteriology at the Central Veterinary Research Laboratory (CVRL), Soba for providing help laboratory facilities for its execution. In addition, I want to express my gratitude to the entire laboratory staff, particularly Dr. Huda Osman Ali and Dr. Mohammed Ahmed Abdel Moneim, for their great support and assistance.

I would like to express my appreciation to my colleagues Dr. Ryan Abdelazim and Dr. Insaf Al-Tom, who assisted me much in collecting samples and completing this work.

I also want to express my gratitude to all of the farmers and owners who allowed me to take samples and provided me with the essential

information. I would really like to thank Babiker Shulluk, the car driver who assisted me in sample collection. Thanks and appreciation to the Ministry of Higher Education for sponsoring this project.

Last but not least, I would like to extend my gratitude to everyone who assisted and helped me in completing this search.

Table of content

List of Tables

List of Figures

List of Abbreviations

AMR	Antimicrobial Resistance
ARGs	Antibiotic resistant genes
NAPs	National Action Plans
WHO	World Health Organization
FAO	Food and Agricultural Organization
ARBs	Antibiotic resistant bacteria
OIE	World Organization for Animal Health
CVRL	Central Veterinary Research Laboratory
OF	Oxidation Fermentation
CIP	Ciprofloxacin
AK	Amikacin
EX	Enrofloxacin
AMX	Amoxycillin
VA	Vancomycin
P	Pencillin
B	Bacitracin
CAZ	Ceftazidime
CL	Colistin
TE	Tetracycline
CN	Gentamicin
IMI	Imipenem
SPSS	Statistical Package for Social Science, version
Hrs.	Hours

Abstract

Antibacterial resistance (AMR) is recognized as a One Health Challenge. In the present study the prevalence of isolated and resistant bacteria to detect antibacterial in dairy farms in Khartoum State was investigated.

A cross sectional study was conducted in dairy farms in Khartoum State between March and November 2019. Samples from workers' hands, workers'shoes, animal milk and feces were collected from 160 dairy farms in seven sub- localities namely: Jabalawlia, Bahri, Umbada, Karari, Khartoum, Umdurman, Sharg-Alnile in Khartoum State. The collected samples were cultured, purified and identified using standard bacteriological methods including primary and secondary biochemical tests. Antibiotic sensitivity test was done for the bacterial isolates using disc diffusion method.

Of 160 samples, 172 isolates that belong to 9 genera were identified. Of these, the most prevalent genus was *Staphylococcus* (n = 92; 53.5%), particularly in the hands (n = 28; 30.4%) and shoes (n = 27; 29.3 %). Followed by Enterobacteria (n = 32; 18.6%) particularly in the feces (n = 12; 37.5) and shoes (n = 11; 34.4%). However, *Staphylococcus* isolates were the most prevalent organism in milk samples (51.3%) compared to other isolates.

In sensitivity test, Gentamicin, Tetracycline and Ciprofloxacin were highly effective drugs for most of Gram positive isolates (n = 123; 100%), followed by Bacitracin (n = 116; 94.3%). However, the majority of the isolates showed resistance to Vancomycin (n = 76; 61.8%).

In Gram negative bacteria Enrofloxacin, Amoxicillin, Amikacin and Ciprofloxacin were highly effective drug for all isolates (n = 49; 100%), followed by Colistin, Tetracycline and Gentamicin (n = 44; 89.8%). However, the majority of the isolates showed resistance to Ceftazidime (n = 20; 40.8%).

Moreover, *Corynebacterium* bacteria displayed multi-drug resistance (100%) for 3 drugs (Vancomycin, Penicillin, and Bacitracin) Bacillus spp showed resistance (100%) for 2 drugs (Amoxicillin and Vancomycin) and Micrococcus spp resistant (85.7%) for vancomycin and (71.4%) for Pencillin.

In Gram negative bacteria *Acintobacter* showed resistance (100%) to 2 drugs namely Ceftazidime and Imipenem.

In conclusion, the most prevalent bacteria in dairy farms in Khartoum State was *Staphylococcus* especially in milk samples. Gentamicin, Tetracycline and Ciprofloxacin were highly potent antibiotic for most Gram positive isolates. While Enrofloxacin, Amoxicillin, Amikacin and Ciprofloxacin were highly effective antibiotics for Gram negative isolates. Resistance to Vancomycin and Ceftazidime was increased. The multi-drug resistance was identified for various antibiotics especially in Gram positive bacteria such as Vancomycin, Bacitracin, Penicillin and Amoxicillin, while in Gram negative bacteria the multi-drug resistance was observed with Ceftazidime and Imipenem.

Arabic abstract

المستلخص

تعتبر مقاومة مضادات الميكروبات (AMR) أحد تحديات الصحة الواحدة . في هذه الدراسة تم التقصى عن إنتشار البكتيريا والمقاومة لمضادات الميكروبات في مزارع الألبان بولاية الخرطوم.

أجريت دراسة مقطعية في مزارع الألبان في ولاية الخرطوم في الفترة بين مارس ونوفمبر 2019. العينات كانت مسحة يدوية للعمال، أحذية العمال، اللبن وروث الابقار جمعت من 160 مزرعة ألبان في سبع محليات تعرف ب:جبل أولياء، بحري، أمبده، كرري، الخرطوم، أم درمان، شرق النيل بولاية الخرطوم .تم زراعة العينات التي تم جمعها وتنقيتها وتحديدها باستخدام الطرق البكتريولوجية القياسية بما في ذلك الإختبارات البيوكيميائية الأولية والثانوية . .كما تم إجراء إختبار حساسية المضادات الحيوية للمعزولات البكتيرية باستخدام طريقة الإنتشار القرصي.

من 160 عينة تم التعرف علي172 معزولة تنتمي الي 9 نوعا، من بين هذه الأنواع كانت البكتيريا الأكثر إنتشارًا هي *Staphylococcus* (العدد=92؛ 53.5%) خصوصاً في عينة اليد (العدد =28؛ 30.4%) وأحذية العمال (العدد=27؛ 29.3 ٪)، تليها *Enterobacteria* (العدد =32؛ 18.6 ٪) خاصة في الروث (العدد =12 ؛ 37.5 ٪) وروث أحذية العمال (العدد 11 = ؛ 34.4٪). علي كل حال *Staphylococcus* كانت البكتريا الأكثر إنتشاراً في عينات اللبن (51.3%) مقارنة ببقية العزلات.

في اختبار الحساسية، كان Gentamicin و Tetracycline و Ciprofloxacin من الأدوية عالية الفعالية لمعظم المعزولات موجبة الجرام (العدد =123 ؛100%) ، يليهم Bacitracin(العدد =116 ؛ 94.3 ٪). ومع ذلك، أظهرت غالبية المعزولات مقاومة ل Vancomycin (العدد =76 ؛61.7%).

في البكتيريا سالبة الجرام كان كل من Enrofloxacin وAmoxicillin وAmikacin و Ciprofloxacin من الأدوية عالية الفعالية لجميع المعزولات (العدد =49؛ 100%) يليهما Gentamicin و Tetracycline وColistin (العدد=44 ؛ % 89.8). ومع ذلك ، أظهرت غالبية المعزولات مقاومة ل Ceftazidime (العدد 20= ؛40.8 %).

بالإضافة إلي ذلك، تم التعرف على مقاومة الأدوية المتعددة، حيث أظهرت Corynebacterium مقاومة دوائية متعددة (100%) لثلاثة عقاقير (Vancomycin و Penicillin و Bacitracin) وال Bacillus أظهرت مقاومة دوائية (100%) لعقارين (Amoxicillin و Vancomycin) و *Micrococcus* أظهرت مقاومة دوائية (85.7%) لل Vancomycin ومقاومة بنسبة (71.4%) لل Penicillin.

في البكتريا السالبة لصبغة جرام أظهرت *Aciintobacter* مقاومة دوائية (100%) لعقارين (Ceftazidime و Imipenem).

في الخلاصة وجد أن أكثر البكتريا إنتشارا في مزارع الألبان في ولاية الخرطوم كانت *Staphylococcus*خاصة في عينات اللبن. كما أن Gentamicinو Tetracyclineو Ciprofloxacinأكثر المضادات الحيوية فعالية في أغلبية البكتريات موجبة لصبغة جرام .بينما Enrofloxacinو Ciprofloxacinو Amoxicillinو Amikacinأكثر المضادات الحيوية فعالية في أغلبية البكتريا سالبة صبغة جرام .المقاومة لل Vancomycinو Ceftazidimeكانت عالية .

بالإضافة الي ذلك تم التعرف علي مقاومة دوائية متعددة لعدد من المضادات الحيوية للبكتريا موجبة الجرام مثل Vancomycin، Bacitracin، Penicillin و Amoxicillin، بينما في البكتيريا سالبة الجرام لوحظت مقاومة الأدوية المتعددة في ال Ceftazidime و Imipenem.

Introduction

The goal of the One Health strategy is to achieve optimum health for humans, animals and the environment (King *et al*., 2008). Due to the rapid development and spread of resistant bacteria and genes among humans, animals and the environment on a worldwide scale, antimicrobial resistance (AMR) is recognized as a One Health challenge (Rousham *et al*., 2018). AMR is a resistance to the effects of drugs used effectively to treat specific microbial infections (Singer *et al*., 2016). It was originally considered to be of clinical concern, but it has been posed as a global challenge by recent findings of its distribution through environmental routes (Sharma *et al*., 2019).

According to one estimate, the number of deaths due to antimicrobial resistance may exceed 10 million annually by 2050 if adequate action is not taken (Waseem *et al*., 2017). The environment is increasingly documented for the participation of antimicrobial resistance (AMR) as a worldwide burden (Rehman *et al*., 2020). The presence of antimicrobial resistant bacteria and related resistant genes (ARGs) in the environment is now well known for its role in the spread of resistance to antimicrobials (Singer *et al*., 2016).

Abuse of antibiotics in humans and livestock and their inappropriate disposal, inadequate hygiene and sanitation, and ineffective infection prevention and control in healthcare settings are the most significant factors contributing in the ever increasing threat of antimicrobial resistance (Boonyasiri *et al*., 2014; Sharma *et al*., 2019 and Waseem *et al*., 2019).

Due to the growing contamination of environmental matrices with pharmaceuticals, evaluation of sewage sludge from the perspective of ARGs

spread in the environment and associated health threats is a major focus of many studies (Waseem *et al*., 2017).

However, an environmental focus is not important only for a water and environmental protection perspective but for avoiding the spread of antimicrobial resistance (Waseem *et al*., 2017).

Global and National Action Plans (NAPs) to challenge antimicrobial resistance (AMR) have been initiated and coordinated through the tripartite alliance of the World Health Organization (WHO), the Food and Agricultural Organization (FAO) and the World Organization for Animal Health (OIE) (Rousham *et al.*, 2018).

Therefore, there is a need for studies to investigate the prevalence of resistant bacteria to antimicrobials in the animals and environment and connections between isolated bacteria in animals and in the surrounding environment.

Objectives

This study aimed to:

- Determine the prevalence of isolated bacteria in the dairy farms.
- Determine the prevalence of antibiotic-resistant bacteria in samples from animal milk, feces, workers' hands and workers 'shoes in dairy farms in Khartoum State.

CHAPTER ONE

1.1 Literature review

1.1 Definition

Antibacterial agents are substances that can destroy (bactericidal action) or inhibit microorganism growth (bacteriostatic action). They may be of natural origin, synthetically or semi-synthetically derived (Kohanski *et al*., 2010). The term "antibiotic" refers to substances that are produced naturally by certain fungi and bacteria and are synthetically formed (Smith *et al*., 2015).

1.2 Classification of antibacterial agents

Many of the antibiotics are the essential metabolites of environmental bacteria and fungi (Kieser *et al*., 2000). The most important groups of antibiotics is classified according to their spectrum, mode of action, route of administration and molecular and chemical structure. Common classes of antibiotics that are based on their molecular and chemical structures are macrolides, quinolones, tetracyclines, aminoglycosides, sulfonamides, oxazolidinones, glycopeptides, and betalactam (Sabundayo and Caldero´n, (2007); Adzitey, (2015); Frank and Tacconelli, (2012); Van Hoek *et al*., (2011) and Ashfaq *et al*., (2020). Carbapenems are also beta-lactam ring containing antibiotics and are very effective against betalactamases. Due to a broader range of activity they are very active against all of gram-negative and positive bacteria, examples of them include meropenem, imipenem, ertepam, and others (Greenwood, 2007 and Walsh, 2003).

1.3 Administration of antibacterial drugs

Antimicrobials are administered by two main routes that include oral route (drenching method, Stomach tube method) and parental route (intramuscular

injection, subcutaneous injection, intravenous injection and intradermal injection. Other route includes intraperitoneal, intra pleural and intra cerebral routes (Brander *et al*., 1991).

1.4 Usage of antimicrobials in animals

Antimicrobials are used in livestock for a variety of reasons, including disease treatment, prophylaxis and growth promotion. Since 2005, the use of antimicrobials for growth promotion, which often involves sub-therapeutic doses, has been prohibited in European Union countries (Wee *et al*., 2020).

Therapeutic usage of antimicrobials is intended to treat existing microbial diseases, commonly used for the treatment of individual animal. It involves testing of each infected animal, which involves laboratory examination, determining the microbes and antimicrobial sensitivity testing. Metaphylaxis usage involves previous treatment that can decrease the number of sick or dead animals for the whole animal population and decrease the antimicrobial dosage needed for the care of large numbers of the population who are symptomatically ill, thereby lowering treatment costs as well. Growth promotion usage also involves antimicrobial usage in food animals that given at small and sub therapeutic dosage in food to stimulate growth in animals (Schwarz *et al*., 2001 and Ibrahim *et al*., 2020).

1.5 Side effects of antibiotics

Antibiotics can cause several side or adverse effects in some people. Common side effects of antibiotics may include allergic reactions, diarrhea or constipation, nausea and fungal infections and may cause weakness in immunity by destruction of good and bad bacteria as well (Igbinosa and Odjadjare, 2015 and Balabanova, 2020).

1.6 Antibiotic resistance

Resistance against antibiotics is an urgent problem because antibiotics are a cornerstone of modern medicine and most medicinal procedures in human and animal health rely on functioning antibiotics (Munita and Arias, 2016). The overuse and misuse of antibiotics in human medicine and in animal agriculture, where the vast majority of antimicrobials are used, contribute to the evolution and spread of antibiotic-resistant pathogens. Antibiotic resistance will develop in five different mechanisms was identified and explained so far as; alterations of the target site of the antibiotic, enzymatic inactivation of antibiotics, reduction of the inner and outer membrane permeability, flush out of the drug and using an alternative metabolic pathway (Manage, 2018).

1.7 Transmission of antimicrobial resistance via livestock

The introduction of antibiotics into the environment causes a selective pressure which results in an increase in the proportion of bacteria that are resistant to antibiotics (Igbinosa and Odjadjare, 2015). Livestock have been considered as a reservoir for antimicrobial resistance (AMR) that can spread to humans. The transmission of AMR has been linked to close proximity and ecological interfaces involving cattle (Wee *et al.*, 2020). The transmission of AMR between humans and livestock can be thought of in two ways: clonal transfer of antibiotic resistance bacteria (ARBs) and horizontal transmission of antibiotic resistance genes ARGs (Chang *et al*., 2015). In addition, transmission can be either direct or indirect. Direct transmission occurs when humans and livestock come into close contact, while involves an intermediate between the two populations. Indirect transmission can either involve an environmental component such as the soil, animal manure,

sewage and surface water, or an intermediate vector such as wild animals, invertebrate vectors or food-borne infections (Wee *et al*., 2020).

1.8 Sources of resistance

1.8.1 Commensals

The development of metagenomics has shed light on the composition of the human gut flora. Numerous resistance genes were discovered in the unculturable fraction, the so-called microbial "dark matter," which comprises the majority of the gut flora, but they were not homologous with clinically relevant resistance genes. The culturable fraction, on the other hand, contained various homologies of pathogenic bacteria resistance genes. However, the pathogens direction of transfer from commensal gut flora to pathogens is unknown (Sommer *et al*., 2009 and Woolhouse *et al*., 2015).

1.8.2 Soil

Soil is also a major reservoir of AMR. Antibiotics resistance is likely to be as natural, widespread and ancient as antibiotic production. The link between resistance to naturally occurring antibiotics in the soil and resistance to antibiotics manufactured in the clinic, on the other hand, is uncertain. One recent metagenomics study, for example, discovered various examples of resistance genes in the soil that were 100 percent identical to those found in clinical isolates across all major antibiotic classes. That research shows that horizontal gene transfer occurs between soil bacteria and pathogens, but it doesn't reveal in which direction(s) it occurs. The discovery of resistance determinants for synthetic quinolones (*qnr* genes) in soil, for example, appears to suggest transfer from the clinic rather than the laboratory (Woolhouse *et al*., 2015)

1.8.3 Farm animals

Animal health and human health are closely linked because of the existence of a large number of zoonotic diseases and the impact of unhealthy livestock on human food (Orand, 2012). Antimicrobials are used on livestock farms for a number of reasons such as therapeutics; metaphylactics, meaning that the presence of clinical illness in one animal triggers drug treatment of the whole herd or flock; prophylactics; and growth promotion. In Europe, antimicrobial usage is particularly high in intensively farmed species such as pigs and poultry and less so in extensively farmed cattle and sheep (Woolhouse *et al*., 2015).

The OIE (World Organization for Animal Health) has compiled a list of antibiotics that are considered "critically important" for farm animals. This list covers antibiotics from all major classes used in human medicine (World Health Organization, 2007 and Woolhouse *et al*., 2015).

Antimicrobial drugs are categorized as critically important, highly important, and important based on two criteria. Antimicrobials are classified as critically important if they were used to treat diseases caused by organisms that could be transmitted through non-human sources or diseases caused by organisms that could acquire resistance genes from non-human sources using these two criteria: (i) sole treatments or one of few options to treat severe human disease, and (ii) used to treat diseases caused by organisms that could acquire resistance genes from non-human sources (World Health Organization, 2007).

1.9 Antibiotics resistance in Sudan

The sensitivity pattern of isolated bacteria from mastitis in Eldamazine locality, Blue Nile State, Sudan were investigated by Babiker *et al.* (2021). The study revealed that the most prevalent bacteria were *Staphylococcus* spp

(73.36%), *Streptococcus* spp (4.4%), *Bacillus* spp (8.9%) *Pseudomonas* spp, and *Escherichia* spp (8.9%). The most effective antibiotics in isolated bacteria were ciprofloxacin, gentamycin, tetracycline, vancomycin and ofloxacin. In addition, the isolated bacteria were resistant to ampicillin and erythromycin, while *Pseudomonas* spp were resistant to all antibiotics used.

The antimicrobial resistance pattern of bacteria isolated from clinical cases of bovine mastitis in Khartoum State, Sudan was evaluated. The majority of the isolates were highly sensitive to gentamycin, ciprofloxacin, norfloxacin and kanamycin, highly resistance to penicillin-G and moderately sensitive to novobiocin, tetracycline and cefalexin (Yasin *et al.*, 2016). Salih (2015) investigated the causative agent of bovine mastitis and the sensitivity of antibiotics to different bacteria in the treatment of bovine mastitis. The isolated genera were found to be 74% *Bacillus* spp., 24% *Staphylococcus* spp., 1% *Corynebacterium* spp. and 1% *Klebsiella* spp. Moreover, 100% of isolates were sensitive for chloramphenicol and ciprofloxacin, 91.6% for gentamycin and piperacillin/ tazobactam, 83.3% for pefloxacin and tetracycline, 75% for amikacin and ofloxacin, 66.6% for ceftizoxime, 33.3% for co-Trimoxazole and cefotaxime and 16.6% for ampicillin/ sulbactam.

CHAPTER TWO

2. Materials and Methods

2.1 Study area

The study was carried out in Khartoum State from March 2019 to November 2019. Khartoum State is the national capital and the largest city of Sudan, it's located at the confluence of the White Nile, the two Niles unite to form the river Nile. The State lies between longitudes 31.5-34E and the latitude 15 to16 N. The Northern region of the State is mostly desert and semi-desert in other regions, average rainfall reaches 100-200mm in north-eastern areas and 200-300mm in north-western. Temp in summer 25-40°C from April to June. 20-35 °C July to October, in winter 25-15°C November to March. Khartoum State include three major localities (Khartoum, Khartoum North and Omdurman).

2.2 Study population

The target population involved dairy farms in the three major localities in Khartoum State (Khartoum, Khartoum North and Omdurman).

2.3 Study design

A cross sectional study was conducted in dairy farms which covered the three major localities in Khartoum State (Khartoum, Khartoum North and Omdurman). In these localities the study covered seven sub-localities, Jabal-awliya, Bahri, Ombadda, Karari, Khartoum, Omdurman and Sharg Al-Nile. Four different types of samples were taken from the workers' hands and workers 'shoes, whereas milk and fecal samples were collected from cattle.

2.4 Sample size

About 160 samples were collected from 40 dairy farms in Khartoum State localities; 24 samples from each of Jabal-awliya, Bahri, Ombadda, Karari

and Khartoum localities and 20 samples from each of Omdurman and Sharg Al-Nile sub-localities. The collected samples were 40 samples from each of workers' hands, workers' shoes, animal milk and feces in the seven sub-localities in Khartoum State (Khartoum, Khartoum North and Omdurman). The percentages of samples taken from the different sub-localities are presented in Fig.1.

2.5 Collection and preservation of the samples

The samples were collected from workers' hands using sterile cotton swabs impregnated in normal saline before taking the sample. Samples from workers' shoes, animal milk and animal feces were collected in sterile plastic containers. Milk samples were collected after washing of animal udder gently with water and taking in sterile plastic containers directly from udder. Samples were then preserved in ice and transported to Bacteriology laboratory in the Department of Pathology, Parasitology and Bacteriology, College of Veterinary Medicine, Sudan University of Science and Technology and Department of Bacteriology, Central Veterinary Research Laboratory (CVRL), Soba.

2.6 Sterilization

2.6.1 Flaming

Sterilization of the metals and loops, which were used in the laboratory to transfer of bacterial colonies or spreading them on glass slides were sterilized by flaming (Barrow and Feltham, 2003).

2.6.2 Steam sterilization

Sterilization of culture media was achieved by autoclave. The temperature that applying for autoclave was 115-121°C for 15-20 minutes under pressure 10-15 pounds per square inch gauge pressure (Barrow and Feltham, 2003).

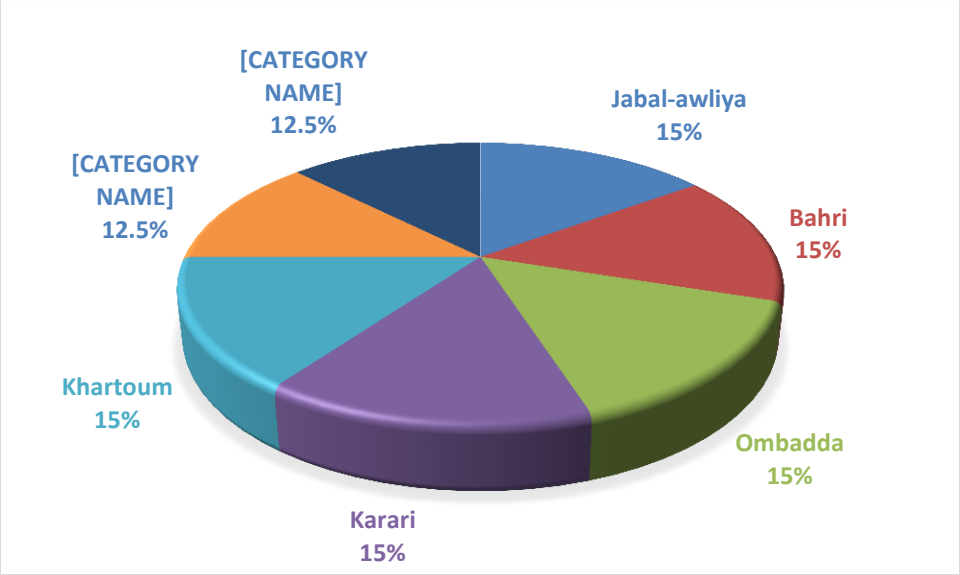

Fig. 1. Percentage of samples taken from seven sub-localities in Khartoum State.

2.6.3 Hot air oven

Glassware such as petri dishes, tubes, and bottles are sterilized by placing them in papers or stainless steel cans and sterilizing them using a hot air oven. The applicable temperature and time is 170 °C for 90 minutes, respectively (Barrow and Feltham, 2003).

2.7 Preparation of media and reagents

All microbiological growth media were prepared according to manufacturer's instructions.

2.7.1 Nutrient agar

Nutrient agar was prepared by adding 28 grams of dehydrated Oxoid nutrient agar to 1000 milliliter of distilled water and boiled. The medium was adjusted to pH 7.4 then it was poured in screw capped bottles each containing 100 milliliters and sterilized by autoclaving at 121°C for 15 minutes in pressure of 15 pounds per square inch.

2.7.2 Blood agar

Forty grams of the powder were dissolved in 1000 milliliter of distilled water and then boiled to dissolve the ingredients. The medium was adjusted to pH 7.4 poured in 100 milliliter amounts and sterilized by autoclaved at 121°C in pressure of 15 pounds per square for 15 minutes. The stock blood agar was heated and cooled to 45-50 °C and poured five per cent of defiberinated sheep or horse blood aseptically. The medium was distributed after mixing.

2.7.3 MacConkey agar

About 52 grams of agar were soaked by boiling in 1000 ml of distilled water and the pH was adjusted to 7.4. The stock medium was sterilized at 115°C for 20 minutes at pressure ten pounds per inch.

2.7.4 Peptone water

The medium was prepared by mix 50 grams of peptone water powder (Oxoid, CM9-CM10) to one liter of distilled water, then dispensed in three ml amount into clean test tubes and autoclaving at 121°C. for 15 minutes.

2.7.5 Sugar test media

About 4.5 grams of sugar with 45 ml of peptone water and phenol red 0.5 ml in 100 ml distilled water were mixed and soaked in conical flasks, and distributed into test tubes. Durham tubes were inserted into all tubes. The media were steaming in 65°C. for 30 minutes for sterilization.

2.7.6 Huge and Leifson's (Oxidation and fermentation medium)

About 5 grams sodium chloride, 0.3 grams dipotassium hydrogen phosphate (K_2HPO_4), 2 grams peptone and 3 grams agar were weighed and mixed. Those ingredients were dissolved in 1000 milliliter of distilled water. The pH was adjusted to 7.1. Then the medium was filtered and added 15 milliliters 2 percent aqueous solution of bromothymol blue and sterilized at 115°C for 15 minutes under pressure of 15 pounds per square inch. Then sterile solution of glucose was added to give the end concentration of 1 percent. The medium was mixed and distributed aseptically in 10 milliliter volumes in sterilize test tubes sealed with cotton plugs.

2.7.7 Muller-Hinton agar

The medium was prepared by mixing 6 grams of meat infusion, 17.5 grams of hydrolyzed casein, 1.5 grams of starch and 10 grams of agar which were dissolved in 1000 ml of water by boiling. The pH was adjusted to 7.4 and then sterilized at 121 °C for 15 minutes under a pressure of 10 pounds per square inch.

2.7.8 Manitol salt agar

The medium was prepared by mixing 11.1 grams of oxoid dehydrated medium in 100 ml of distilled water and sterilized. The medium cooled to 50-55°C. The medium was mixed well and distributed in sterile petri dishes.

2.7.9 Motility media (Semi-solid medium)

About 0.2 percent New Zealand agar with nutrient broth was mixed and dispense in sterile test tubes containing Graigie tubes and sterilized in autoclave at 121°C for 15 minutes under pressure of 15 pounds per square inch.

2.7.10 Arginine broth

The medium was prepared by weighing five grams of peptone, five grams yeast extract, two grams dipotassium hydrogen phosphate (K_2HPO_4), 0.5 grams of glucose and three grams arginine monohydrochloride. The ingredients were mixed in 1000 milliliter distilled water and dissolved by heating. The medium adjusted to pH 7.0 filtered and sterilized at 115°C and pressure of ten pounds per square inch for 20 minutes.

2.7.11 Nessler's reagent

Five grams of potassium iodide in five milliliter distilled water were mixed then added cold saturated mercuric chloride solution and shaking till given precipitation. Added forty milliliter sodium hydroxide. The solution was diluted to 100 milliliters with distilled water and use after 24 hrs.

2.7.12 Mercuric chloride reagent

About 12 grams of mercuric chloride in distilled water were mixed and adding 16 milliliters of concentrated hydrochloric acid to the solution and shaking until the solution was dissolved.

2.8 The bacterial culture and purification

Each sample was streaked onto 5% blood agar plates (Oxoid, CM 271, UK) using sterile cotton swab and incubated aerobically and anaerobically at 37°C for 24 hrs. in an aerobic incubator (Scott Science, Model LIB 080M, serial no. 08101705, UK). The incubation was further continued to 48 hrs. if no growth was observed after 24 hrs. Before discarded as negative for growth. The growing isolates were then sub cultured onto blood agar and nutrient agar plates (Oxoid, CM 1, UK) and incubated at 37°C for 24 hrs. The obtained pure cultures were further sub cultured on blood agar slant, incubated at 37°C for 24 hrs. Then stored in a refrigerator (Coldair, Model H.P, Serial no. 06-207538, Sudan) at four °C for further analysis.

2.9 Methods for bacterial isolation and identification

The purified isolates were identified by conventional bacteriological methods as described in medical laboratory manual for tropical countries Cheesbrough, (2005) and (Barrow and Feltham, 2003). The bacterial isolates were characterized and identified based on their motility, morphological and biochemical characteristics of the tested bacteria.

2.9.1 Gram stain and microscopy

A thin smear was made of each bacteria and fixed with heat. Then stained with Gram stain. The method involves covering the slide with crystal violet stain for two minutes, then rinsing it with water, and covering it again with Lugol's iodine solution for one minute. Then the slide is washed with water, and the color is removed using acetone, which is washed immediately. Then immerse them in diluted carbolfuchsin for 30 seconds and rinse them off at the end. The smear was then dried using filter paper and examined under an immersion oil lens (100 X).

2.9.2 Testing of aerobic growth of the isolates

Bacterial isolates are inoculated onto nutrient agar plates aerobically at 37 °C for 24 to 48 hours. Aerobics are classified depending on the appearance of bacterial growth on the surface of the media.

2.9.3 Catalase test

A drop of hydrogen peroxide (H_2O_2) was added on a clean microscope slide. Using a sterile glass rod, a small colony of isolate was taken and dissolved in a drop of hydrogen peroxide. The appearance of gas bubbles indicates a positive reaction.

2.9.4 Oxidase test

A blot was taken from culture-fresh colonies of the tested organism and applied to oxidase strips, which is performed on filter paper impregnated with 1% tetramethyl-p-phenylene dihydrochloride (oxidase reagent). A positive result appears by developing a dark purple color within five to ten seconds.

2.9.5 Huge and Leifson's (OF media)

After inoculation of media, one of the tubes of OF medium was topped with liquid paraffin layer. The tubes were incubated at 37°C for up to 15 days and examined for change in colour to the yellow that is means of acid production. If the change was in the non-sealed the reaction is oxidative. If the yellow in the sealed or both of the tubes it was said to be fermentative.

2.9.6 Motility medium

Small colony was inserted using straight wire inside a Graigiẻs tube containing semi-solid agar. The test tubes were putted in a rack and incubated at 37°C for 24-48hrs. The tubes were tested for migration of the bacteria outside the Graigiẻs tube.

2.9.7 Arginine broth

The isolated bacteria were inoculated in five milliliter of arginine broth and incubated at 37°C for 24hrs. Then added 0.25 milliliter of Nissler's reagent. Observation of brown colour indicates arginine hydrolysis.

2.10 Antibiotics sensitivity tests

Antibiotic sensitivity was performed for each isolate using the method of Kirby-Bauer (disc diffusion method). This was performed on Mueller–Hinton agar with the following antibiotic discs (Ciprofloxacin CIP 5µg, Amikacin AK 30µg, Enrofloxacin EX 5µg, Amoxycillin AMX 10µg, Vancomycin VA 30µg, Pencillin P 10 units, Bacitracin B 10units, Ceftazidime CAZ 30µg, Colistin CL 10µg, Tetracycline TE30 µg, Gentamicin CN 10µg and Imipenem IMI 10 µg, HiMedical Laboratories Pvt. Limited, India). Sensitivity was read after incubation for 24 hrs. At 35ºC.

2.11 Data analysis

Descriptive statistics were performed using SPSS software (Statistical Package for Social Science, version 22; Inc, USA). The qualitative data were summarized by frequencies (Gomez *et al.*, 1984).

Chi-square tests were used to verify the existence of a possible association between the bacterial isolates and the source of samples or the variation in antibiotic resistance.

CHAPTER THREE

3. Results

In this study, samples were taken from workers' hands, workers' shoes, animals' milk and feces in dairy farms in seven sub-localities in Khartoum State. These sub-localities include Jabalawlia, Bahri, Umbada, Karari, Umdurman, Khartoum and Sharg-Alnile. The samples were investigated to identify the prevalence of isolated bacteria and antibiotic resistance in dairy farms in Khartoum State.

3.1 Bacterial isolates

Bacterial isolates from 160 samples were identified. The frequency and percentages of these bacteria are listed in Table 1. These bacteria belong to 9 different genera of Gram negative and Gram positive rods and cocci namely; *Staphylococcus*, *Enterococcus*, *Micrococcus*, *Corynebacterium*, *Bacillus*, *Enterobacteria, Pasteurella*, *Moraxella and Acintobacter* spp. The most prevalent organism overall was *Staphylococcus* (n = 92; 53.5%) followed by *Enterbacteria* (n = 32; 18.6%). *Corynebacterium spp* was found to be the least prevalent bacterial species (n = 1; 0.6%).

Results of the present study revealed that 50 (29.1%) organisms isolated from workers' shoes, 42 (24.4%) from feces, 41 (23.8%) from workers' hands and 39 (22.7%) from milk samples (Table 2). Results indicated that 23 (13.4%) of samples had at least two different organisms, 2 (8.7 %) from workers' hands, 10 (43.5 %) from workers' shoes, 6 (26.1 %) from feces and 5 (21.7 %) from milk. Out of 172 isolates, there are 123 (71.5%) Gram positive bacteria and 49 (28.5%) Gram negative bacteria (Table 3).

Table 1: Isolated bacteria from workers' hands and shoes, animals' feces and milk samples collected from dairy farms in Khartoum State, Sudan

No	Bacterial isolates	Isolate No (%)	Samples No (%)			
			Hands (n= 40)	Shoes (n= 40)	Feces (n= 40)	Milk (n= 40)
1	***Staphylococcus spp***	92(53.5)	28(30.4)	27(29.3)	17(18.5)	20(21.7)
2	***Enterococcus spp***	8 (4.6)	1 (12.5)	2 (25.0)	3 (37.5)	2 (25.0)
3	***Micrococcus spp***	7 (4.1)	3 (42.8)	2 (28.6)	1 (14.3)	1 (14.3)
4	***Corynebacterium spp***	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)
5	***Bacillus spp***	15 (8.7)	3 (20.0)	5 (33.3)	3 (20.0)	4 (26.7)
6	***Enterobacteria spp***	32(18.6)	2(6.2)	11(34.4)	12(37.5)	7 (21.9)
7	***Pasteurella spp***	2 (1.2)	1 (50.0)	0 (0.0)	0 (0.0)	1 (50.0)
8	***Moraxella spp***	3 (1.7)	0 (0.0)	1 (33.3)	2 (66.7)	0 (0.0)
9	***Acintobacter spp***	12 (6.9)	3 (25.0)	2 (16.7)	4 (33.3)	3 (25.0)
	Total	172	41(23.8)	50(29.1)	42(24.4)	39 (22.7)

Table 2: Percentages of bacterial organisms isolated from different samples of workers' hands and shoes and animals' feces and milk in seven sub-localities in Khartoum State, Sudan

Source of sample	Frequency	Percentages (%)
Workers hands	**41**	**23.8**
Workers shoes	**50**	**29.1**
Feces	**42**	**24.4**
Milk	**39**	**22.7**

Table 3: Percentages of Gram negative and Gram positive bacteria isolated from different samples of workers and animals in seven sub-localities in Khartoum State, Sudan

Bacterial isolates	Frequency	Percentages (%)
Gram negative	49	28.5
Gram positive	123	71.5

3.2 Antimicrobial susceptibility of the Gram positive bacterial isolates

The antimicrobial susceptibility tests of the bacterial isolates were obviously variable. In Gram positive bacteria, two bacterial isolates namely *Staphylococcus spp* (vancomycin; 58.7%) and *Enterococcus spp* (enrofloxacin; 100%) showed resistance to only one antibiotic. While *Micrococcus spp* showed resistance to vancomycin (85.7%) and penicillin (71.4%) and *Bacillus spp* displayed 100% resistant to vancomycin and amoxicillin. *Corynebacterium* were 100% resistant to 3 different types of antibiotics (vancomycin, penicillin and bacitracin). Antibiotic sensitivity pattern of Gram positive bacteria in samples collected from dairy farms in Khartoum State is presented in Table (4).

Table 4: Antibiotic sensitivity pattern of Gram positive bacteria in dairy farms in Khartoum State, Sudan

Bacterial isolates	*EX*		*AMX*		*VA*		*P*		*B*		*GEN*		*CIP*	
	R (%)	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R(%)**	**S (%)**	**R (%)**	**S (%)**
Staphylococcus	0(0)	92(100)	6(6.5)	86(93.5)	54(58.7)	38(41.3)	24(26.0)	68(73.9)	6(6.5)	86(93.5)	0(0)	92(100)	6(6.5)	86(93.5)
Enterococcus	8(100)	0 (0)	0 (0)	8(100)	0(0)	8(100)	0(0)	8(100)	0(0)	8(100)	0(0)	8(100)	0(0)	8(100)
Micrococcus	1(14.3)	6(85.7)	1(14.3)	6(85.7)	6(85.7)	1(14.3)	5(71.4)	2(28.6)	0(0)	7(100)	0(0)	7(100)	0(0)	7(100)
Corynebacterium	0(0)	1(100)	0(0)	1(100)	1(100)	0(0)	1(100)	0(0)	1(100)	0(0)	0(0)	1(100)	1(100)	0(0)
Bacillus	0(0)	15(100)	15(100)	0(0)	15(100)	0(0)	0(0)	15(100)	0(0)	15(100)	0(0)	15(100)	0(0)	15(100)

Keys: Enrofloxacin EX 5µg, Amoxicillin AMX 10µg, Vancomycin VA 30µg, Pencillin P 10 units, Bacitracin B 10units, Gentamicin CEN 10µg, Ciprofloxacin CIP 5µg and Tetracycline TE30 µg.

3.3 Antimicrobial susceptibility of the Gram negative bacterial isolates

In Gram negative bacteria, the isolated *Pasteurella* were sensitive (100%) to enrofloxacin, ceftazidime, colistin, tetracycline, gentamicin, imipenem, amoxycillin, amikacin and ciprofloxacin. While *Enterobacteria* bacteria showed highly sensitivity (100%) to enrofloxacin, amikacin, imipenem, amoxycillin and ciprofloxacin only.

Acintobacter spp showed 100% resistant to ceftazidime and imipenem. While the isolates of *Moraxella* were 100% resistant to ceftazidime only. Antibiotics sensitivity pattern of Gram negative bacteria in samples collected from dairy farms in Khartoum State is presented in Table (5).

3.4 Multi-drug resistance

In Gram positive isolates, *Corynebacterium* showed multi-drug resistance for 3 drugs (vancomycin, penicillin and bacitracin). *Bacillus* (vancomycin and amoxicillin) and *Micrococcus* (vancomycin and penicillin) showed multi-drug resistance for 2 drugs. In Gram negative bacteria *Acintobacter* showed multi-drug resistance to 2 drugs (ceftazidime and imipenem). See table (6).

Table 5: Antibiotic sensitivity pattern of Gram negative bacteria in samples collected from dairy farms in Khartoum State

Bacterial isolates	*EX*		*CAZ*		*CL*		TE		AK	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Enterobacteria spp	0 (0)	32(100)	5 (15.6)	27(84.4)	5(15.6)	27(84.4)	5(15.6)	27(84.4)	0(0)	32(100)
Pasteurella spp	0 (0)	2(100)	0(0)	2(100)	0(0)	2(100)	0(0)	2(100)	0(0)	2(100)
Moraxella spp	0 (0)	3(100)	3(100)	0(0)	0(0)	3(100)	0(0)	3(100)	0(0)	3(100)
Acintobacter spp	0 (0)	12(100)	12(100)	0(0)	0(0)	12(100)	0(0)	12(100)	0(0)	12(100)

Bacterial isolates	GEN		IMI		CIP		AMX	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Enterobacteria spp	5(15.6)	27(84.4)	0(0)	32(100)	0(0)	32(100)	0(0)	32(100)
Pasteurella spp	0(0)	2(100)	0(0)	2(100)	0(0)	2(100)	0(0)	2(100)
Moraxella spp	0(0)	3(100)	0(0)	3(100)	0(0)	3(100)	0(0)	3(100)
Acintobacter spp	0(0)	12(100)	12(100)	0(0)	0 (0)	12 (100)	0(0)	12 (100)

Keys: Enrofloxacin EX 5µg, Ceftazidime CAZ 30µg, Colistin CL 10µg, Tetracycline TE30 µg, Gentamicin CN 10µg, Imipenem IMI 10 µg, Ciprofloxacin CIP 5µg, Amoxicillin AMX 10µg and Amikacin A2K 30µg

Table 6: Multi-drug resistance of isolated bacteria from dairy farms in Khartoum State

Bacterial isolates	Resistant drugs
Corynebacterium spp	Vancomycin * Penicillin * Bacitracin
Bacillus spp	Vancomycin * Amoxycillin
Micrococcus spp	Vancomycin * Penicillin
Acintobacter spp	Ceftazidime * Imipenem

CHAPTER FOUR

4. Discussion

Antimicrobial resistance (AMR) is increasingly acknowledged as a serious health problem in all countries. According to reports, overusing or abusing antibiotics has contributed to the development and spread of antibiotic-resistant germs, rendering therapy ineffective and posing a serious risk to the public's health (Prestinaci *et al.*, 2015).

In the present study prevalence of isolated bacteria and antibiotic resistance in dairy farms in Khartoum State were investigated. Samples from workers' hands, workers' shoes, animal milk and feces were taken from dairy farms located in seven sub-localities in Khartoum State: Jabalawlia, Bahri, Umbada, Karari, Khartoum, Umdurman and Sharg-Alnile.

160 samples were used in this study to obtain about 172 isolates from 9 different bacterial genera. *Staphylococcus* (53.5%) and *enterobacteria* (18.6%) were discovered to have the highest occurrence of isolated bacteria, according to the findings of the current analysis. *Corynebacterium* (0.6%) was the least prevalent type of bacteria that isolated from milk sample. *Corynebacterium spp* are considered as minor contagious mastitis causative pathogens (Kasozi, *et al*., 2014).

However, in Gram positive bacteria (n=123; 71.5%) the most prevalent bacteria were *Staphylococcus* (n = 92; 53.5%), especially in the hands (n = 28; 30.4%) and shoes (n = 27; 29.3 %). These findings are consistent with those of Tondo *et al.* (2000), who reported that *Staphylococcus* was isolated from 90.4% of raw milk samples, and Balemi *et al*., (2021) who found that *Staphylococcus* (n = 11, 17.2%) was present in higher percentages in milk samples from cows.

Moreover, *Staphylococcus spp*. was found in high percentage (73.36%) in milk samples from cows infected with mastitis in the Damazin locality, Blue Nile State, Sudan and in high percentage also (53.2%) when isolated from cows infected with mastitis in Khartoum State, Sudan, according to Babiker *et al*. (2021) and Yasin *et al.* (2016) respectively.

The high prevalence of *Staphylococcus* in hands and shoes in this study might be associated with the absence of hygienic practices, and consistent hand-milking practices throughout the dairy herds. Since *Staphylococcus* is usually found on the udder or teat skin surface of infected animals, the primary source of transmission from infected udders to uninfected is usually by the milkers' hands during hand-milking (Balemi *et al*. 2021).

It was found that the percentage of *Enterobacteria* was the highest bacteria among the Gram negative bacteria (n=49; 28.5%), especially in the feces and shoes samples, in agreement of the results of Massé *et al*. (2021). Presence of entero-microorganisms especially *E. coli* in milk samples (22.2%) indicates the low hygienic milking practices in the dairy farms, which constitute a public health hazard. *E. coli* frequently contaminates food and it is a good indicator of fecal pollution (Ali and Abdelgadir, 2011). In addition, the higher prevalence of *Staphylococcus* and *Enterobacteria spp* over other isolates in this study can be attributed to the natural flora present in animals.

In Gram positive bacteria gentamicin, tetracycline and ciprofloxacin were effective drugs for most of isolates (n = 123; 100%) and followed by Bacitracin (n = 116; 94.3%). However, the majority of the isolates showed resistance to vancomycin (n= 76, 61.7%) in agreement of the results of (Beyene *et al*., 2017). In Gram negative bacteria enrofloxacin, amoxicillin, amikacin and ciprofloxacin were effective drugs for all isolated bacteria (n =

49; 100%), followed by colistin, tetracycline and gentamicin (n = 44; 89.7%). On the other hand, the most of the isolates showed resistance to ceftazidime (40.8%). Results of the current study revealed that the majority of bacteria are still sensitive to antibiotics, which is consistent with findings of Mahlangu *et al.* (2018). According to Yasin *et al.* (2016), the majority of the Gram positive and Gram negative isolates from milk samples were highly susceptible to the antibiotics gentamycin, ciprofloxacin, norfloxacin, and kanamycin, highly resistant to penicillin-G, and only moderately susceptible to novobiocin, tetracycline, and cefalexin.

Multiple antibiotic resistance (MDR) refers to microorganisms that do not respond to more than two kinds of antibiotics when used therapeutically (Najeeb *et al.*, 2013). Regarding to multidrug resistance, the study reflects that only one isolate (*Coryenbacterium)* shows multidrug resistance pattern (100%) for 3 drugs (vancomycin, penicillin, and bacitracin). However, *Bacillus* spp exhibited resistance for 2 drugs (vancomycin and amoxycillin). *Micrococcus* resistance for 2 drugs (vancomycin and penicillin). While *Actinobacter* showed resistance to 2 drugs (ceftazidime and imipenem).

Conclusion and Recommendations

Conclusion

In conclusion, *Staphylococcus spp* were found to be the most prevalent bacteria in dairy farms in Khartoum state. It was isolated in high percentages from workers' hands and workers' shoes samples. This might be the indication of low hygienic milking practices, and consistent hand-milking practices throughout the dairy farms.

Moreover, presence of *Enterobacteria* in feces and shoes samples indicates low hygienic milking practices in the dairy farms, which produce a public health hazard.

In Sensitivity tests, Gentamicin, Tetracycline and Ciprofloxacin exhibited highly effective action for most Gram positive isolates. For Gram negative isolates Enrofloxacin, Amoxicillin, Amikacin and Ciprofloxacin were found highly effective antibiotics for most isolates.

Resistance to Vancomycin and Ceftazidime were increased in Gram positive and negative bacteria.

In addition, the multi-drug resistance was identified for various antibiotics in Gram positive bacteria such as Vancomycin, Bacitracin, Penicillin and Amoxycillin, while in Gram negative bacteria the multi-drug resistance was observed in Ceftazidime, Imipenem, Colistin, Teteacycline and Gentamicin.

Recommendations

- In recommendation, high and strict hygienic practice and measures such as regular washing milkers' hands, animal's udders, cleaning and sterilization of dairy equipment, utensils, and treatment and isolation of diseased animals from the herd are highly recommended.
- Following the guidelines for ethical antibiotic usage to avoid antibiotic resistance.

References

Adzitey, F. (2015). Antibiotic classes and antibiotic susceptibility of bacterial isolates from selected poultry; a Mini Review. *World's Veterinary Journal*, 5 (3): 36-41.

Ali, A. A. and Abdelgadir, W. S. (2011). Incidence of *Escherichia coli* in raw cow's milk in Khartoum State. *British Journal of Dairy Sciences*, 2 (1): 23-26.

Ashfaq, M., Hashmi, M. Z., Mumtaz, A., Javed, D., Ain, N. U., Shifaqat, S and Rehman, M. S. U. (2020). Environmental risk assessment of antibiotics and AMR/ARGs. In Antibiotics and Antimicrobial Resistance Genes in the Environment, *Elsevier*: 331-349.

Babiker, M. Ali., Fangama, M. I. M., Suliman, S. E. and Abdalla, M. A. (2021). Bovine mastitis in Eldamazine locality – Blue Nile State. *International Journal of Current Microbiology and Applied Sciences*, 10 (9): 1-7.

Balabanova, B. (2020). Antibiotics and antimicrobial resistance mechanism of entry in the environment. In Antibiotics and Antimicrobial Resistance Genes in the Environment, *Elsevier*: 126-137.

Balemi, A., Gumi, B., Amenu, K., Girma, S., Gebru, M.U., Tekle, M., Ríus, A.A., D'Souza, D.H., Agga, G.E. and Kerro Dego, O. (2021). Prevalence of mastitis and antibiotic resistance of bacterial isolates from CMT positive milk samples obtained from dairy cows, camels, and goats in two pastoral districts in Southern Ethiopia. *Animals*, 11(6): 1-17.

Barrow, G. I. and Feltham, R. K. A. (2003). Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd edition, Cambridge university press. British.

Beyene, T., Hayishe, H., Gizaw, F., Beyi, A.F., Abunna, F., Mammo, B., Ayana, D., Waktole, H. and Abdi, R.D. (2017). Prevalence and antimicrobial resistance profile of *Staphylococcus* in dairy farms, abattoir and humans in Addis Ababa, Ethiopia. *BMC Research Notes*, 10 (1): 1-9.

Boonyasiri, A., Tangkoskul, T., Seenama, C., Saiyarin, J., Tiengrim, S. and Thamlikitkul, V. (2014). Prevalence of antibiotic resistant bacteria in healthy adults, foods, food animals, and the environment in selected areas in Thailand. *Pathogens and Global Health*, 108 (5): 235-245.

Brander, G. G., Pugh, D. M., Bywater, R. J. and Jenkins, W. L. (1991). Applied veterinary pharmacology and therapeutics. BaiIliere, Tindall, London, 364.

Chang, Q., Wang, W., Regev-Yochay, G., Lipsitch, M. and Hanage, W.P. (2015). Antibiotics in agriculture and the risk to human health: how worried should we be? *Evolutionary Applications*, 8 (3): 240-247.

Frank, U. and Tacconelli, E. (2012). The Daschner Guide to in-hospital antibiotic therapy: European Standards. Springer Science and Business Media.

Gomez, K.A. and Gomez, A. A. (1984). Statistical procedures for agricultural research, John Wiley & Sons.

Greenwood, D., Finch, R. and Davey, P. (2007). Antimicrobial chemotherapy. Oxford University Press.

Ibrahim, M., Ahmad, F., Yaqub, B., Ramzan, A., Imran, A., Afzaal, M., Mirza, S.A., Mazhar, I., Younus, M., Akram, Q. and Taseer, M.S.A. (2020). Current trends of antimicrobials used in food animals and aquaculture. In Antibiotics and antimicrobial resistance genes in the environment. *Elsevier*: 39-69.

Igbinosa, E. O. and Odjadjare, E. E. (2015). Antibiotics and antibiotic resistance determinants: an undesired element in the environment. *Formatex*, 2: 858-866.

Kasozi, K.I., Tingiira, J.B. and Vudriko, P. (2014). High prevalence of subclinical mastitis and multidrug resistant *Staphylococcus aureus* are a threat to dairy cattle production in Kiboga District (Uganda). *Open Journal of Veterinary Medicine*, 4 (4): 35-43

Kieser, T., Bibb, M. J., Buttner, M. J., Chater, K.F. and Hopwood, D.A. (2000). Practical *Streptomyces* Genetics. Norwich: John Innes Foundation, Vol 291, p. 397.

King, L. J., Anderson, L. R., Blackmore, C. G., Blackwell, M. J., Lautner, E. A., Marcus, L. C., Meyer, T. E., Monath, T. P., Nave, J. E., Ohle, J. and Pappaioanou, M. (2008). Executive summary of the AVMA one health initiative task force report. *Journal of the American Veterinary Medical Association*, 233 (2): 259-261.

Kohanski, M. A., Dwyer, D. J. and Collins, J. J. (2010). How antibiotics kill bacteria: from targets to networks. *Nature Reviews Microbiology*, 8 (6): 423-435.

Mahlangu, P., Maina, N. and Kagira, J. (2018). Prevalence, risk factors, and antibiogram of bacteria isolated from milk of goats with

subclinical mastitis in Thika East Subcounty, Kenya. *Journal of Veterinary Medicine*, 1-8.

Manage, P. M. (2018). Heavy use of antibiotics in aquaculture; emerging human and animal health problems–A review. *Sri Lanka Journal of Aquatic Sciences*, 23 (1): 13-27

Massé, J., Lardé, H., Fairbrother, J.M., Roy, J. P., Francoz, D., Dufour, S. and Archambault, M. (2021). Prevalence of antimicrobial resistance and characteristics of Escherichia coli isolates from fecal and manure pit samples on dairy farms in the province of Québec, Canada. *Frontiers in Veterinary Science*, 8: 1-14.

Munita, J.M. and Arias, C.A. (2016). Mechanisms of antibiotic resistance. *Virulence Mechanisms of Bacterial Pathogens*, 4(2): 481-511.

Najeeb, M.F., Anjum, A.A., Ahmad, M.U.D., Khan, H.M., Ali, M.A. and Sattar, M.M.K. (2013). Bacterial etiology of subclinical mastitis in dairy goats and multiple drug resistance of the isolates. *Journal of Animal Plant Science*, 23(6):1541-1544.

Orand, J. (2012). Antimicrobial resistance and the standards of the World Organisation for Animal Health. *Revue Scientifique et Technique-OIE*, 31(1): 335-342.

Prestinaci, F., Pezzotti, P. and Pantosti, A. (2015). Antimicrobial resistance: a global multifaceted phenomenon. *Pathogens and Global Health*, 109 (7); 309-318.

Rehman, K., Jabeen, K., Chohan, T.A. and Akash, M.S.H. (2020). Databases, multiplexed PCR, and next-generation sequencing technologies for tracking AMR genes in the environment. In

Antibiotics and Antimicrobial Resistance Genes in the Environment, *Elsevier*, 1: 223-233.

Rousham, E.K., Unicomb, L. and Islam, M.A. (2018). Human, animal and environmental contributors to antibiotic resistance in low-resource settings: integrating behavioural, epidemiological and One Health approaches. Proceedings of the Royal Society B: *Biological Sciences*, 285 (1876): 1-9.

Sabundayo, B.P. and Caldero´n, C.B. (2007). Antimicrobial Classifications: Antimicrobial susceptibility testing protocols, p.363.

Salih, R.R.M. (2015). Update on bovine mastitis etiological, clinical and treatment aspects in Khartoum State, Sudan. *Journal of Animal and Feed Research,* 5 (6): 153-159.

Schwarz, S., Kehrenberg, C. and Walsh, T.R. (2001). Use of antimicrobial agents in veterinary medicine and food animal production. *International Journal of Antimicrobial Agents*, 17 (6): 431-437.

Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M. and Zheng, B. (2019). Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules*, 24 (13): 1-22

Singer, A.C., Shaw, H., Rhodes, V. and Hart, A. (2016). Review of antimicrobial resistance in the environment and its relevance to environmental regulators. *Frontiers in Microbiology*, 7, 1728.

Smith, R.A., M'ikanatha, N.M. and Read, A.F. (2015). Antibiotic resistance: a primer and call to action. *Health Communication*, 30 (3): 309-314.

Sommer, M.O., Dantas, G. and Church, G.M. (2009). Functional characterization of the antibiotic resistance reservoir in the human microflora. *Science*, 325 (5944): 1128-1131.

Tondo, E.C., Guimarães, M.M., Henriques, J.A. and Ayub, M.A. (2000). Assessing and analysing contamination of a dairy products processing plant by *Staphylococcus aureus* using antibiotic resistance and PFGE. *Canadian Journal of Microbiology*, 46 (12): 1108-1114.

Van Hoek, A.H., Mevius, D., Guerra, B., Mullany, P., Roberts, A.P. and Aarts, H.J. (2011). Acquired antibiotic resistance genes: an overview. *Frontiers in Microbiology*, 2, 203.

Walsh, C. (2003). Antibiotics: actions, origins, resistance. American Society for Microbiology (ASM).

Waseem, H., Ali, J., Sarwar, F., Khan, A., Rehman, H.S.U., Choudri, M., Arif, N., Subhan, M., Saleem, A.R., Jamal, A. and Ali, M.I. (2019). Assessment of knowledge and attitude trends towards antimicrobial resistance (AMR) among the community members, pharmacists/pharmacy owners and physicians in district Sialkot, Pakistan. *Antimicrobial Resistance and Infection Control*, 8 (1): 1-7.

Waseem, H., Williams, M.R., Stedtfeld, R.D. and Hashsham, S.A. (2017). Antimicrobial resistance in the environment. *Water Environment Research*, 89 (10): 921-941.

Wee, B.A., Muloi, D.M. and van Bunnik, B.A. (2020). Quantifying the transmission of antimicrobial resistance at the human and livestock interface with genomics. *Clinical Microbiology and Infection*. 26 (12):1612-1616.

Woolhouse, M., Ward, M., Van Bunnik, B. and Farrar, J. (2015). Antimicrobial resistance in humans, livestock and the wider environment. Philosophical Transactions of the Royal Society B. *Biological Sciences*, 370 (1670): 1-7.

World Health Organization. (2007). Critically important antimicrobials for human medicine: categorization for the development of risk management strategies to contain antimicrobial resistance due to non-human antimicrobial use: report of the second World Health Organization Expert Meeting, Copenhagen, 29-31.

Yasin, W.M., Sabiel, Y.A., El-Gaddal, A.A. and Mansour, M.E. (2016). Antimicrobial resistance of pathogenic bacteria isolated from mastitis cows in Khartoum State, Sudan. *British Microbiology Research Journal*, 17 (6): 1-6.