EFFECTS OF CO-TRIMOXAZOLE AND AMOXICILLIN THERAPY ON GUT MICROBIOTA POPULATION, PHYSIOLOGICAL, BIOCHEMICAL AND PATHOLOGICAL PARAMETERS IN SWISS MICE

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A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science in Biochemistry of Chuka University.

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DECLARATION AND RECOMMENDATIONS

Declaration

This thesis is my original work and has not been presented for an award of diploma or conferment of degree in any other University.

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Recommendation

This thesis has been examined, passed and submitted with our approval as University Supervisors.

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DEDICATION

I dedicate this work to my family at large, my father, mother, sisters and brothers and friends for their unwavering support and encouragement towards achieving my master's degree in Biochemistry.

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ABSTRACT

Antibiotics have been utilized in treatment of bacterial infections since their discovery. Despite being beneficial in managing infections, antibiotics have significant implications on health by disrupting the gut microbiota. Gut microbiota comprises of wide range of microorganisms that inhabit the gut, including fungus, archaea, bacteria, and viruses. The gut microbiota plays pivotal role in health by influencing metabolic processes, immunological and neurobehavioral functions. This study investigated the impact of amoxicillin and co-trimoxazole on the gut bacterial population of mice, using three-week-old Swiss mice models simulating six-month-old human babies. The experiment aimed to assess physiological, biochemical, immuno-pathological changes, and the induction of oxidative stress. Male swiss mice were randomly assigned to five groups: normal control, amoxicillin group, septrin group, amoxicillin+septrin group, and amoxicillin+co-trimoxazole+probiotics. Over 63 days, mice were monitored, weighed after each antibiotic dosage. Euthanasia was performed using isoflurane, and blood samples was collected via cardiac puncture for hematological analysis. The liver, spleen, kidney, lungs and heart were harvested and weighed for determination relative organ weight (ROW), liver, brain and kidneys were harvested for histo-pathological examination. Serum obtained from whole blood underwent further analysis for various markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine, urea and cytokines. Tissue glutathione (GSH) and malondialdehyde (MDA) levels, along with serum nitric oxide (NO), were determined to gauge oxidative stress. Numeric data underwent analysis using one-way ANOVA followed by Tukeys' post hoc test, with significance reported at $p<0.05$. Results, in form of graphs and images, revealed amoxicillin and septrin administered singly or in combination resulted in reduced gut microbiota population resulting in gut microbiota dysbiosis. Probiotics administration ameliorated the gut microbiota dysbiosis. There were no significant changes in body weight as well as relative organ weight (ROW) of the selected organs. Hematological exams revealed significant drop in the red blood cells (RBCs) count, hematocrit level and hemoglobin especially in amoxicillin+septrin treated group. White blood cells count (WBCs) was significantly elevated in septrin group compared to control, amoxicillin group, amoxicillin+septrin treated group and amoxicillin+septrin+probiotics treated group. Liver function test markers aspartate aminotransferase (AST), alanine aminotransferase (ALT), AST:ALT ratio and alkaline phosphate (ALP), were significantly ($p<0.05$) elevated indicating liver damage. Kidney function markers showed elevated levels of creatinine, urea, uric acid and significant drop in the levels of albumin indicating kidney damage. Gut microbiota dysbiosis results in electrolyte imbalances noted by a drop in the levels of serum electrolytes; sodium, chloride and potassium. There were significant ($p<0.05$) elevated levels of interferon gamma (IFN-γ), tumor necrotic factor alpha (TNF-α) indicating active inflammation, histological exams revealed tissue damage in the liver and kidneys, and oxidative stress indicated by elevated malondialdehyde (MDA) and glutathione (GSH) levels in target organs. There were significant ($p<0.05$) elevated nitric oxide (NO) levels in the serum indicating active inflammation or damage to organ functions. Probiotics administration alongside antibiotics showed promising outcomes, by restoring gut microbiota population and consequently protecting the body from induction of immunological responses and inflammation, protection from oxidative stress and organ damage suggesting a potential avenue for ameliorating complications associated with antibiotic-induced dysbiosis. This comprehensive study highlights the intricate effects of antibiotics on gut microbiota and associated health parameters, emphasizing the need for cautious antibiotic use to mitigate potential adverse outcomes.

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LIST OF ABBREVIATIONS AND ACRONYMS

AD:	Alzheimer Disease.
ALP	Alkaline Phosphatase
ALT:	Alanine aminotransferase.
AST:	Aspartate aminotransferase.
BM:	Bone Marrow.
CDC:	Center for disease control.
DCs:	Dendritic cells.
DTNB:	5,5'- dithiobis-(-2 nitro benzoic acid).
EDTA:	ethylene diamine tetra-acetic acid.
EEC:	Entero-endocrine cells.
ELISA	Enzyme Linked Immunosorbent Assay
ENS:	Enteric nervous systems
FDA:	Food and drug administration.
GABA:	Gamma-aminobutyric acid.
GF:	Germ free.
GGT	Gamma-glutamyl Transferase
GIT:	Gastrointestinal tract.
GSH:	Glutathione.
GSSH	Oxidized Glutathione.
HDL	High Density Lipoprotein
HG:	Hemoglobin.
HIV:	Human Immunodeficiency virus.
HPC:	Hematopoietic progenitor cells.
HSC:	Hematopoietic stem cells.
HSPC:	Hematopoietic stem and progenitor cells.
IBD:	Inflammatory Bowel Disease.
IFN-γ	Interferon Gamma.
IL:	Interleukin.
IPR	Institute of Primate Research
LDL	Low Density Lipoprotein.
LPS	Lipopolysaccharide
MCH:	Mean corpuscular hemoglobin.

MCHC	Mean Corpuscular Hemoglobin Concentration
MCV:	Mean cell volume.
NACOSTI	National Commission for Science, Technology and Innovation
NADH	Nicotinamide Adenine Dinucleotide + Hydrogen
NAFLD	Non-alcoholic fatty liver disease
NCBI:	National Center for Biotechnology Information.
NO	Nitric Oxide.
OS:	Oxidative stress.
PBPS:	Penicillin-binding proteins.
PBS	Phosphate-Buffered Saline
PCV	Packed Cell Volume.
P-LCR	Platelet-Large Cell Ratio
RMCBS:	Rapid murine coma and behavior scale.
RNS	Reactive Nitrogen Species.
ROS:	Reactive oxygen species.
SCFAs:	Short chain fatty acids.
SPF:	Specific Pathogen free.
SSA:	Sulfosalicylic acid solution.
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid Reactive Substance
Th1:	Type 1 helper cells.
TLR:	Toll-Like receptors.
TNF-α	Tumor Necrotic Factor Alpha
WBC	White Blood Cells.
WHO:	World Health Organization.

CHAPTER ONE
INTRODUCTION

1.1 Background to the Study

Antibiotics are broadly used drugs in management of bacterial infections. They are grouped into two primary categories, that is, bactericidal and bacteriostatic (Nemeth *et al*., 2015; D'Agate *et al*., 2020). Antibiotics are further classified into aminoglycosides, macrolides, tetracyclines, sulfonamides, penicillins, carbapenems (Etebu and Arikekpar, 2016). Antibiotic consumption globally has been on the rise (Klein *et al*., 2018). Approximately 25% of pediatric medications issued in the USA are antibiotics (Chai *et al.,* 2012). Antibiotics are mainly prescribed in confirmed cases of bacterial infections in patients. These includes treatment of tuberculosis, cholera, syphilis, gonorrhea, urinary tract infections (UTIs), bacterial food poisoning among other bacterial infections (Cheesbrough, 2005; WHO, 2016).

Amoxicillin and co-trimoxazole are among commonly prescribed broad-spectrum antibiotics globally. Amoxicillin antibiotics is one among the class of penicillin antibiotics, which is frequently used in the primary care context (Shah *et al*., 2023). Amoxicillin misuse has been associated with jaundice, colitis and hypersensitive reactions. Co-trimoxazole market name septrin, belongs to class sulfonamides and is formed by combination of trimethoprim and sulfamethoxazole in the ratio 1:5 respectively. Co-trimoxazole is usually prescribed to patients with underactive immune system such as patients with cancer and HIV/AIDS patients. Its misuse has been associated with stevens-Johnsons syndrome and allergic reactions (Acharya *et al*., 2020).

Gut microbiome plays important function in general health and wellbeing. Numerous facets of human health, such as immunological, metabolic, and neurobehavioral characteristics, are significantly influenced by gut bacteria (Cummings & Macfarlane, 1991). About 100 trillion microbes, including viruses, bacteria, fungus, and protozoa, are present in the human gut (Bull & Plummer, 2015) and studies on humans as well as animal models provide varying degrees of evidence that the gut microbial composition has a role in overall wellness (De Palma, 2017). Infants are prescribed for antibiotic treatments during bacterial infections, including meningococcal disease, pneumonia,

bacterial sinusitis, sore throat, skin infections, ear pain and those born to Human Immunodeficiency virus (HIV) positive mothers (WHO, 2000). In contemporary obstetric and neonatal care, the utilization of prenatal broad-spectrum antibiotics has become widespread. Numerous indicators have demonstrated the link between early-life usage of antibiotics and its impact on the gut microbiome and a host of health conditions, including inflammatory bowel conditions, diabetes, atopy and obesity (Eck *et al*., 2020).

Ecological balance and stability in gastrointestinal microbiota are essential to gastronomic functionality and, hence, to human and animal health (Rinninella *et al*., 2019). The gut microbiota and the host have developed a symbiotic relationship where the microbes depend on the host to provide a habitat for their growth and survival. In contrast, the host depends on the gut microbiota, which plays vital roles in food absorption enhancement, cell production in the host intestinal tract, gut endocrine regulation, and immunological function (Ouwehand *et al*., 2002). Such factors influencing bacterial population density include pH, redox activity, peristalsis, bacterial cell adhesion, microbial symbiosis, mucin production, nutritional source, diet, and bacterial interference within different regions of the GI tract (Hao *et al*., 2004).

The intestinal gut microbiome of developing infants is dynamic and readily perturbed by external stimuli such as antibiotic exposure (Gibson *et al*., 2015). Infant gut microbiota differs from adults in composition because the adult gut is densely inhabited. The first colonization in newborns' gastrointestinal tract represents the infant's central gut microbiota, having a long-lasting effect on its diversity and activity. This is an essential determinant of health and immunity later in life. Therefore, it is of prime importance to establish good gut microbiota in the early period of life (Bharadia *et al*., 2020). Disruption of gut microbiota promotes an infectious and septic condition through various modes of action, including making space for the replication of pathogenic gut bacteria, impaired generation of important microbial metabolites, and priming of the immune system toward high pro-inflammatory activity such as short-chain fatty acids (Adelman *et al*., 2020).

This antibiotic use disrupts the gut microbial composition, creating an imbalance that causes overproduction of oxidative free radicals. This overproduction will induce oxidative stress on the tissues and subsequent inflammation, affecting intestinal neurons (Dumitrescu et al., 2018). The antibiotic-induced gut microbiota may result in inflammatory responses resulting from a dysregulation in metabolic processes within the host complex system that integrates the various interactions within the gut microbiota, metabolic activities, and immunological response (Zheng *et al*., 2020). In this regard, the antibiotic-induced modifications to the gut microbiota provided new insight into the complex system, which is integrated with the various interactions at the gut microbiota level, metabolic activities, and immunological responses in the host. In interaction with enteric gut microbiota bacteria, reactive oxygen species are rapidly produced by gut epithelia and similarly induced in diverse cell types by microbial cues (Jones *et al*., 2012). This implies that the disturbance of the gut microbiota as a result of implication results in an unregulated release of ROS, thus leading to tissue stress and inflammation of the organs.

This research was used to simulate the implications of antibiotics on human infants using three weeks old mice as experimental animal model. The study in the mouse model is expected to elucidate effects of co-trimoxazole and amoxicillin induced dysbiosis on infants and children such as effect on the immune responses, inflammation of tissues, the brain and liver function, effects on the kidney function, hematopoiesis and oxidative stress, and studying changes following administration with probiotics.

1.2 Statement of the Problem

There is an increasing public health concern on antibiotic misuse globally. Antibiotics are major disruptors of gut microbial composition causing gut microbiota dysbiosis and the most commonly prescribed broad-spectrum antibiotics includes amoxicillin and co-trimoxazole. The gut microbiota dysbiosis could result in changes in physiological, biochemical and pathological parameters in an individual. Dysbiosis of the gut microbiota has been linked with a number of illnesses and disorders which includes neurodegenerative disorders, inflammatory bowel diseases, type 2 diabetes, asthma, hypertension, anemia, atherosclerosis and many other health issues. Many studies have been conducted to ascertain the cause of these health issues. In Kenya, there is growing

concern regarding prescription of antibiotics especially on children, and most of these antibiotics are obtained over the counter or prescribed unnecessarily in healthcare institutions. Therefore, there is need for more research regarding the toxicological effects of use and misuse of antibiotics on the gut microbiota at an early age and subsequent health effect associated with it. The developing gut microbiota is highly dynamic and can easily be disrupted by external factor, one of the major factors include antibiotic exposure that causes gut dysbiosis linked to wide range of diseases and disorders.

1.3 Objectives of the Study

1.3.1 General Objective

To elucidate the impact of co-trimoxazole and amoxicillin antibiotic therapy on gut microbiota population and subsequent changes in physiological, biochemical and pathological parameters in Swiss mice.

1.3.2 Specific Objectives

i. To determine the effects of co-trimoxazole and amoxicillin antibiotic therapy on gut microbiota population and physiological changes in Swiss mice model.
ii. To assess the effect of co-trimoxazole and amoxicillin induced gut microbiota dysbiosis in the induction of pathological changes in Swiss mice model.
iii. To evaluate the effect of co-trimoxazole and amoxicillin induced gut microbiota dysbiosis in induction of oxidative stress in Swiss mice model.

1.4 Hypotheses

$H0_1$: There is no significant effect of co-trimoxazole and amoxicillin antibiotic therapy on gut microbiota population and physiological changes in Swiss mice model.

$H0_2$: There is no significant effect in pathological changes in Swiss mice model, co-trimoxazole and amoxicillin induced gut microbiota dysbiosis.

$H0_3$: There is no significant effect in co-trimoxazole and amoxicillin induced gut microbiota dysbiosis in induction of oxidative stress in Swiss mice model.

1.5 Significance of the Study

A 2020 survey conducted in Kenyan public hospitals indicated the overuse of antibiotics, especially in children, in both inpatient and outpatient admissions (Maina *et al*., 2020). Much concern has been raised regarding the irresponsible prescription and over-the-counter selling of broad-spectrum antibiotics. The uninformed members of the public are oblivious to the effects of antibiotics on gut microbiota, which play a vital role in maintaining health. They, therefore, stand the risk of dysbiosis. This dysbiosis might lead to immune-pathological responses, physiological changes, and oxidative stress. The study aims to examine the consequences of early antibiotic exposure on developing gut microbiota and evaluating the impact of administering antibiotics with probiotics.

CHAPTER TWO

LITERATURE REVIEW

2.1 Gut Microbiota Population and Effect of Antibiotic Induced Dysbiosis

The gut microbiota represents diverse microorganisms inhabiting the gastrointestinal tract of humans and is usually associated with various health functions, including normal digestion, immunity, and metabolic activity (Guinane & Cotter, 2013). However, these beneficial microbes may be disturbed by using antibiotics, which results in dysbiosis. Such an imbalance may be associated with adverse health conditions, such as inflammation and susceptibility to infection. Thus, antibiotic-induced dysbiosis's impact is importance in management and restoration of gut health.

2.1.1 Gut Microbiota

Gut microbiota are microscopic organisms that inhabit the intestinal tract of humans, animals and even arthropods (insects) (Vázquez *et al*., 2012). The human gut harbors trillions of important bacteria which are beneficial to the health of their host by taking part in degradation of toxins, synthesis of vitamins and defending host against infections (Friedrich, 2008). The gut microbiota has established symbiotic relationship in which they both benefit from each other. This relationship is always interrupted by changes in environmental conditions in the gut such as host immune reaction, entry of a parasite and even introduction of antibiotics into the gut (Zhu *et al*., 2017). Disruptions in microbiome colonies results in alterations of functional features linked to metabolites processed in the intestines, which in turn cause a variety of bacteria-related illnesses and persistent enteric inflammatory conditions (Yoon & Yoon, 2018). Previous studies have demonstrated that the gut microbial composition is an essential component of gut and the brain circuit since the gut microbiota develops nearly concurrently with the brain (Aziz *et al*., 2012; Shan *et al*., 2018). These gut bacteria provide essential features for fermenting indigestible substrates, such as food fibers and endogenous intestinal mucus, promoting the growth of specialized microorganisms that produce gasses and short-chain fatty acids (SCFAs) (Kendall *et al*. 2006).

2.1.2 Human-Mice Relation by Age and Changes in Gut Microbiota

The development of the age relationship between mice and humans is important in coming up with medications which are specific to certain age brackets in experimental

murine models. Therefore, there is growing need for more accurate research results for humans of a certain age bracket. Research has been done to ascertain more precise relation by age between humans and mice models (Dutta & Sengupta, 2016). While weaning in mice takes place between twenty-one to twenty-eight days postnatal (Curley *et al*., 2009). Weaning in human usually begin at the age of six months (Wright *et al*., 2011). The figure below (Fig.1) shows a comprehensive summary of human to mice relation by age.

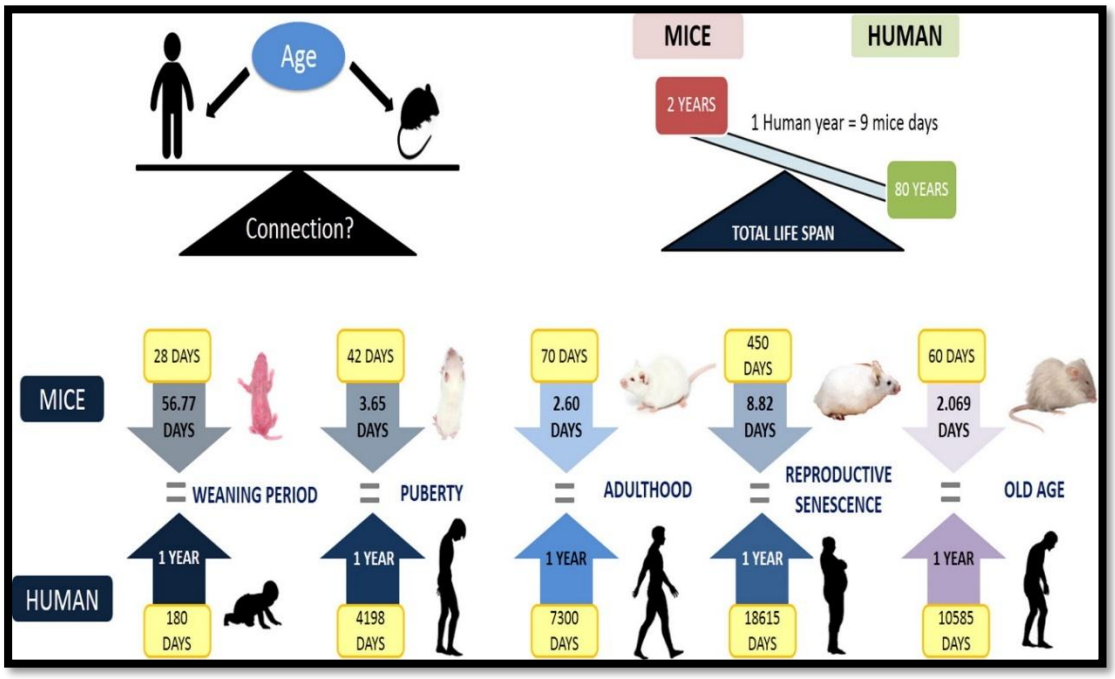


Figure 1: Man, and mice relation by age. The image illustrates the lifespan between mice and human from weaning period to old age. Milestone development in gut microbiota takes place at the weaning period. Image adopted from (Dutta and Sengupta, 2016).

2.1.3 Infant Gut Microbiota and Factors for Variation

During the perinatal period, a variety of microorganisms, primarily from the mother and the immediate surroundings, first invade the gut of an infant (Wang *et al*., 2024). However, profound milestone in gut microbiota is realized at the weaning stage when the infant begins to feed on solid foods. The intimate relationship between gut microbiota function and health is now well recognized (Kim *et al.,* 2020). The infant gut microbiota undergoes dynamic changes with time depending on the environmental exposure, diet, health status, antibiotics introduction and age (Sugino *et al*., 2021). The bacteria in the alimentary canal interact with the host and develop symbiotic relationship in which both benefit from each other. The bacteria depend on the host to

provide nutritive environment and conducive environment for their growth and development and the host benefits from the microbial organisms by fighting pathogenic microorganisms, breakdown of food, adsorption of ions and production of vitamin K (Penders *et al*., 2006). However, a study shows that infants alimentary canal microbiome varies depending on mode of delivery and feeding method. Neonates born by caesarean section differs from those delivered vaginally both in timing of colonization and composition. This is due to presence of vaginal microbiome which may colonize the neonatal gut microbiome (Palmer *et al*., 2007). Studies are still underway to ascertain the possible long-term effects of disruption of the infant gut microbiome to a person at an older age.

2.1.4 Effects of Antibiotics on the Gut Microbiota

Antibiotics are medications that slows down bacterial growth or kills bacteria. They are produced as secondary metabolites by bacteria such as *Aspergillus nidulans* and *Penicillium chrysogenum* or artificially developed using science to kill or block the growth of bacteria. However, the drug's improper use over the years has led to the emergence of bacteria that are resistant to drugs (Magiorakos *et al*., 2012). Despite their importance in treatment of bacterial infection, antibiotics have adverse implications to the body such as disruption of the gut microbiota resulting in gut dysbiosis (McDonnell *et al*., 2021). Antibiotics treatment on infants impacts numerous physiological factors of growth and may lead to prolonged gut microbiota disturbance (Uzan *et al*., 2021). In contemporary obstetric and neonatal care, the utilization of perinatal broad-ranging antibiotics has become standard practice. Growing research indicates that early usage of antibiotics is linked to significant changes in the gut microbiome and a number of conditions, such as atopy, IBD, diabetes, and obesity (Eck *et al*., 2020). Antibiotics are classified into two main categories based on the mode of action that is bactericidal and bacteriostatic. Bactericidal kills the bacteria while bacteriostatic antibiotics inhibits growth of bacteria (Giguère, 2013).

2.1.5 Amoxicillin and its Pharmacological Effects

Amoxicillin is among commonly prescribed antibiotics globally especially in healthcare care institutions. It is an amino-penicillin antibiotic, which is developed through addition of an amino group to penicillin to alter resistance to the antibiotic

(Shah *et al*., 2023). Amoxicillin binds with proteins called penicillin-binding proteins (PBPs) on the underlying surface of the microbial cell membrane, inhibiting bacterial cell wall formation (Miyachiro *et al*., 2019). The inactivation of PBPs hinders the cross-linking of peptidoglycan, compromising the structural integrity and rigidity of the bacterial cell wall. This suppresses the productive activities that constitute bacterial cell-wall formation, resulting in the depreciation of the cell wall of bacterial cells subsequently causing cell rupture (NCBI, 2022). Amoxicillin is a member of a class of antibiotics used to treat dental infections and respiratory infections, among other chest infections. It is also used for managing stomach ulcers in addition to other medications and antibiotics. It is a pediatric medication used to treat infections of the ears and chest. Pseudomembranous colitis can result from treatment with amoxicillin which results in massive growth of bacterium *Clostridioides difficile* in the colon. Responses to hypersensitivity consist of irritation, toxic epidermal necrolysis, angioedema, erythematosus multiforme, Stevens-Johnson condition, and serum sickness-like symptoms (Castle, 2007).

Amoxicillin is broad-spectrum antibiotic and belonging to class of antibiotics known as penicillin. This medication is approved for the treatment of infections like otitis media, rhinopharyngitis, gastritis, *H. pylori* infection, lower respiratory tract infections caused by β-lactamase-negative organisms such as *Pneumococcus species*, *Streptococcus species*, *Staphylococcus species*, and *H. influenzae*, acute bacterial sinusitis, ailments of the skin and urinary tract infections (Shah *et al*., 2023).

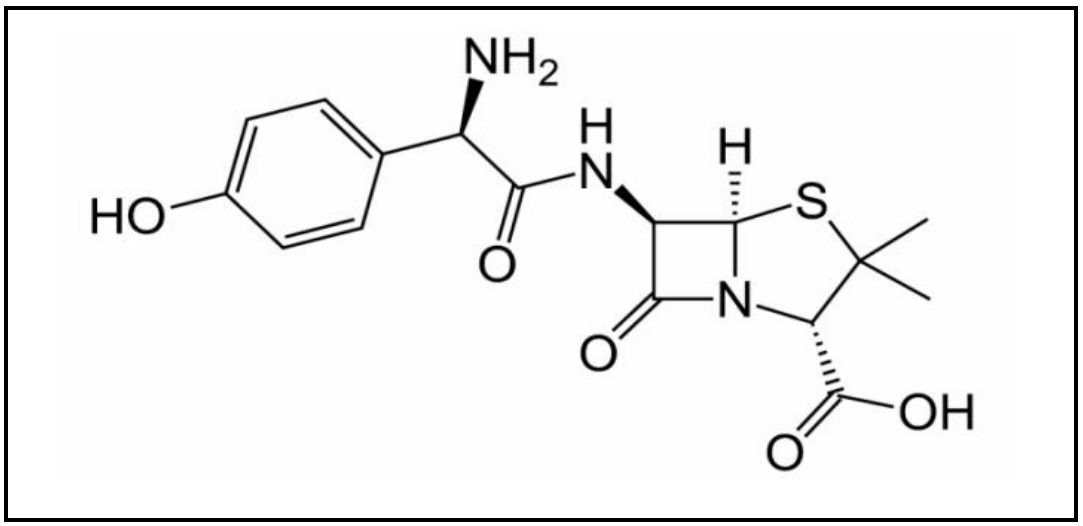


Figure 2: Image showing chemical formula of Amoxicillin ($C_{16}H_{19}N_3O_5S$) (Adapted from Ramos *et al*., 2012).

2.1.6 Cotrimoxazole and its Pharmacological Effects

Co-trimoxazole is broad-spectrum antibiotic belonging to a class of antibiotics called sulfonamides. It is formed by combination of antibiotics trimethoprim and sulfamethoxazole (Sunil et al., 2017). Co-trimoxazole works by inhibiting growth of bacteria and used in treatment of bacterial infections including pneumonia, bronchitis, and infection of urinary tract, ears, and intestines and traveler's diarrhea (Evans, 2020). World Health Organization recommends that people living with or exposed to HIV should be administered with co-trimoxazole depending on their CD4 cell count to reduce risk of opportunistic diseases and also reduces risk of toxoplasmosis an infection affecting the brain (WHO, 2016). However, despite its vital role in treatment of bacterial infection if used improperly it causes some health side effects such as skin reactions like rash, ulcers of mouth or genitals and even anemia. The side effects are more pronounced when taken with other drugs such as Zidovudine or ganciclovir (Blumenthal et al., 2019).

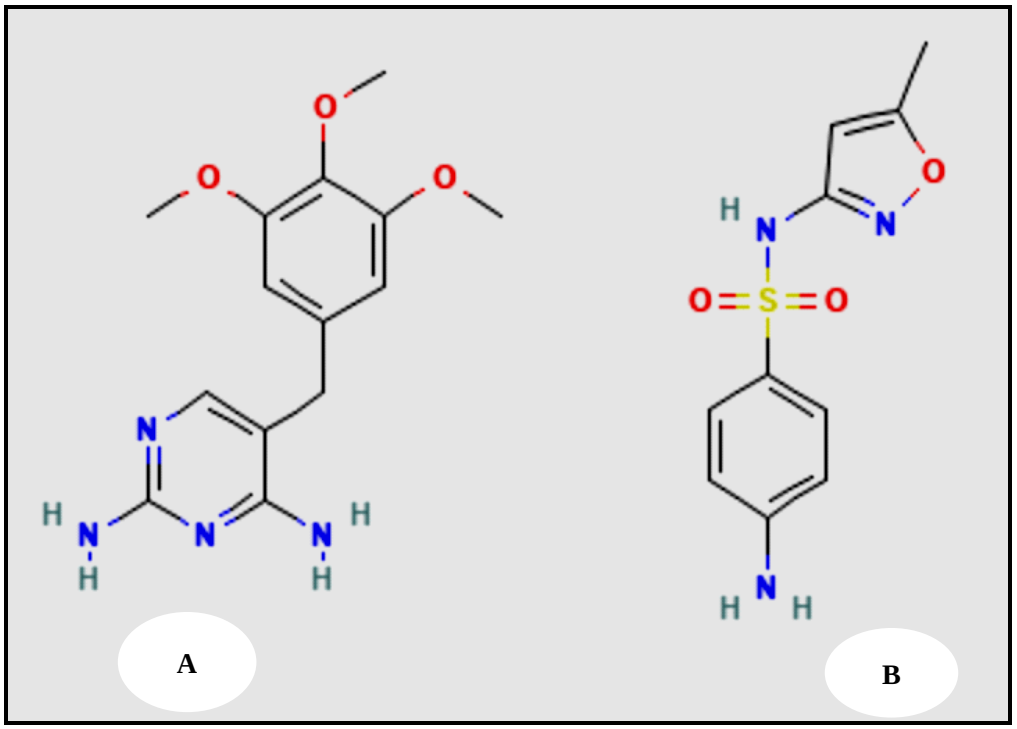


Figure 3: Chemical structures of (A) trimethoprim and (B) Sulfamethoxazole which are combined together to form co-trimoxazole ($C_{24}H_{29}N_7O_6S$). (Image adapted from NCBI, 2021).

2.2 Dysbiosis and its Associated Health Effects

Dysbiosis is a condition in which the gut microbial population is altered resulting in altered symbiotic balance between the gut microbiome and the host is interrupted due to exogenous or endogenous modifications or both. A disruption in the gut microbiota produces disturbances among numerous species of bacteria, which could give rise to the introduction of pathogenic bacteria that may invade the host's cells and result in an illness. The gut microbiota also plays an important function in displaying the activation of signaling molecules and identifying bacterial epitopes by gastrointestinal lining and mucosal immune cells (Meng *et al*., 2020). The gut microbiota assists in conveying hormonal signals, immune system modulation, metabolism of dietary nutrients, and production of end metabolic products like vitamins and short-chain fatty acids (SCFAs), including acetate, butyrate and propionate (Meng *et al*., 2020).

These SCFAs are involved in multiple roles, including managing inflammation and peripheral immune defense of adipose tissue, immune responses, and oral tolerance (Yoo *et al*., 2020). Proteases produced by intestinal microbiota can also promote health and protect against diseases. However, not all gut microbiota benefit host survival, as some bacteria like *H. pylori*, *E. coli* and *F. nucleatum* can give rise to pathogenic reactions within the host (Meng *et al*., 2020). Gut dysbiosis is present in many intestinal disorders, such as inflammatory bowel disease, rectal cancer and osmotic diarrhea (Dixit *et al*., 2021).

2.2.1 Gut Dysbiosis in Infants and Disease Risk

Infant gastrointestinal dysbiosis is defined as an extreme imbalance of helpful and potentially infectious bacteria in an infant's gut system. The most common pathogenic organisms found in the newborn gut which might prevail during dysbiosis comprise *E. coli*, *K. pneumoniae*, and *C. difficile* (Gao *et al*., 2019). Damage to an organ when it is in a morphogenetic stage can have impacts on the capability of the organ to regenerate and grow, as well as impacting the functioning of other systems, depending on the organ. The development of the gut microbiota is most crucial during early childhood with the three initial years of life with age-related changes in taxa prevalence due to the environment, diet, and medications (Underwood *et al*., 2020). Excessive use of antibiotics leads to a drastic decrease in the amounts of good bacteria in the gut such as

Lactobacillus and *Bifidobacterium* (Yoon & Yoon, 2018). Therefore, dysbiosis of the gut is a potent risk factor for neonatal sepsis, and alteration in the earliest time frame of microbiota may also act as a sign that indicates sepsis (Lee *et al*., 2021). Gut microbes evolve in tandem with the host immunological system; thus, a disruption in the gut microbial influences an infant's immune response. Treatment with antibiotics, probiotics, prebiotics, and fecal transplants can all help to reverse infant dysbiosis (Valdes *et al*., 2018).

2.2.2 Impacts of Antibiotics on Gut Microbiota and Host Health

The human gut hosts a wide variety of microbes, which include archaea, fungi, bacteria and viruses. The process of gut microbiota colonization is assumed to begin in the early postnatal period after birth when the infant is bearing contact with the maternal microbiota and other aspects of the environment. Microbes have coexisted with mammals over a long period of time and have developed a relationship in which the host animal harbors the microbiota in its gut providing them with conducive environment to grow and multiply. Microorganisms help the host in production of important metabolites, in breakdown of food materials and help the host by inhibiting growth of pathogens hence boosting host immune system (Zhang & Chen, 2019).

Disturbance to this interdependence between the microbes may lead to disease to the host since pathogenic microorganisms may develop due to competition for nutrients in the gut hence causing infection. Disruption may be due to environmental stimuli or administration of antibiotics into the gut. Despite the huge benefits noted when using antibiotics in treating infections, it has been realized that these compounds exert significant negative impacts on the intestinal microbiota and the host immune response (Konstantinidis *et al*., 2020). Antibiotics lead to gut dysbiosis and disrupt gut microbiota in children and also in adults, which causes several illnesses including diabetes, inflammatory bowel conditions, obesity, asthma and superinfection in sickly patients (Zhang & Chen, 2019).

Other adverse consequences of misuse of antibiotics in the gut include degradation of the cells lining the intestines which includes endocrine cells and enterocytes (Konstantinidis *et al*., 2020). Different research works have demonstrated that

antibiotics-induced intestinal dysbiosis increases the innate intestinal immunological system, alters the landscape of the pain-related sensory system, and decreases visceral pain-associated responses. The latter is linked with modifying gut neuro-immune sensory systems depending on commensal microbiota and is associated with viscera sensitivity (Aguilera *et al*., 2015). Therefore, the study of the effects of administering antibiotics and subsequent effects on the gut microbiota to establish cause of observed host health is an important field to dwell into especially on the infants.

2.2.3 Effects of Antibiotic-Induced Dysbiosis on Weight of Infants

Research has been conducted to determine the association between infant consumption of antibiotics and weight gain (Gerber *et al*., 2016). Study also established that shifts in gut microbiota composition can explain overweight clinical manifestations (Davis, 2016). Prenatal antibiotic use can lead to developmental disorders throughout infant growth, even if the fetus is exposed only to the antibiotics used by the mother (Turta & Rautava, 2016). For instance, the research conducted showed that if pregnant women are treated with antibiotics while at the second and even at the third trimester of pregnancy, their children have an 84% enhanced risk of developing obesity in childhood up to the age of 7 years (Mueller *et al*., 2015).

The exact mechanisms behind the causative role of the composition of the microbiota in host metabolic function are not known yet. However, several pieces of evidence have identified the link between the initial change in the microbiota due to the use of antibiotic treatments with long-lasting metabolic effects, including the microbial changes in the copies of genes in mice involved in carbohydrate metabolism into SCFAs and increased levels of colonic SCFAs following the early-life usage of antibiotics (Cho *et al*., 2012; Neuman *et al*., 2018). Tian *et al*. (2019), demonstrated that low doses of antibiotics given to infant mice led to increased adiposity, altered the liver homeostasis of cholesterol and lipids, and increased predisposition in a high-fat diet. Hence, evidence supports a link between changes in gut microbiota population, obesity, and antibiotic prescriptions (Angelakis *et al*., 2018).

2.2.4 Impacts of Gut Microbiota Dysbiosis on the Immune System

The symbiotic link between the microbes in the gut and the host is resulting in a mutually beneficial partnership between gut microbiota and the host's immune system, both innate and adaptive, in controlling the equilibrium of the gut and suppressing inflammation (Yoo *et al*., 2020). The microbiome in the gut has a major function in the training and development of major aspects of immune system and the spleen, on the other hand, assists the host immune system in sustaining the microbe-host relationship (Zheng *et al*., 2020). The gut microbiota takes part in the degradation of proteins and complex carbohydrates, biosynthesis of vitamins and putting out many numerical metabolic products that can bridge gut epithelial and immune cells. The defense mechanism end product includes the mucosal barrier production by gut epithelial cells to isolate microbiota from host immune cells and lower the gate permeability. Studies have indicated that disturbance in the composition of gut microbial communities can cause inflammation (Yoo *et al*., 2020).

Depression in microbiota diversity has been related to the high prevalence of gastrointestinal diseases, pro-inflammatory properties, and the low gut bacterial density is a feature of chronic disease (Liu *et al*., 2020). Changes in the symbiotic relation with the mucosal immune system results in rise number of disease-causing bacteria and related metabolic implications due to impaired intestinal barrier and enhanced susceptibility to infections (Pickard *et al*., 2017). Thus, gut dysbiosis or microbiota imbalance may also impair immune signaling, promoting inflammation, oxidative stress, and insulin resistance (Yoo *et al*., 2020). Long-term gut dysbiosis and progressive translocation of bacteria and their metabolites through the mucosal barrier may enhance the risk for type 2 diabetes, autoimmune diseases, cardiovascular disorders, and inflammatory bowel disease (Yoo *et al*., 2020). Some bacteria in the gastrointestinal system ferment complex carbohydrates to produce SCFAs, which are used as sources of energy by colon epithelial cells and influence the immunological reaction of the host through interactions with immune cells and signaling pathways.

Short chain fatty acids are involved in activating, recruiting, and differentiating immune cells, such as macrophages, neutrophils, T-lymphocytes, and dendritic cells. In addition, SCFAs reduce the production of pro-inflammatory cytokines. IL-6, IL-12, and

TNF-α reduce their output by activating macrophages and dendritic lymphocytes; SCFAs can alter the physiological functions of immune cells (Yoo *et al*., 2020).

Interleukin-10 (IL-10) is a cytokine, a hormone-like substance that lowers or reduces inflammation by inhibiting the release of cytokines such as TNF- α, IL-6, and IL-1 by activated macrophages. Interleukin-10 may further increase endogenous anti-cytokine levels while reducing the levels of pro-inflammatory cytokine receptors (Zhang & An, 2007). Interferon (IFN)-γ is an interleukin and a critical inflammation and immune response modulator. This cytokine may play a role in orchestrating the polarization of inflammation by inducing other pro-inflammatory cytokines, including TNF-α and IL-6 (Biolo *et al*., 2006).

2.2.5 Impact of Gut Dysbiosis and Effect on Metabolism and Induction of Metabolic Acidosis

Gut microbiota has been found to influence the metabolic pathway of lipids and alter the levels of lipids in the blood and tissues of the host (Ridler *et al*., 2019). The gut microbiota is involved in the regulation of bile acid and SCFAs. In this regard, bile acids are extensively emulsified during fat digestion (Chattopadhyay *et al*., 2022). In contrast, the gut microbiome mediates the modulation of lipid balance and conversion of conjugated bile acids. The gut microbiota actively contributes to the biosynthesis of secondary bile acids, such as deoxycholic and lithocholic acid, the choline metabolite, and short-chain fatty acids (Sitkin *et al*., 2016). Disruption of microbiota is associated with metabolic disorders, including diabetes, obesity, and non-alcoholic fatty liver disease (Hur & Lee, 2015).

The evidence from mouse studies indicates that the gut microbiota, in conjunction with the diet, controls the host lipid homeostasis and lipid concentrations in sera and tissues (Schoeler & Caesar, 2019). Alterations in the composition of the gut microbiome have recently been linked to dyslipidemia, including non-alcoholic liver disease and atherosclerosis (Lei *et al*., 2022). A cross-sectional study by Fu *et al*. (2015) highlights the significant relationship between gut microbiota composition and serum triglycerides, HDL cholesterol, and LDL cholesterol.

Metabolic acidosis is when acid levels in the body rise because of increase in production or consumption of acids increases or when there is a decrease in levels of bicarbonate through gastrointestinal loss or kidney excretion (Kraut & Madias, 2010). This leads to a decreased blood pH since there is a reduction in serum bicarbonate levels (Lim, 2007). The most common causes of acute types of metabolic acidosis include overproduction of ketoacids or lactic acids, while chronic metabolic acidosis usually implies bicarbonate loss and renal tubular acidosis. The values calculated for anion gap, obtained by subtracting the sum of [HCO3 −] and [Cl −] from [Na +], help in enabling the categorization of the disorders into the normal hyperchloremic anion gap or with an elevated anion gap (Kraut & Madias, 2010). A critical component of the gut microbiota is involved in the fermentation process and the synthesis of SCFAs. These SCFAs take part in in regulating the absorption and secretion of electrolytes in the gut. Sodium and water absorption are influenced by the gut microbiota ensuring proper fluid balance in the body. Dysbiosis can lead to various gastrointestinal issues and electrolyte imbalances.

2.2.6 Effect of Gut Microbiota Dysbiosis on Gut-Brain Axis

The brain and gastrointestinal system communicate bidirectionally through a network called the microbiota-gut-brain axis (Sherwin *et al*., 2016). The gut microbiota plays a huge role in the bidirectional relation between the brain and gut and is essential in host health and at immunological responses. Metabolic disorders, including diabetes, obesity, and major depressions, as well as neuropsychiatric disorders like schizophrenia and autism, are all modified by alterations in gut flora. Actually, recent research has demonstrated that gut microbiota, including commensalism and gut pathogens, control the immunological and central neurological systems. Gut bacterial commensals are in contact with the brain (Evrensel & Ceylan, 2015).

These communications occur through the stimulation of the vagus nerve, through factors such as cytokines, the traffic of metabolites including short-chain fatty acids, and enteroendocrine cell activation. Through these routes of communication, the microbiota gut-brain axis modulates other key body functions such as neurotransmission, neurogenesis, neuroinflammation, and neuroendocrine signalling (Sherwin *et al*., 2016). Gut bacteria are capable and have the capacity for synthesizing

and transporting neurochemicals, including serotonin and gamma-aminobutyric acid, to affect the gut-brain axis. These bacterial products induce several factors in enteroendocrine cells, which may involve several neuropeptides produced in the bloodstream and the ENS, trigger immune cells, further releasing cytokines or stimulating the vagal nerve (Evrensel & Ceylan, 2015).

It might influence the structure, connectivity, and function of neurons through neurotransmission and neurogenesis and is associated with neuro-inflammation (Ceppa *et al*., 2020). Animal studies indicate that intervention in gut microbiota balance increases phenylalanine and isoleucine in the periphery and the production of pro-inflammatory Th1 cells. Both phenylalanine and isoleucine have been reported to be able to pass through the brain barrier from blood and to promote neuroinflammation (Wang *et al*., 2019; Ceppa *et al*., 2020).

2.2.7 Impacts of Gut Microbiota Dysbiosis on Hematopoiesis

The gut microbiota represents a complex viral, bacterial, archaeal, and fungal community inhabiting the human body and playing an essential role in human health. Most of the research currently conducted aims to establish the involvement of microbiome in hematopoiesis using murine models (Yan *et al*., 2018). This is evidenced by germ-free mice, known to have abnormalities in bone marrow cell populations. Germ-free mice have reduced populations of hematopoietic cells and progenitor cells, abnormal numbers of splenic myeloid cells, and diminished T-cell activity compared to SPF mice (Yan *et al*., 2018).

Studies have shown that oral antibiotics administration depletes gut bacteria causing suppressive effects on hematopoiesis (Zhang *et al*., 2015). Therefore, dysbiosis of the gut by antibiotics is linked to hematopoiesis suppression in humans. Many antibiotics have been linked to cytopenia, which includes anemia, clotting disorders, pancytopenia, and neutropenia (Anderson *et al*., 2007; Yan *et al*., 2018). According to Zhang *et al*. (2015) the gut microbiota sends signals through toll-like receptors (TLRs) and MyD88 gene that activates neutrophils to maintain the immunity bringing about a balanced activation. Josefsdottir *et al*. (2017) provides a clear illustration of the impact of broad-spectrum antibiotics treatment on hematopoiesis in mice, showing that microbiota

contributes to steady-state hematopoiesis through the regulation of Stat1 signaling. Steady-state hematopoiesis is also affected because antibiotics reduce microbial density in the intestine (Yan *et al*., 2022). As for the phenomena associated with hematopoiesis, it has been demonstrated that replenishment of the intestinal microbiota reverses the depression of this function in mice that were administered antibiotics (Josefsdottir *et al*., 2017; Yan *et al*., 2022).

2.3 Gut Dysbiosis Role in Induction Oxidative Stress and Organ Damage

Oxidative stress is a relative shift in balance between antioxidants and oxidants with significant elevated levels of oxidants leading to disturbance of redox signaling and balance and molecular changes (Dumitrescu *et al*., 2018). Reactive oxygen species can be divided into two groups: free-radical ions and non-radical ions. Free radicals refer to molecules that are characterized by the presence of one or more unpaired electrons that make the molecule reactive. When two free radicals get a chance to share their unpaired electrons, the formation of non-radical species occurs (Birben *et al*., 2012). Enteric commensal bacteria-induced signals trigger the release of reactive oxygen species (ROS) in gut epithelia and other adjacent cells. In addition to causing damage, physiologically generated ROS act as second messengers in multiple signaling pathways induced by cytokines and growth factors and are involved in cellular communication (Francesca & Pietro, 2017).

At higher levels, though, the oxidative species is potentially neurotoxic, leading to bio-molecular alteration such as lipid, protein and deoxyribonucleic acid (DNA) oxidation that may lead to a range of cellular pathologies including cellular death (Massaad & Klann, 2011; Dumitrescu *et al*., 2018). However, the human body has a mechanism to counter balance the rising levels of oxidants by producing antioxidants. Some of the antioxidant molecules produced by the body includes catalases, glutathione (GSH) and oxidases (Birben *et al*., 2012). Glutathione is a major marker for oxidative stress in the body, because it is highly abundant in cellular compartments and is a highly soluble antioxidant. Decreased levels of glutathione in tissues are an indication of vulnerability of cells to oxidative stress (Gawryluk *et al*., 2011).

Malondialdehyde (MDA), which is produced when reactive oxygen species break down polyunsaturated lipids, is a sign of oxidative stress in tissues. The majority of free radical production occurs in red blood cells. Multiple intracellular signaling is known to be activated by oxidative stress, resulting in apoptosis or cell overgrowth and subsequent organ malfunction, including failure of the kidneys, liver, brain, lungs, and spleen, among other essential organs (Ogura & Shimosawa, 2014).

2.3.1 Nitric Oxide as Measure for Oxidative Stress

Nitric oxide (NO) is a signal molecule that is critically involved in a variety of cell types and tissues. Nitric oxide synthase (NOS) is the enzyme that takes part in the for production of NO, an essential signal molecule that participates in many physiological processes (Förstermann & Sessa, 2012). NOS catalyzes a reaction where it takes L-arginine and converts it into L-citrulline with the help of such cofactors as Nicotinamide adenine dinucleotide phosphate (NADPH), tetrahydrobiopterin, and oxygen (Förstermann & Sessa, 2012). There are three categories of isoforms of NOS involved in the formation of NO via the oxidation of L-arginine into citrulline (Griendling & FitzGerald, 2003). Many cells of the body actively produce the neuronal NO and endothelial nitric oxide. Inducible NO is elicited in response to inflammatory cytokines and other inflammatory mediators and can be trans-scripturally regulated. Macrophages also synthesize nitric oxide in cytotoxic concentrations in some cases. A pink azo colour develops when nitrite reacts with sulfanilamide and N-(1-naphthyl) ethylene diamine dihydrochloride (NED). The azo dye formed in this reaction can be determined using the absorbance of the compound at 550 nm with the help of a UV-visible spectrophotometer (Laythan & Water, 2009).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Site

This study was performed at the Technical University of Kenya School of Health and Biomedical Science laboratories, Nairobi, Kenya.

3.2 Experimental Animals

The experimental animals utilized in this study were laboratory-bred three-week-old naive male Swiss mice with an average weight of 17.5 + 2 g, acquired from the Institute of Primate Research (IPR), Kenya. They were kept in the animal care facility at the Technical University of Kenya and fed on mouse chow and water provided *ad libitum*. They were kept in a well-controlled environment with temperatures ranging between 21 - 25°C and controlled humidity, with an equal hour cycle of day and night of 12 hours each day. The mice were given three days to adapt to the new environment before the treatment began. The mice weight was taken before antibiotic treatment and recorded once every week before they were administered antibiotic treatment.

3.3 Experimental Design

A complete randomized design (CRD) was used in which male Swiss mice were selected randomly and placed into five separate cages, each cage with six mice (n=6). The mice were kept in polypropylene cages of size 43x27x15 cm and beddings made of wood shavings. The group one was the normal control group and only took distilled water and mice chow. The second group was administered 9.62 mg/Kg of amoxicillin; group three was administered 15 mg/Kg of septrin. Group four was administered the alternate treatment of amoxicillin and septrin, and group five were administered the alternate treatment of amoxicillin and septrin, followed by the administration of a probiotic two consecutive days post-drug administration. The probiotic administered was avalife probiotics each capsule containing 3 billion colony forming units (CFUs) comprising of *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Bifidobacterium longum*, *Bifidobacterium bifidium*, and *Streptococcus thermophilus*. Administration was done orally via gastric gavage, and each mouse was marked with a visible yellow dye for identification.

The mice were given a single dose per day for four days in a row, followed by a five-day break. The antibiotics were chosen based on their preference for treating bacterial infections. They are mainly prescribed by pediatricians and also act as an indicator of the general impact of antibiotics (Rajesh & Singhal, 2013). The mice were put in similar environmental conditions, in the cages, and were fed with the same meal and water. The same people also handled them to normalize the gut microbiota (Caputi *et al*., 2017). The experimental design is summarized as shown in the table below (Table 1);

Table 1: Table showing a summary of the experimental design

Groups of Mice (n=6)	Treatment	Study Period
Group 1 (Normal control)	Distilled water	63 days
Group 2 (Experimental group)	9.62mg/kg Amoxicillin	63 days
Group 3 (Experimental group)	15mg/kg Septrin	63 days
Group 4 (Experimental group)	Alternate administration of 9.62mg/kg Amoxicillin and 15mg/kg Septrin	63 days
Group 5 (Experimental group)	Alternate administration of 9.62mg/kg Amoxicillin and 15mg/kg Septrin followed by a Probiotic	63 days

3.4 Determining Total Body Weight of Mice and Weight of Selected Organs

The weight measurement of mice was recorded once every week at the beginning of antibiotic treatment. After 63 days of administration of amoxicillin and septrin, the study period elapsed, and each mouse's total body weight was measured using an analytical weighing balance (Mettler PM34, DoltaRange®). The mice were euthanized using 10% ketamine, and the mice were dissected. Their selected organs, including the brain, lungs, liver, kidneys, and spleen, were obtained, and the weight of each organ was measured using analytical weighing balance and values recorded to determine relative organ weight. The following formula was utilized in determining ROW:

Relative organ weight = [organ weight/body weight] ×100 (Mossa *et al*., 2015)

3.5 Determination of Microbial Diversity on Treatment with Antibiotics

3.5.1 Preparation of Culture Media

MacConkey agar powder (LabMal MacConkey Agar 500 g, Oxoid, Hampshire, United Kingdom), 30.1 g dissolved in 600 ml of distilled water. The solution was heated gently until MacConkey agar powder was completely dissolved. The next step is sterilization

by autoclaving at 15 lbs pressure for 15 minutes. The media was allowed to cool at room temperature. The procedure was done following manufacturer protocol. The sterilized MacConkey solution was then poured into sterile glass plates on a flat sterile bench and allowed to solidify.

3.5.2 Culturing and Determination of Colony-Forming Units

The gut swab was taken from the lower end of the alimentary canal using a sterile swab. Colony forming units was determined by taking a gut swab and performing a five-fold serial dilution (Budding *et al*., 2014). The sample was then spread on a media plate and incubated at 37°C for 24 hours. The microbes were examined to determine their microbial colony population and colony-forming units (CFU). Formula utilized:
CFU= (Number of colonies x Dilution factor/Volume of culture plate) x 100.

3.6 Blood Sample Collection

The blood sample collection was collected via cardiac acupuncture following Parasuraman *et al*., (2010) protocol of blood collection in small animals. The whole blood sample for complete blood count (CBC) was collected into EDTA tubes. It was put on an automated shaker for 20 minutes, waiting CBC to be done. For blood serum analysis, the blood was placed at room temperature and allowed to settle for an hour, after which it was centrifuged at speed of 1000 rpm at 4 °C for a period of 5 minutes. The supernatant obtained was collected and stored at -20°C as it awaited analysis.

3.6.1 Biochemical Analysis of Blood Serum

The blood collected via cardiac acupuncture was transferred into sterile Eppendorf tubes. The procedure for collecting blood via cardiac puncture was done following Kaur *et al*. (2019) protocol. The blood was to clot for one hour at room temperature, then centrifuged at 1000 rpm for 5 minutes at 4°C. The serum formed the supernatant, which was transferred into sterile Eppendorf tubes and then analyzed using an auto-analyzer. The serum auto-analyzer was able to determine the levels of Alanine aminotransferase (ALT), direct bilirubin, Aspartate aminotransferase (AST), total proteins, alkaline phosphatase (ALP) and gamma-glutamate transferase (GGT) markers for the liver function. It also indicated levels of creatinine (CREAT) and urea, which are markers of kidney function.

3.6.2 Determination of hematological indices

The blood was drawn using a 26-gauge 1 ml syringe via cardiac puncture and transferred into EDTA tubes, after which they were sealed and put in an automated shaker while awaiting analysis. The blood samples from each of the mice were analyzed using a blood auto-analyzer, which provided a complete blood count (CBC) and their results were recorded accordingly. The results that were obtained from the complete blood count were given values for packed cell volume (PCV), hematocrit, mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), hemoglobin levels (HGB), mean corpuscular hemoglobin (MCH), and White blood cell count (WBC) (Rathkolb *et al*., 2013).

3.7 Evaluation of Pathological Changes in Mice

3.7.1 Cytokine-Specific Sandwich ELISA

In this technique, biomarkers for pro and anti-inflammatory Cytokines were utilized by cytokine-specific ELISA kits according to the manufacturer's recommendation (Thermo Fisher Scientific, 900-T00). The method used antigen-antibody reactions where Streptavidin-HRP concentrate was diluted as the secondary antibody for IFN-y, whereas Avidin-HRP concentrate was diluted as the secondary antibody for TNF-α and IL-10 (Chiswick *et al*., 2012; Ge *et al*., 2017).

3.7.2 Histopathological Examination of Selected Organs

The following organs were harvested; the brain, lungs, liver, kidneys, heart and spleen and washed using phosphate-buffered saline (PBS), fixed using 10% formalin and deep frozen at -70°C. Before analysis, the samples were dehydrated in changing ethanol concentrations in ascending order of 50%, 70%, 90%, 95%, and 100% for 30 minutes. Tissues were then embedded in paraffin wax using an automatic tissue processor. In each thin sections of 5 µm thick slices were processed using HM 310 microtome. The sample slices were mounted onto Mayer's egg albumin-coated glass slides.

The slides were further dewaxed using two changes of xylene for two minutes. This was followed by rehydration by changing ethanol concentration in descending order grades of 100%, 95%, 90%, 80%, 70% and 50% for 30 minutes and washed using tap water. Gradual removal of water was done to avoid sudden shrinkage of tissues and

lysis of cells. The slices were stained using hematoxylin, followed by 1% eosin for 2 minutes (Slaoui *et al*., 2011). The slices were then dehydrated in ascending grades of Ethanol 70%, 80%, 90%, 95 and 100% for 30 minutes. The slices were cleaned three times using xylene; then, the slices were mounted using Dibutylphthalate polystyrene xylene (DPX) for microscopic examination (Bloch *et al*., 1990).

3.8 Effect of Antibiotic-Induced Dysbiosis on Induction of Oxidative Stress

3.8.1 Determination of Nitric Oxide Levels in Serum

Griess Reagent Kit for Nitrite Determination (G-7921) (Thermo Fisher) was used to determine Nitric oxide levels in the serum. Nitric oxide was determined by adding 200 µl of 1x reagent diluent into the blank wells and 50 µl of nitrate standards into the appropriate wells. Followed by adding 50 µl of 1x reagent diluent into the zero standard wells. Further, 50 µl of the samples were added into the appropriate wells. Followed by adding 25 µl of diluted NADH into all zero standard, standard, and sample wells. 25 µl of diluted nitrate reductase was added into all zero standard, standard, and sample wells. The plates were tapped gently to mix the contents. The plate was then sealed and incubated at 37°C for 30 minutes. Then 50 µl of Griess reagent I was added into each well, except the blank wells. This was followed by adding 50 µl of Griess reagent II, except for the blank wells, into each well. The plate was tapped gently to mix the contents. At room temperature the plate was incubated for 10 minutes. The plate reader was blanked against the blank wells, and the optical density read at 540 – 570 nm according to the manufacturer protocol. The mean optical density was manually subtracted from the blank wells from all readings.

3.8.2 Evaluation of Oxidative Stress on Tissues

Body organs were harvested, included the lungs, spleen, brain, kidney, heart and liver (Mahassni *et al*., 2017). The organ samples, 0.2 g was homogenized in ice water (4°C) in 0.5 mM Tris buffer at pH 7.5. This was done in the presence of a protease inhibitor cocktail to a final concentration of 100% (W/V). The homogenate was then transferred into 1.5 ml micro-centrifuge tubes and stored in liquid nitrogen, awaiting further analysis (Höring *et al*., 2021).

3.8.3 Determination of Glutathione (GSH) Concentration

Glutathione concentration was determined by the addition of 20 microliters to the standard solution and the specific 3 replicates of selected organs supernatants in a 96-well plate in triplicates, followed by the addition of 100 microliters of 5,5-Dithio-Bis (2-Nitrobenzoic Acid) (DTNB) (Caito & Aschner, 2015). The plates were then incubated at 37°C for 10 minutes, and their absorbance was taken at 405 nm on a microplate reader (R and D systems, Minneapolis, MN) at 30, 60, 90, and 120 seconds. From the time intervals selected, the time interval that produced the best GSH standard graph was used to determine GSH concentrations of the organs (Browne *et al*., 1998). High levels of GSH indicates active oxidative stress.

3.8.4 Determination of Malondialdehyde (MDA) Levels

The removal of MDA attached to phospholipids found in the membrane, organ homogenates underwent hydrolysis using a 3.4 mol/l NaOH solution. For the elimination of proteins, 3.4 mol/l of hydrochlorate (HClO4) was added and centrifuged at 3000 rpm for 10 minutes. The resulting mixture was allowed to react with a 0.4% solution of 2-thiobarbituric acid (TBA) at 95^{o}C for 40 minutes (Musalmah *et al*., 2005). Malondialdehyde levels were evaluated using thiobarbituric acid reactive species (TBARS) tests and diene-conjugated species output (Janero, 1990).

In a 10-mL test tube, 1 mL of standard MDA solution and 1 mL of TBA was added. Three replicates of organ homogenates (0.5 ml each) were combined with an identical volume of 0.67% thiobarbituric acid. Heat for 30 minutes at 92-96oC. The test tubes were cooled to room temperature, and the generation of thiobarbituric acid reactive species was measured at 535nm with a spectrophotometer. The calibration standards were repeated (n = 3) using the above approach. A blank sample (n = 5) was redone, this time using acetic acid instead of the standard. (Lovric *et al*., 2008; Zeb & Ullah, 2016). The production of diene-conjugate was assessed by isolating lipids from an assortment of chloroform: methanol (2:1, v: v) mixtures. The resultant organic phase was measured at 234 nm. The findings were presented as malondialdehyde and diene equivalents per milligram of protein.

3.9 Statistical Analysis

The raw quantitative data of colony forming units, body weight, relative organ weight, hematological indices, AST, ALT, ALP, creatinine, urea, albumin, total protein, cytokines, GSH, MDA and NO obtained were captured in an Excel sheet and transferred to Graph pad prism software for analysis. Data was presented as means ±standard error (SE). One-way ANOVA was used in the analysis of data involving different groups and also in the study of various parameters, including relative organ weight, behavioural changes, blood hemogram profiles, cytokine analysis, glutathione, MDA levels and AST/ALT levels with acceptable significance level at $P<0.05$ followed by Tukey post hoc tests. The results obtained were presented in the form of graphs and tables. Histopathological results were presented in images, comparing those administered with treatments and the control group.

3.10 Ethical Consideration

The principles regarding research ethics were keenly observed during the research activity. This study sought approval from the Chuka University Ethics Committee and obtained a research permit from the National Commission for Science, Technology and Innovation (NACOSTI). The animals were handled strictly according to the protocol for lab animal handling by the Institute of Primates' research. The integrity of the research was upheld by avoiding plagiarism and acknowledging the work done by others through proper citations and references.

CHAPTER FOUR

RESULTS

4.1 Determination of the Effects of Amoxicillin and Septrin on the Gut Bacterial Population

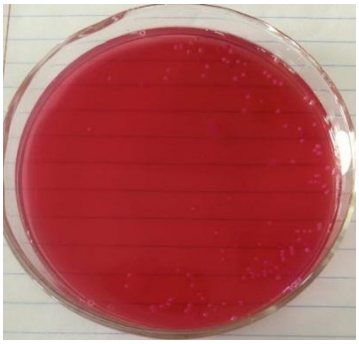
The impact of antibiotic treatment was clearly demonstrated by varying population of bacterial colonies exhibited on the MacConkey agar plates. The control group plate showed massive growth of bacteria, which was marked as normal bacterial growth. The amoxicillin group showed significantly reduced growth of bacterial colonies comparison to the control group. The septrin treated group exhibited dispersed growth of bacteria while the group receiving alternate amoxicillin and septrin group showed extensively reduced with few sparse colonies on the plate (Fig. 4). The amox +sept +probiotic group showed massive growth compared to other test groups but lower than the control group.

(A). WT- Control

(B). Amoxicillin

(C). Septrin

(D). Amoxicillin+Septrin



(E). Amoxicillin+Septrin+Prob

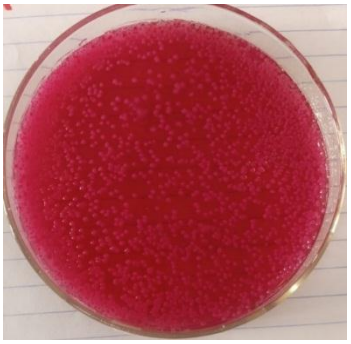


Figure 4: Photographs of culture plates showing effects of antibiotics on gut microbial colony population after 24 hours of incubation at 37^{o} C. WT-Wild Type control (A); the other plates show respective antibiotics treatment indicated against them; Amoxicillin (B), Septrin group (C), Amoxicillin+Septrin (D), and Amoxicillin+Septrin+ Probiotic (E).

The impact of antibiotics on the population of gut microbiota was assessed by measuring colony-forming units (CFU). The administration of antibiotics led to a decrease in bacteria colonies in mice treated with amoxicillin CFU/g < $1.0x10^5$, septrin CFU/g < $1.0x10^5$, and a combination of amoxicillin-septrin CFU/g < $1.0x10^5$, compared to the control CFU/g = $8.0 x10^5$, (Fig. 5). Importantly, exposure to probiotics reversed the dysbiosis caused by antibiotics as it shows an increase in the bacteria colonies CFU/g >$3.0x10^5$.

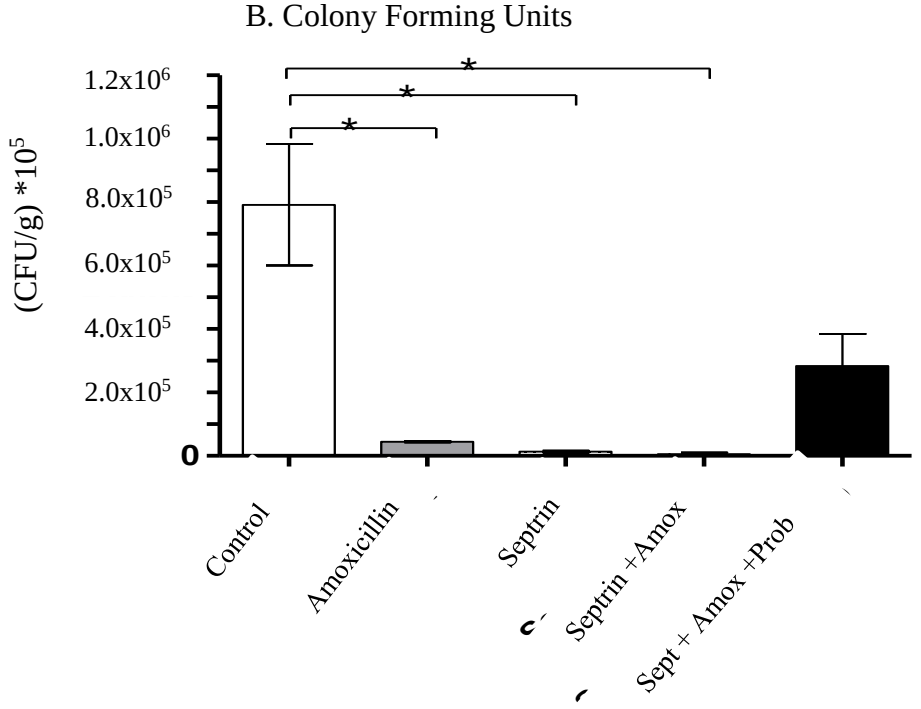


Figure 5: Antibiotic treatment resulted in significant reduction in bacterial colony forming units. Bar graphs indicate Control group, amoxicillin group, septrin group, amoxicillin+ septrin group and amoxicillin+ septrin+ probiotics group. One-way ANOVA was utilized followed by Tukey post hoc test. n=6. Bars represent mean +SEM. (Significance was reported at $p<0.05$).

4.1.1. Effects of Antibiotic-Induced Gut Microbiota Dysbiosis on General Body Weight and Relative Organ Weight

The study revealed consistent rise in weight across all groups during the 63-day study period (Fig. 6). There was no significant change in progressive weekly body weight change in relation to the control group across all the test groups (Fig. 6). There was also no significant no significant ($p<0.05$) effect on body weight across all the test groups in comparison to the control group (Fig.7). There were no significant differences in

relative organ weight changes in the heart (A), liver (B), spleen (C), brain (D), lungs (E), and kidneys (F) compared to the control groups across all test groups (Fig. 8A-F).

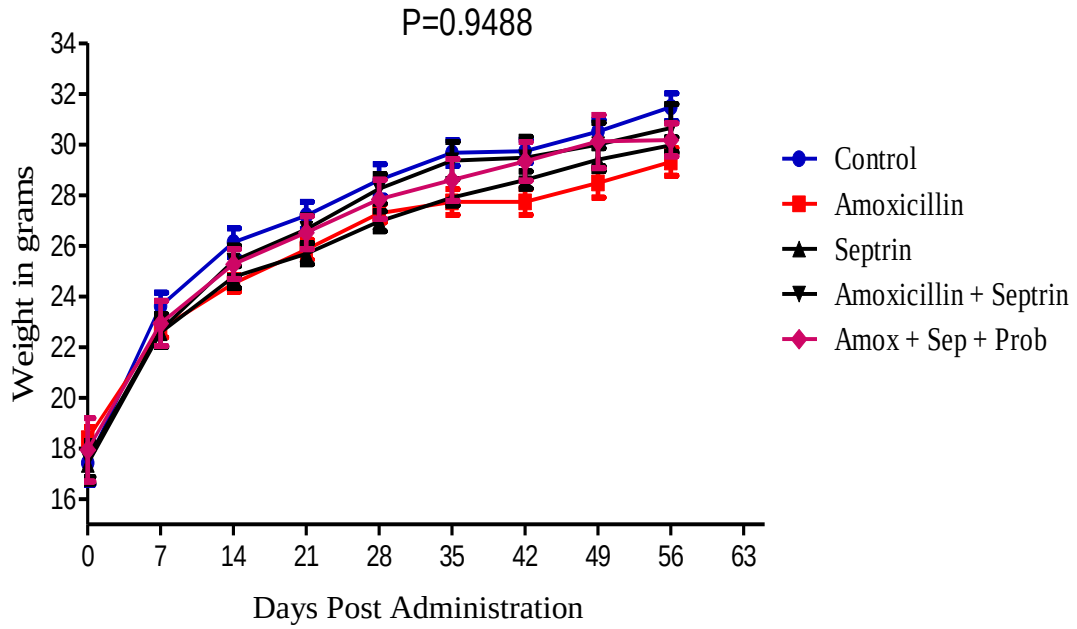


Figure 6: Antibiotics-induced dysbiosis on weekly progressive changes in body weight. The data was analyzed using one-way ANOVA. Significance was reported at $p<0.05$.

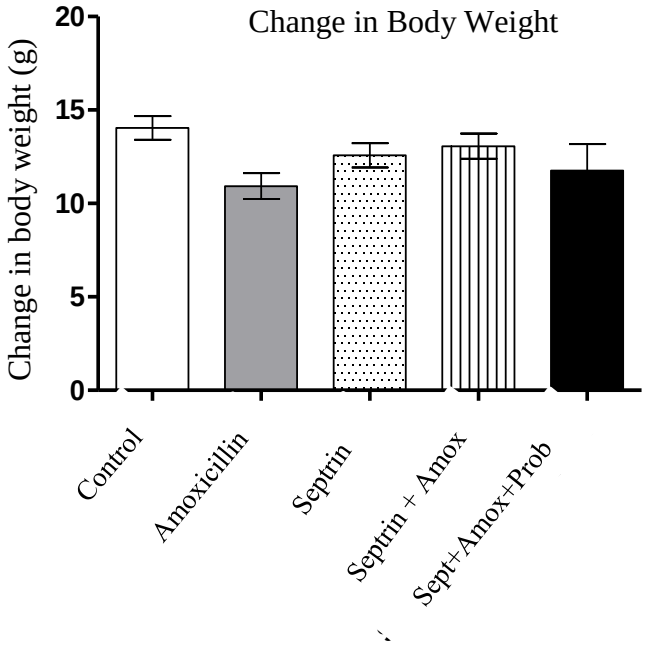


Figure 7: Effect of antibiotics-induced dysbiosis on body weight. There was no significant change in the body weight. The data was analyzed using one-way ANOVA. Bars represent mean +SEM. Level of significance was recorded at $p<0.05$.

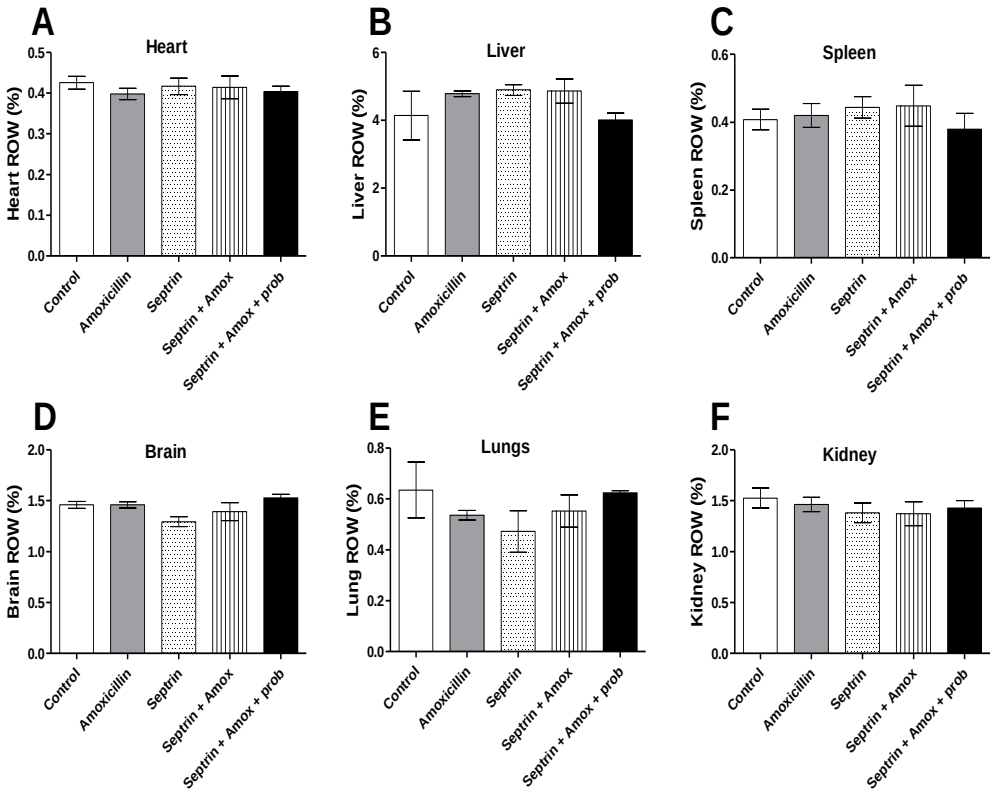


Figure 8: Antibiotics-induced dysbiosis had no significant effect on relative organ weights; Heart (A), Liver (B), Spleen (C), Brain (D), Lungs (E), Kidney (F). The data was analyzed using one-way ANOVA. Bars represent mean +SEM. ($p<0.05$).

4.1.2 Effects of Antibiotics Induced Gut Microbiota Dysbiosis on Red Blood Cells Count and Red Cell Indices

Mice exposed to amoxicillin and septrin antibiotic exhibited significantly low packed cell volume (PCV) levels ($p<0.01$), a condition that was aggravated when both amoxicillin and septrin were administered relative to the control (Fig. 9A). Additionally, haemoglobin (Hb) counts (Fig. 9B) and RBC (Fig. 9C) in mice treated with amoxicillin and septrin solely or in combination were significantly reduced ($p<0.01$), indicative of active anemia. Interestingly, administration of probiotics significantly blocked the reduction in hemoglobin counts and red blood cells count. Levels of mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), red cell distribution width - coefficient of variation (RDW-CV) and red cell distribution width - standard deviation RDW-SD was unaffected by the treatments (Fig. 9B-H).

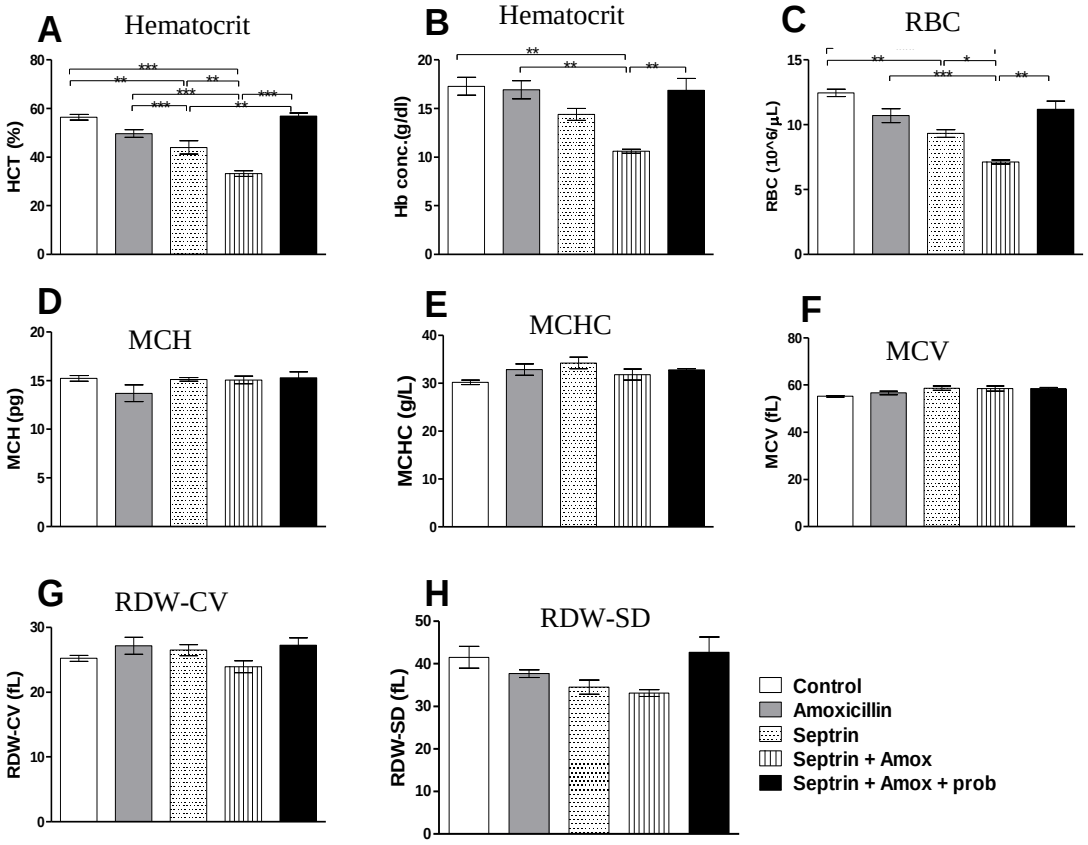


Figure 9: Antibiotics induced-dysbiosis resulted in significant drop in the levels of hematocrit (A), red blood cell count (B), hemoglobin levels (C), and no significant change in red cell indices, MCV (F), MCH (D), RDW-SD (H) and RDW-CV (G) and MCHC (E) in mice. Data analysis performed using one-way ANOVA. Bars correspond to mean ±SEM. ($p<0.05$)

4.1.3 Effects of Antibiotics Induced-Dysbiosis on White Blood Cells Count and Subtypes

Mice exposed solely to septrin exhibited a statistically significant ($p<0.05$) increase in white blood cell (WBC) levels compared to the control, amoxicillin, and amoxicillin-septrin groups (Fig. 10A). Conversely, the administration of probiotics to mice did not reverse septrin-induced leukocytosis. Analysis of WBC subtypes revealed a significant elevation of neutrophils ($p<0.001$) (Fig. 10B), lymphocytes ($p<0.001$) (Fig. 10C), and monocytes ($p< 0.05$) (Fig. 10D) following exposure to septrin alone. Furthermore, co-administration of septrin and amoxicillin led to notable suppression of Neutrophils ($p< 0.001$) and an increase in both lymphocytes and monocytes ($p<0.001$, $p< 0.01$ respectively). Eosinophils levels were significantly increased ($p<0.05$) (Fig. 10E) and a decrease in basophils ($p<0.05$) (Fig. 10F) upon exposure to septrin, and co-exposure with septrin and amoxicillin resulted in a significant elevation of eosinophils ($p<0.05$),

while basophils remained within the normal range. Supplementation with probiotics resulted in a marginal restoration in the altered levels of the WBC and its subtypes due to antibiotic exposure.

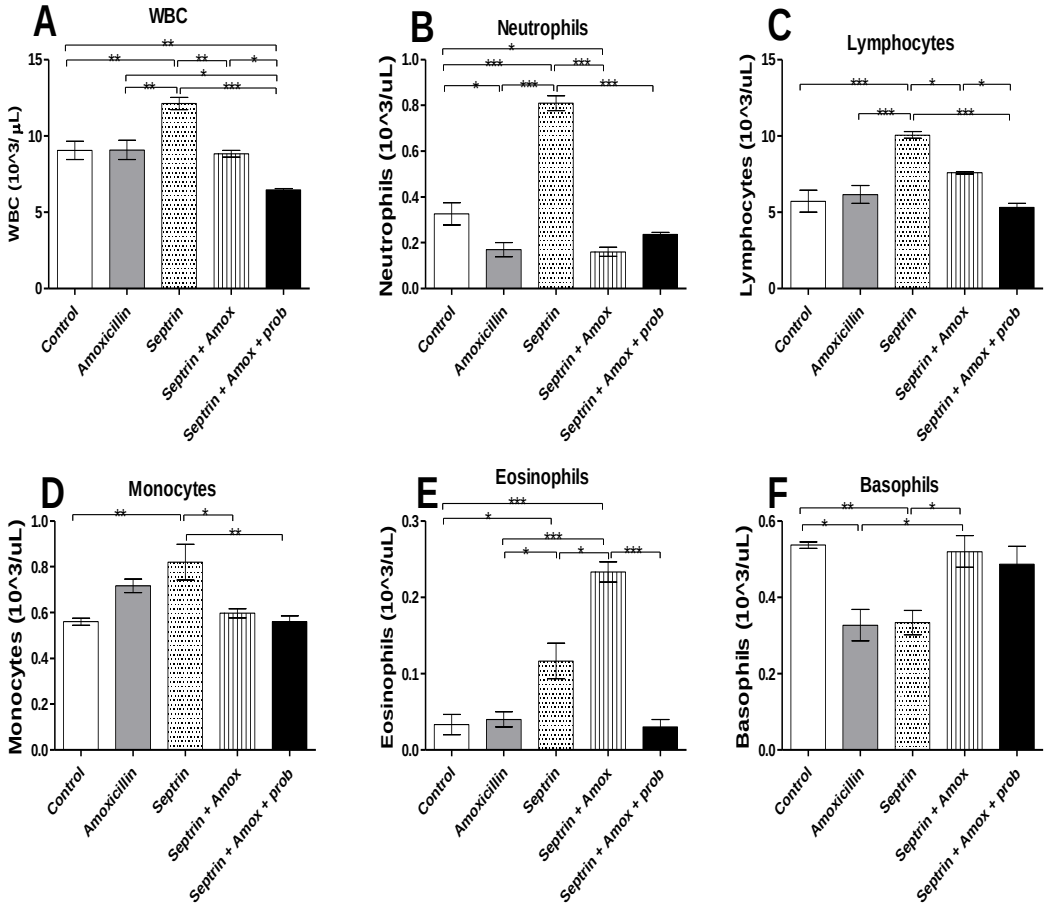


Figure 10: Antibiotic-induced gut microbiota dysbiosis resulted in significant changes in the levels of White Blood Cells counts (WBC) and subtypes; lymphocytes, Neutrophils, basophils, eosinophils and monocytes. The data was analyzed using one-way ANOVA followed by Tukey post hoc test. Bars represent mean +SEM. (*p≤0.05, **p≤0.01, ***p≤0.001).

4.1.4 Impact of Antibiotics Induced-Dysbiosis on Platelet Count and Indices

Septrin exposure alone led to thrombocytopenia, as evidenced by decreased platelet levels. However, administration of amoxicillin alone or in conjunction with septrin notably increased platelet levels in reference to the control (Fig. 11A). Notably, exposure to probiotics led to the restoration of platelet levels. An analysis of platelet indices revealed that administration of amoxicillin and septrin, either alone or in alternate, led to the reduction of platelet large cell ratio (P-LCR) (Fig. 11B), platelet

distribution width (PDW) (Fig. 11C), while mean platelet volume (MPV) remained unaffected by the treatments relation to the control group (Fig. 11D).

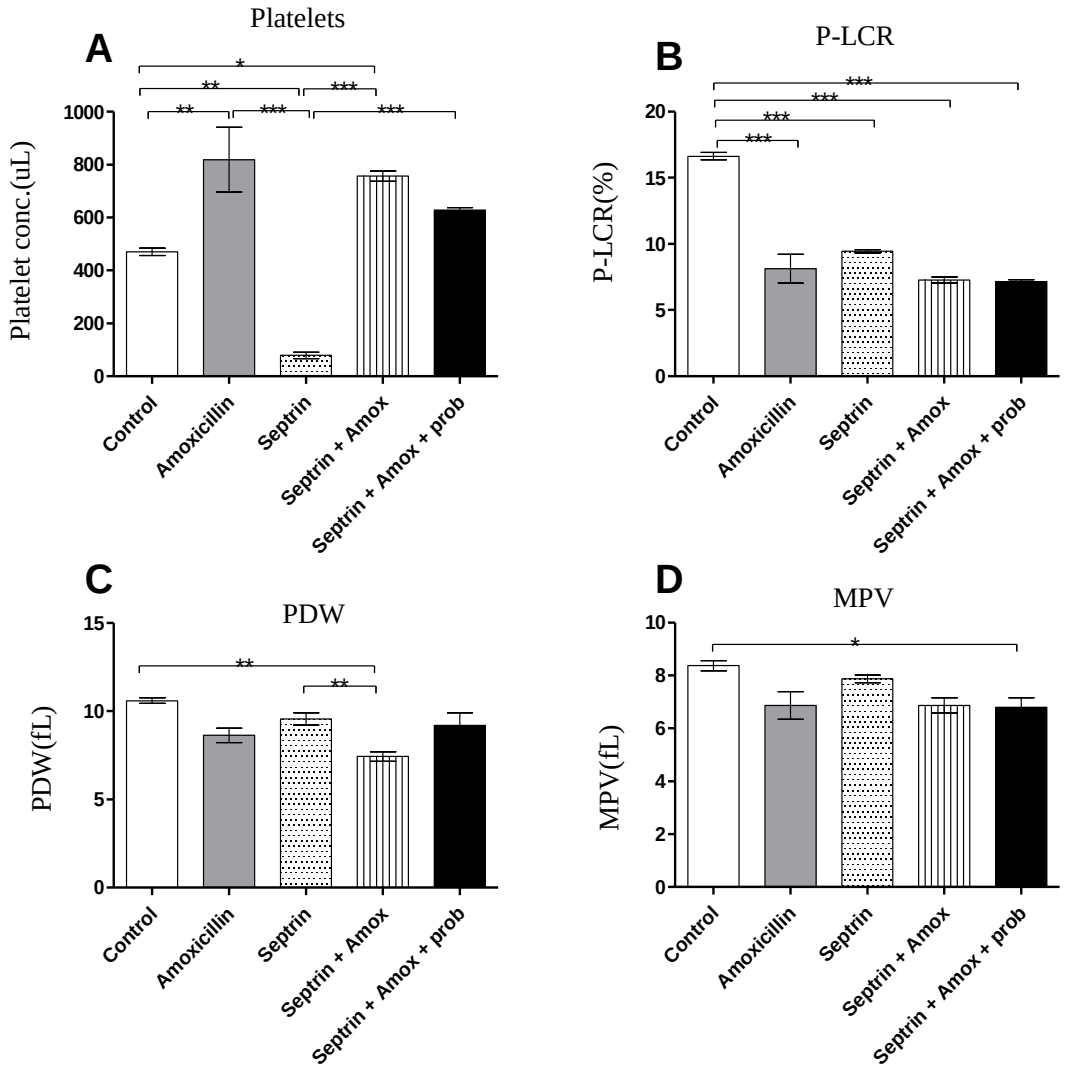


Figure 11: Antibiotic-induced dysbiosis resulted in significant changes in the levels of platelets (A) and significant drop in platelets indices; P-LCR (B), PDW (C) and MPV (D). The data was analyzed using one-way ANOVA followed by Tukey post hoc test. Significance was reported at $p<0.05$. (*$p\leq0.05$, **$p\leq0.01$, ***$p\leq0.001$).

4.1.5 Effect of Antibiotic-Induced Dysbiosis on Serum Electrolytes Levels

The impact of antibiotic induced gut microbiota dysbiosis on electrolytes balance was assessed by determining the levels of electrolytes in the serum. It was noted that there was a drop in the levels of sodium (A), chlorine (B) and potassium(C) in the serum upon treatment with amoxicillin or septrin singly or in combination in relation to the

control (Fig. 12). Significant ($p<0.05$) drop in the levels of sodium in Amoxicillin group, septrin group and amoxicillin-septrin group in relation to the control group (Fig. 12 A). Probiotics treatment restored the sodium levels in the serum (Fig. 12A).

There was significant drop in the levels of chlorine in septrin group and amoxicillin-septrin group in comparison to control group (Fig. 12B). Probiotics treatment restored the chlorine levels in the serum (Fig. 12B). There was a significant drop in the levels of potassium in septrin group, amoxicillin+septrin group and amoxicillin+septrin+probiotics in relation to the control group (Fig. 12C).

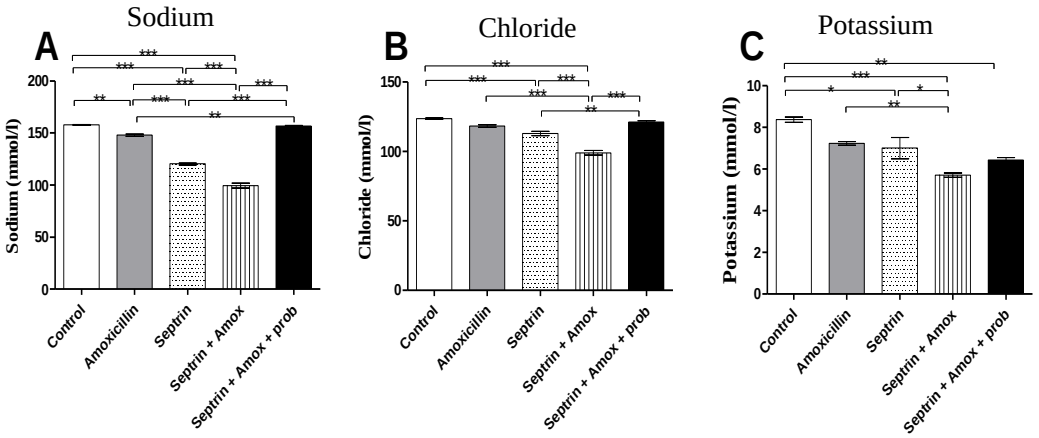


Figure 12: Antibiotic induced dysbiosis resulted in significant drop in serum electrolyte components; Sodium (A), Chloride (B) and Potassium (C). Analysis of data was done using one-way ANOVA followed by Tukey post hoc test. n=6. (Significance: *$p\leq0.05$, **$p\leq0.01$, ***$p\leq0.001$).

4.2 Effects of Antibiotic-Induced Gut Microbiota Dysbiosis on Induction of Pathological Changes

Following oral antibiotic treatment on swiss mice, the study analyzed the effect of gut microbiota dysbiosis on various pathological changes. The study evaluated immunological responses by analyzing the levels of cytokines, examine kidney and liver function markers and histological examination of the brain, the liver and the kidneys.

4.2.1 Effects of Antibiotic-Induced Dysbiosis on Cytokines

The administration of amoxicillin and septrin singly or in conjunction resulted in elevated levels of TNF-α (Fig. 13A). There was also recorded significant elevated levels of INF-γ in septrin treated group and amoxicillin-septrin group which indicates active

inflammation (Fig. 13B). This is evidenced by increased levels of pro-inflammatory cytokines in relation to the control group TNF- α and IFN- γ (Fig. 13 A, B). The levels of IL-10 were comparable across all the test groups (Fig. 13C). Notably, the ratio of inflammatory cytokines to anti-inflammatory cytokines IFN-γ: IL-10 (E) and TNF-α: IL-10 (D) revealed elevated levels in septrin group and amoxicillin-septrin group indicative of imbalance between anti-inflammation cytokines and pro-inflammatory cytokines hence suggesting active inflammation. However, treatment with probiotics alleviated inflammatory responses.

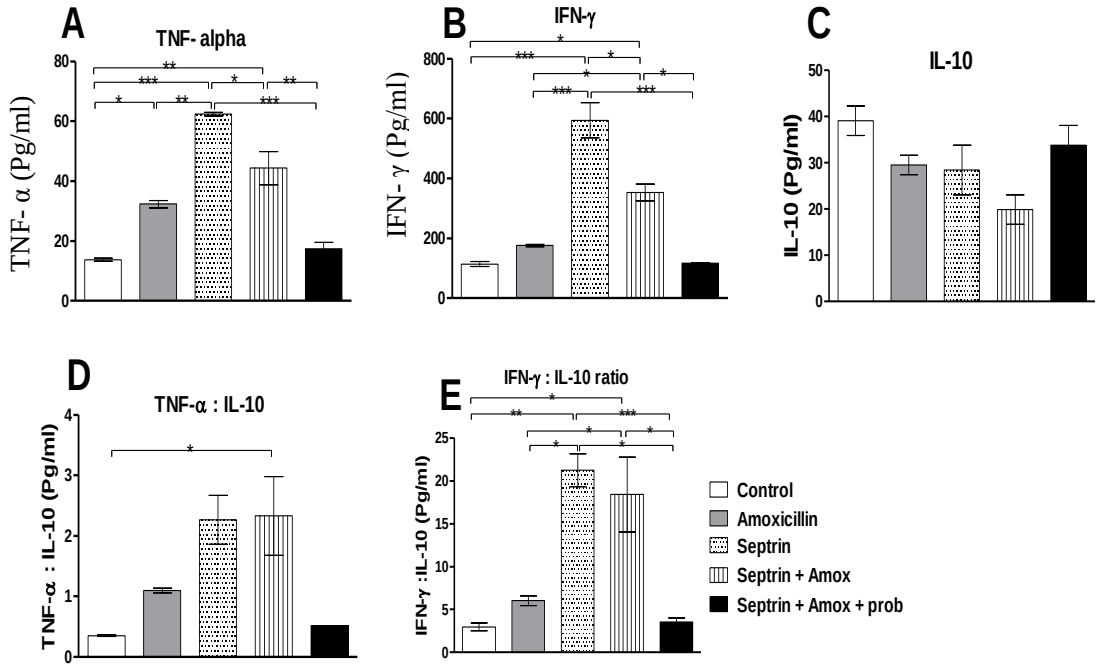


Figure 13: Antibiotic-induced gut microbiota dysbiosis resulted in significant elevated levels of proinflammatory cytokines; TNF-α, (A) and IFN- γ, (B). The were no notable changes in the levels of IL-10 (C). There were significant elevated levels in the ratio between proinflammatory cytokines to anti-inflammatory cytokines; IFN-γ: IL-10 (E) and TNF-α: IL-10 (D). Analysis was done using one-way ANOVA preceded by Tukey post hoc test. Bars represent mean +SEM. (Significance noted at: $p<0.05$).

4.2.2 Effects of Antibiotic-Induced Gut Dysbiosis on the Liver Function

The effects of antibiotic treatment on the liver function were evaluated by determining the levels of markers for liver function including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, the proportion of AST to ALT (AST: ALT) and direct bilirubin. AST, ALT, ALP and AST: ALT ratio. There were

elevated levels of ALT across all the test groups but significance $p<0.05$ reported in Amoxicillin group, septrin group and amoxicillin-septrin group, while probiotics restored the levels of ALT (Fig. 14A). There were elevated levels of AST (B), AST: ALT (C), ALP (D) across all the test groups in relation to the control group suggesting liver damage. The levels of direct bilirubin were unaffected across all the test groups in relation to the control group (Fig. 14 E).

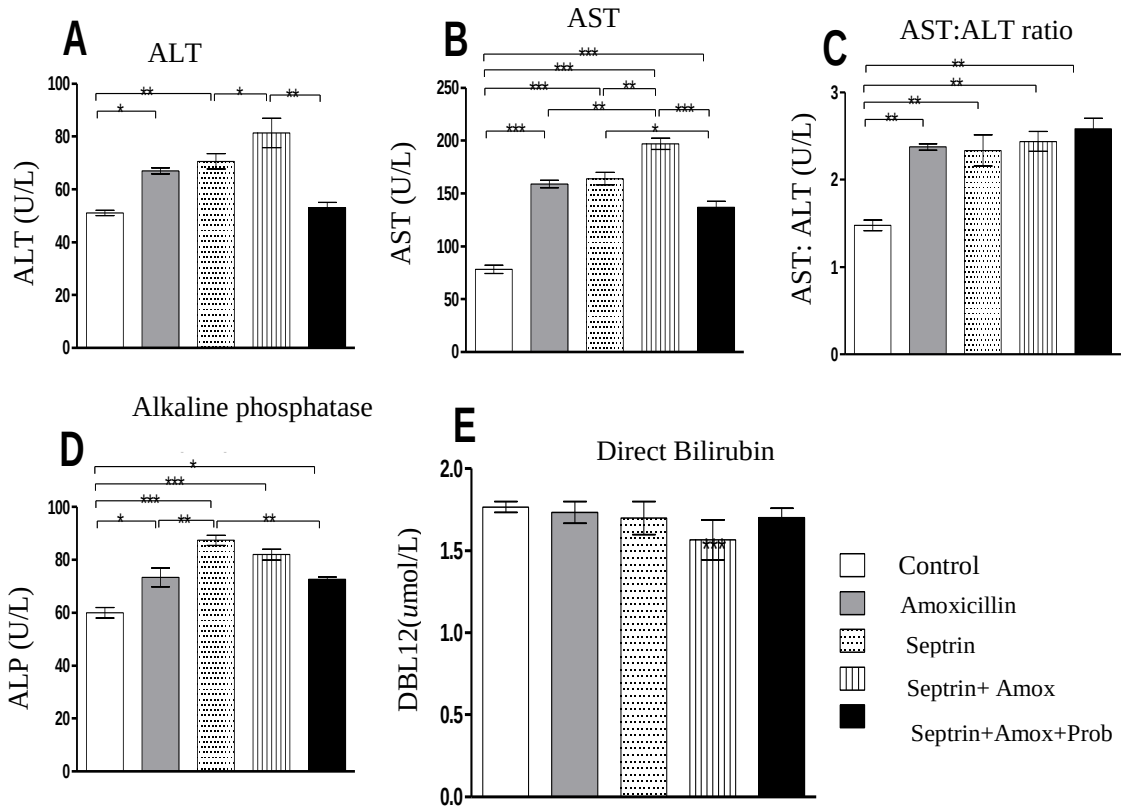


Figure 14: Antibiotic-induced gut microbiota dysbiosis resulted in significant elevated levels of ALT (A), AST (B), ALT: AST ratio (C), Alkaline phosphatase (ALP) (D) and no significant change in the levels of direct bilirubin (E) was noted in relation to the control group. The data was analyzed using one-way ANOVA followed by Tukey post hoc test. Bars represent mean +SEM. (Indicated level of significance: *p≤0.05, **p≤0.01, ***p≤0.001).

4.2.3 Impacts of Antibiotic-Induced Gut Microbiota Dysbiosis on the Kidney Function

Amoxicillin and septrin either singly or in combination Amoxicillin+septrin treatment had deleterious effect on the kidney function. This is evidenced by the increased levels of creatinine (Fig. 15A), Urea (Fig. 15B) and Uric acid (Fig. 15C) in reference to the control group. There was a notable reduction in albumin levels which suggests kidney

damage (Fig. 15D). Probiotics protected the kidneys from damage this is shown by the levels of uric acid, creatinine and albumin and urea, in relation to the control group.

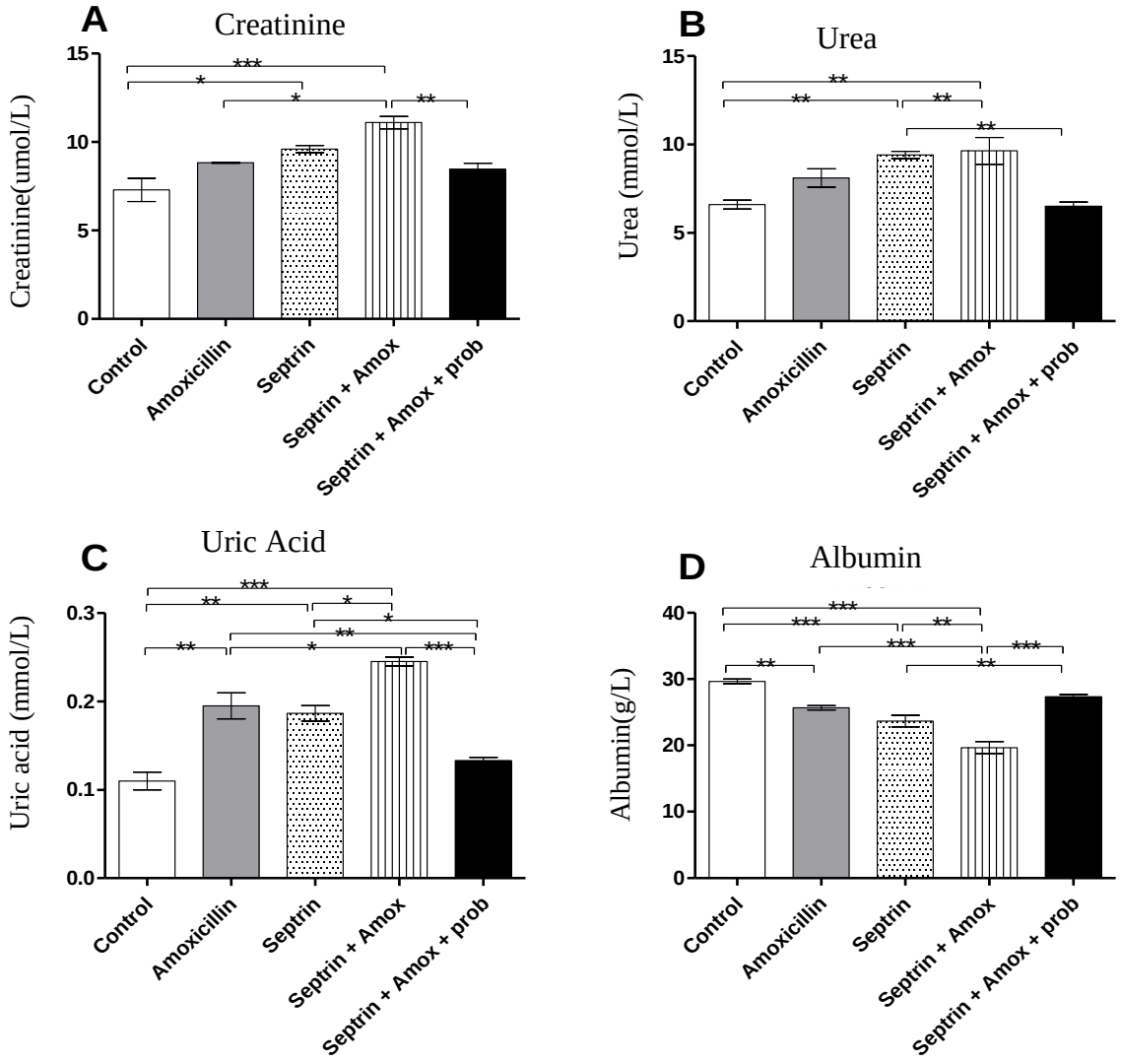


Figure 15:Antibiotic-induced gut microbiota dysbiosis resulted in significant increased levels of creatinine (A), Urea (B), Uric acid (C) and a significant drop in the levels of Albumin (D) was noted. One-way ANOVA followed by Tukey post hoc test was used in data analysis. (significance recorded at: *p≤0.05, **p≤0.01, ***p≤0.001).

4.2.4 Histopathological Effects on the Brain

There was no notable significant neuropathological evidence to indicate pathological damage of the brain. The brain tissues appeared normal on performing histological examination across all the groups (Fig. 16).

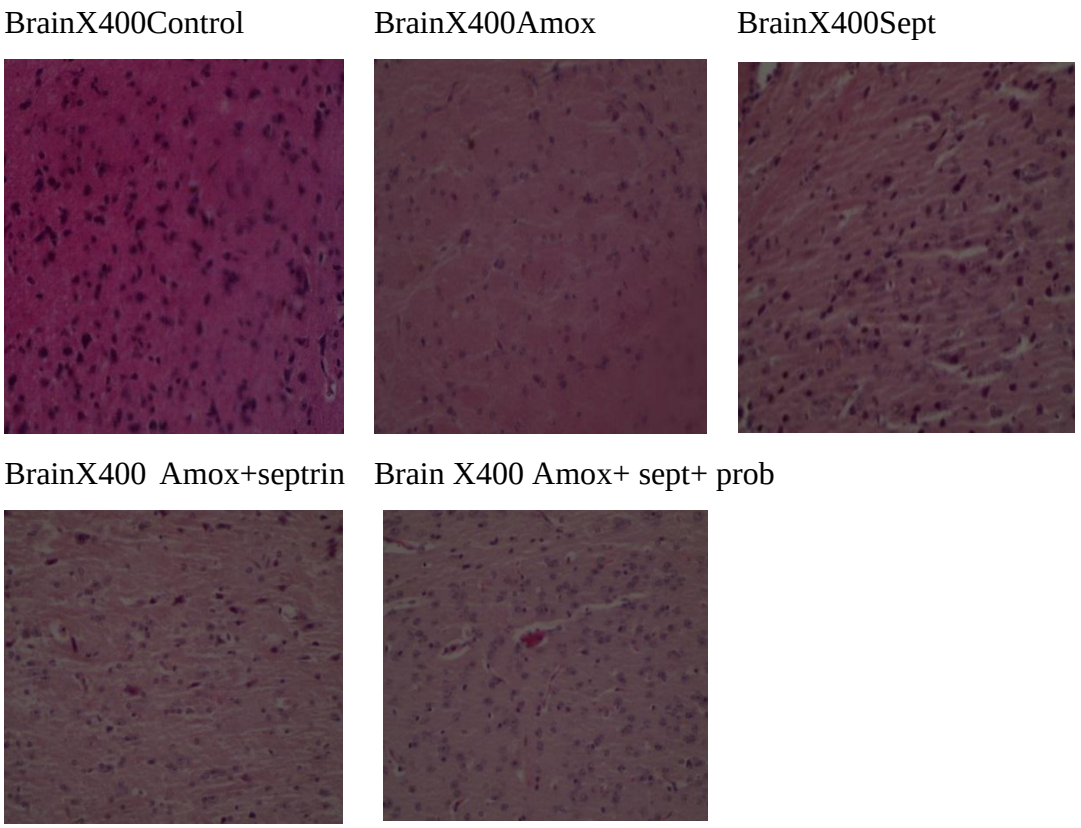


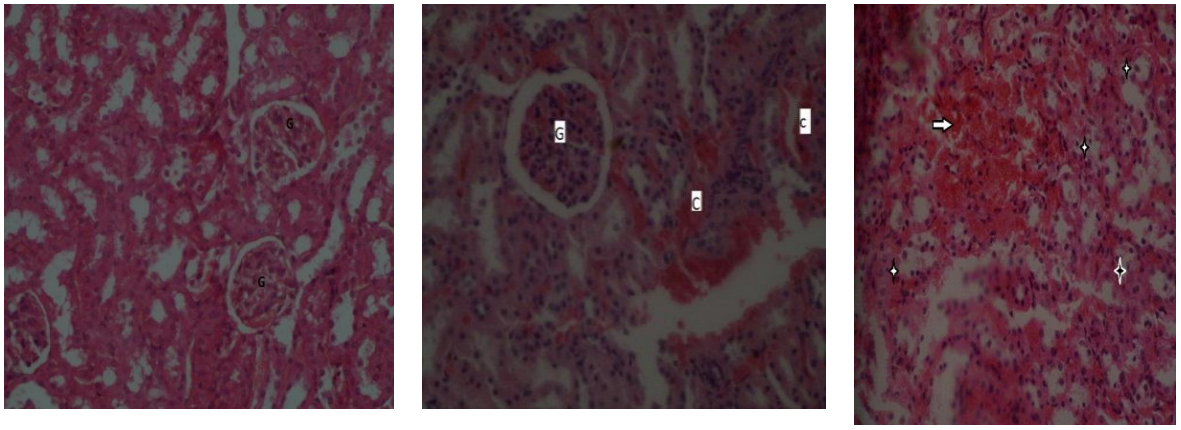
Figure 16:Photomicrographs showing the effects of antibiotic-induced dysbiosis on brains sections of mice. The brain sections appeared normal. Magnification x400. Hematoxylin and Eosin staining.

4.2.5 Histopathological Effects on the Kidney

Histological examination of the kidney with hematoxylin and Eosin sectioning showed sections of renal congestion in group of mice administered with 9.62 mg/kg of amoxicillin but there were no notable glomerular structural abnormalities (Fig. 17). The group of mice administered with 15mg/kg of septrin kidney tissue sections showed diffuse interstitial hemorrhage as indicated by the arrow. There were also areas of tubular epithelial cell swelling indicated by star (Fig. 17 C). This indicates cellular damage and lysis. Similarly, the group of mice administered with both amoxicillin and septrin showed sections of diffuse interstitial hemorrhage indicated using an arrow. There were also sections of sloughing off of tubular epithelium indicating cell necrosis marked using stars (Fig. 17D). This indicates inflammation and kidney injury. The group of mice administered with 9.62 mg/kg amoxicillin and 15 mg/kg of septrin with probiotic showed some sections of renal congestions which indicates impediment of

blood flow however, it was evident that glomerular structure appeared normal (Fig.17 E). This indicates that probiotics protects kidney from structural damage.

A. kidneyX400 control group B.kidney X400 Amoxicilln C kidney X400sept



D. kidneyx400 amox+sept E kidneyx400amox+sept+prob

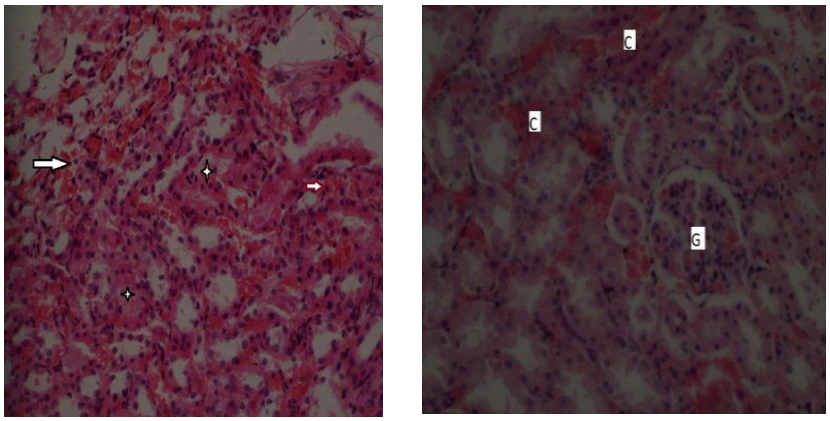


Figure 17:Photomicrographs showing the effects of antibiotic-induced dysbiosis on kidney sections of mice. Magnification x400. Hematoxylin & Eosin staining. C-congestion, G-glomerulus. Arrows indicate diffused interstitial hemorrhage and stars show sections of sloughing off of tubular epithelium. Magnifications are indicated against respective image.

4.2 6 Histopathological Effects on the Liver Tissue

Histological examination was also conducted on the liver to ascertain whether antibiotic-induced dysbiosis has an effect on liver tissues. The group of mice administered with amoxicillin showed sections of congestion indicated by **C** (Fig. 18 B). This shows impaired blood flow, venous obstruction or inflammation of the liver and indicates hepatocellular damage. The group of mice administered with 15 mg/kg of septrin showed sections of congestion indicated by **C** and other sections showed hepatocyte swelling indicated using an arrow head (Fig. 18 C). This shows

inflammation and hepatocellular necrosis. The group of mice administered with both amoxicillin and septrin showed sections of hepatocyte swelling indicated by an arrow (Fig. 18D). This shows that there is hepatocellular damage. The group of mice administered with both amoxicillin and septrin showed sections of congestion indicated by **C**, it was observed that there were traces of blood in the bile duct labeled **BD**, this shows that there is venous obstruction an indication of hepatocellular damage and inflammation.

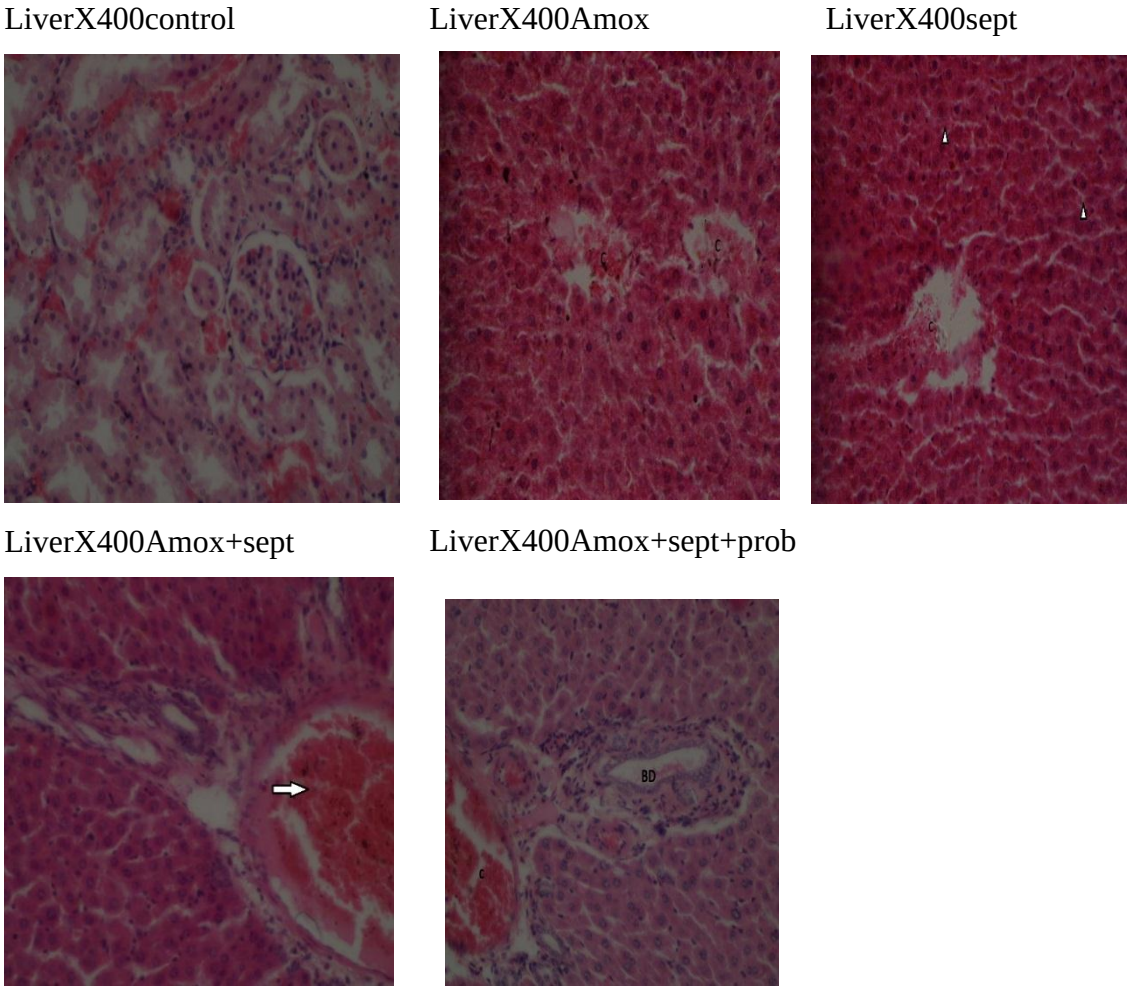


Figure 18: Photomicrographs showing the effects of antibiotic-induced dysbiosis on liver sections of mice. Magnification x400. Hematoxylin & Eosin staining. C-congestion, BD-bile duct. Arrow head indicate hepatocyte swelling. Magnifications are indicated against respective image.

4.3 Effects of Antibiotic-Induced Gut Microbiota Dysbiosis on Induction of Oxidative Stress

The study assessed the impact of antibiotic treatment in inducing gut microbiota dysbiosis on induction of oxidative stress and cellular damage. The study achieved this by evaluating the levels of serum nitric oxide which ascertains the induction of

inflammation and cellular damage. It also assessed the levels of Glutathione (GSH) which elevated levels indicates induction of oxidative stress. Another parameter used is the levels of Malondialdehyde (MDA) which indicates lipid peroxidation.

4.3.1 Effects of Antibiotic-Induced Dysbiosis on the Levels of Nitric Oxide in the Serum

To assess impact of antibiotic induced dysbiosis in inducing oxidative stress, the levels of nitrites in the serum were determined. Amoxicillin and septrin when administered singly or in conjunction, Amoxicillin-septrin resulted in significant $p<0.05$ elevated levels of nitrites in the blood serum across all the test groups in relation to the control group (Fig. 19). These findings suggest antibiotic induced dysbiosis results in oxidative stress.

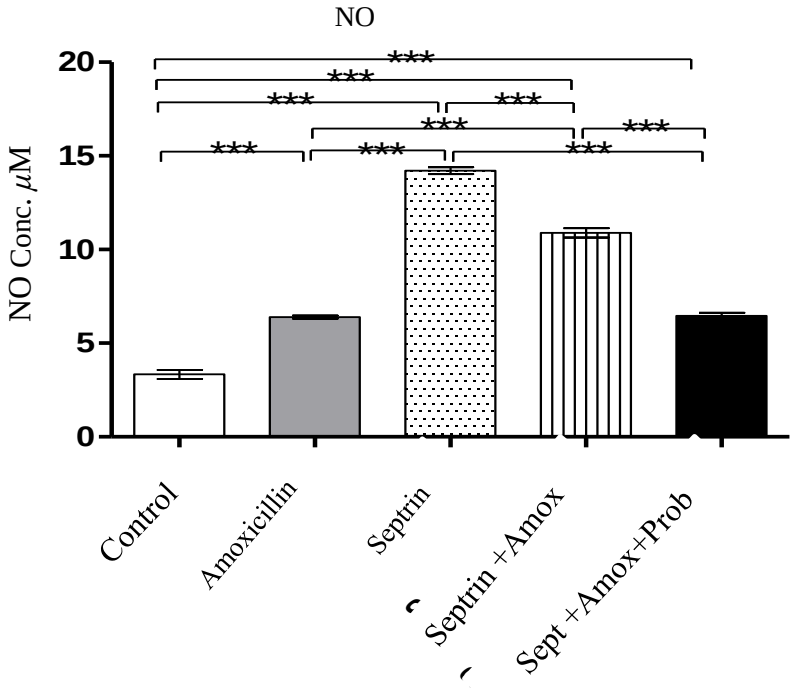


Figure 19: Antibiotic-induced gut microbiota dysbiosis resulted in significant elevated levels of serum nitric oxide. The data was analyzed using one-way ANOVA followed by Tukey post hoc test. Bars represent mean +SEM. (Significance recorded at: *$p\leq0.05$, **$p\leq0.01$, ***$p\leq0.001$).

4.3.2 Effect of Antibiotic-Induced Dysbiosis on Organ Reduced-Glutathione Levels

Amoxicillin and septrin when administered singly or in combination resulted in significant changes in organ levels of Glutathione (GSH). Induced oxidative stress on the liver (A) and the brain (B) is evidenced by significant $p<0.05$ drop in the levels of

glutathione (GSH) in relation to the control group (Fig. 20). Interestingly, probiotic treatment restored the levels of GSH in the liver and the brain suggesting it has counter-protective properties against oxidative stress (Fig. 20 A, B). There were notable elevated levels of GSH in the spleen (C), kidney (D), lungs (E) and heart (F) across all the test groups of mice in reference to the control group. This indicates active oxidative stress in the spleen, kidney, heart and lungs.

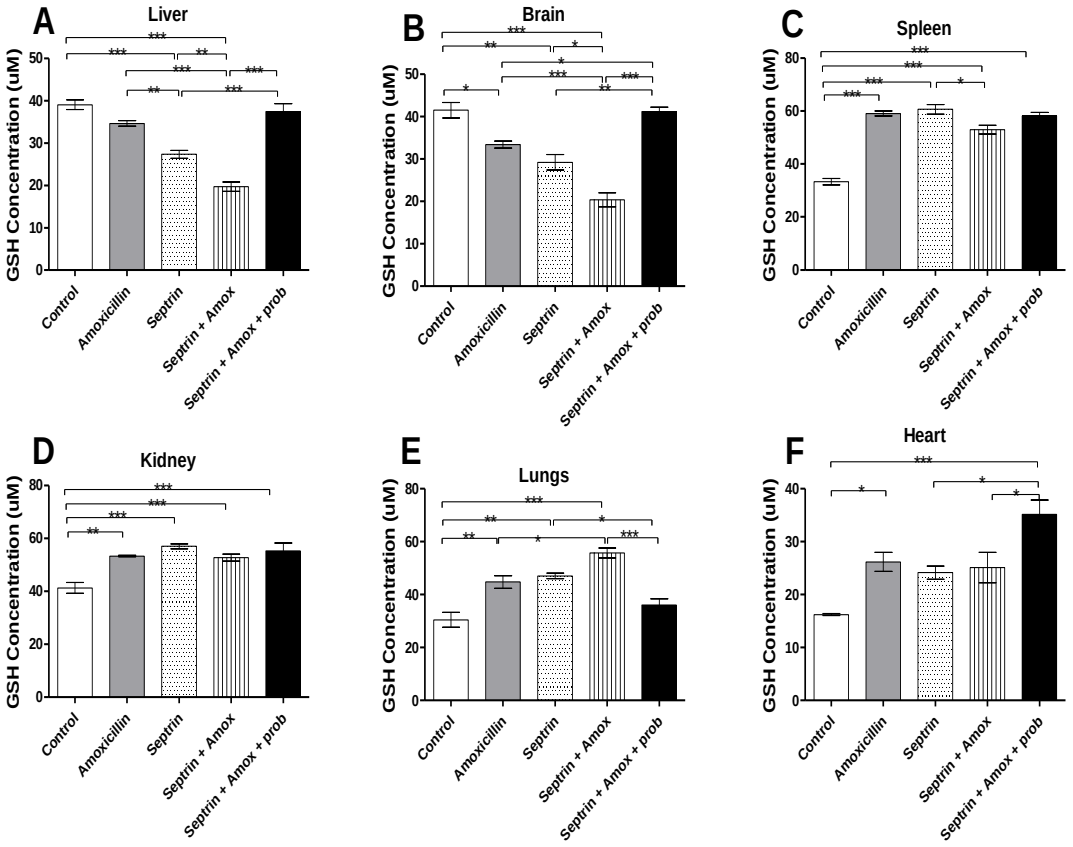


Figure 20: Antibiotic induced gut microbiota dysbiosis resulted in significant changes in the levels of GSH; Liver (A), Brain (B), Spleen (C), Kidney (D), Lungs (E) and Heart (F). The data was analyzed using one-way ANOVA followed by Tukey post hoc test. n=6. Bars represent mean +SEM. (Indicated level of significance: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$).

4.3.3 Determination of Oxidative Stress Using Organ Malondialdehyde Levels

The levels of tissue malondialdehyde (MDA) was assessed to ascertain antibiotic induced dysbiosis on causing lipid peroxidation in organs. The administration of amoxicillin and septrin alone or in conjunction resulted in significant $p<0.05$ elevated levels of MDA in the brain (A) across all the test groups in relation to the control group, probiotic treatment protected the brain from lipid peroxidation. There were significant

elevated levels of MDA in the liver (B), spleen (C) and kidney (D) across all the test groups in reference to the control group (Fig. 21). There were significant elevated levels of MDA in the lungs (E) in Amoxicillin group, septrin group and Amoxicillin+septrin group in relation to the control group. Probiotic treatment restored the levels of MDA in the Lungs (E). There were significant elevated levels of MDA in the heart (F) in in Amoxicillin group and septrin group in comparison to the control group (Fig. 21).

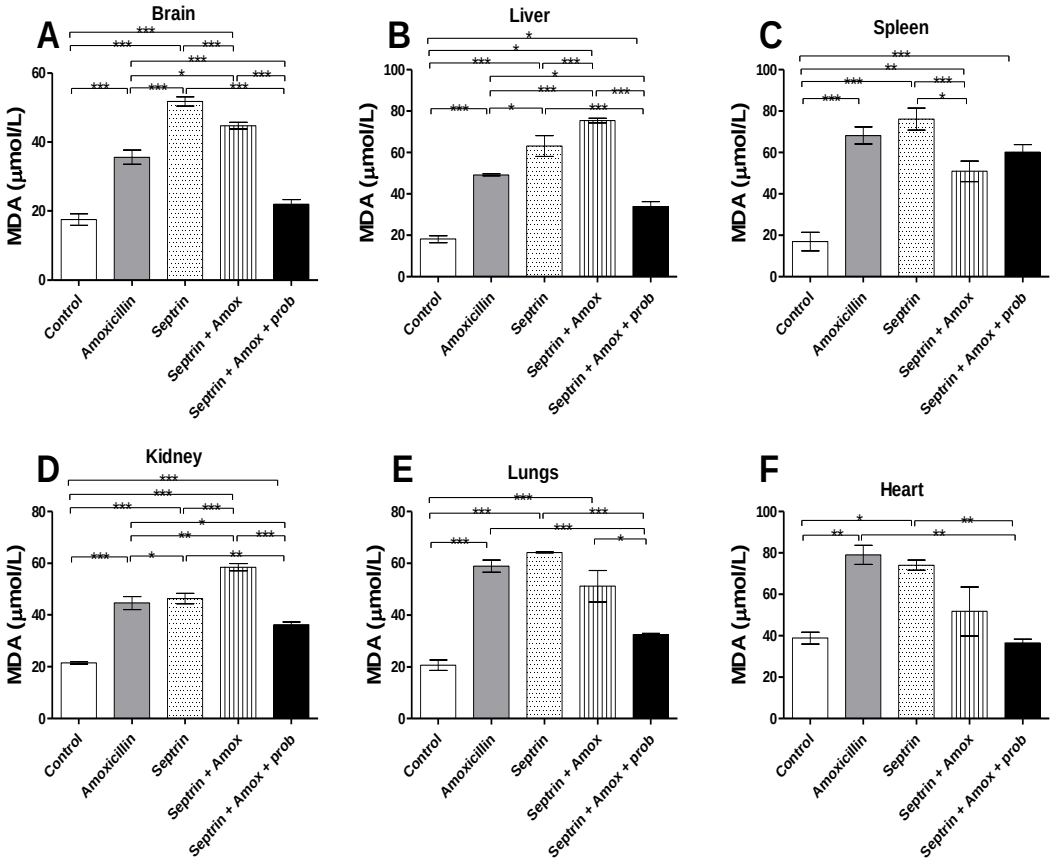


Figure 21: Antibiotic-induced gut microbiota dysbiosis resulted in significant elevated organ MDA levels; brain (A), liver (B), spleen (C), kidney (D) and lungs (E). The data was analyzed using one-way ANOVA followed by Tukey post hoc test. Bars represent mean +SEM. (Indicated level of significance: *p≤0.05, **p≤0.01, ***p≤0.001).

CHAPTER FIVE

DISCUSSION

5.1 Effects of Antibiotic on the Gut Microbiota, Physiological and Biological Changes

Antibiotics are beneficial in managing various bacterial infections by killing the bacteria or inhibiting their growth and multiplication (Nankervis *et al*., 2016). Despite the beneficial use of antibiotics in managing diseases various studies have linked its use and misuse to detrimental impacts on the gut bacterial population (Ramirez *et al*., 2020). This study assessed the impacts of amoxicillin and septrin treatment on the gut bacterial population and subsequent impacts on physiological and biological changes in male swiss mice and potential impacts of using probiotics in conjunction with antibiotic treatment.

5.1.1 Effects of Antibiotics on the Gut Microbiota Population

Several studies have linked alteration on the gut microbiota composition to induction of various health conditions including neurodegenerative disorders, inflammatory bowel diseases, type 2 diabetes, asthma, hypertension, anemia, atherosclerosis and many other health issues (Sarkar *et al*., 2021; Saeed *et al*., 2022).

The current study, assessed the effect of antibiotics use and impact on causing gut microbiota dysbiosis using laboratory bred male swiss mice models. Furthermore, examination was performed to evaluate the effect of antibiotic induced dysbiosis on general health, immunological responses, inflammation and oxidative stress.

The study's validation entailed assessing gut microbial populations through taking a gut swab from distinct mouse groups and spread on sterile MacConkey agar plates. The observed depletion of gut bacterial colonies supports the study's hypothesis. The study affirms that broad-spectrum antibiotics, like amoxicillin and Septrin, can indiscriminately reduce both pathogenic and non-pathogenic gut bacteria (Lekang *et al*., 2022). Antibiotic treatment either singly or in combination resulted significant reduction in the gut microbiota population. The results obtained is consistent with study done by Lange *et al*. (2016) which indicated that antibiotic treatment negatively alters the gut microbiota population. Probiotics treatment replenished the gut microbiota as

exhibited in microbial plates showing massive growth. These findings are in agreement with various studies done to ascertain the impact of probiotics in replenishing gut microbiota population (Hemarajata & Versalovic, 2012; Stavrou, 2016).

Various studies have suggested that antibiotics have deleterious effects on the gut microbiota population (Patangia *et al*., 2022; Yang *et al*., 2021). The findings from the current study affirm this, as evidenced by the evaluation of the bacterial colonies, indicating that amoxicillin and septrin singly or in conjunction impact the bacterial colonies, resulting in gut microbiota dysbiosis. Massive CFUs was observed in the group administered with probiotics suggest its ability to restore gut microbiota population (Hemarajata & Versalovic, 2012). Probiotics are live microorganisms that can grow and proliferate in the gut environment, thereby restoring the gut microbiota balance hence alleviating the gut microbiota dysbiosis induced by antibiotics (Dahiya & Nigam, 2022; Ji *et al*., 2023).

The study monitored changes in body weight weekly to assess the impact of amoxicillin and septrin administration on mice general body weight. The findings of the study suggested that the antibiotic induced dysbiosis resulted in no significant reduction in the body weight in all test groups in comparison to the control group. Several studies have attributed that antibiotic treatment can create a shift in gut microbiota, which results in dysbiosis and resulting in disruptions of metabolic pathways, nutrient uptake, and energy homeostasis which could result in significant changes in body weight (Langdon *et al*., 2016; Kesavelu & Jog, 2023). The findings from the current study, however, contradict from other studies that attribute antibiotic exposure and long-term microbiota alteration to obesity and increased body weight (Cox *et al*., 2014; Zhuang *et al*., 2023). However, other studies reveal insignificant or no effects when antibiotics are administered for a short period (Thuny *et al*., 2010). According to Kesavelu & Jog. (2023), long term effect of antibiotic induced dysbiosis which can persist for as long as 2 years could result in obesity, allergic responses and even asthma, however, probiotic treatment or dietary supplements could reverse the antibiotic induced gut microbiota dysbiosis. The findings showed no significant reduction in the ROW in the heart, brain, lungs and kidney in comparison to the control group. The findings however, are not consistent with other studies, which indicate that antibiotic induced gut microbiota

dysbiosis can impact organ function and morphology of various organs, especially the liver, since dysbiosis results increase in lipid accumulation (Zhang *et al*., 2022). This phenomenon can be attributed to the fact that although dysbiosis can influence organ morphology, the degree of microbiota disruption caused by the antibiotics may not have been extreme enough to result in measurable changes in organ size or weight within the duration of the study. The use of antibiotics along with probiotics had no impact on body weight, which can be attributed to metabolic resilience in mice (Turnbaugh *et al*., 2006; Ouwehand *et al*., 2010).

5.1.2 Effects of Antibiotic-Induced Dysbiosis on Red Blood Cell and Indices

The study assessed the impact of antibiotics induced gut microbiota dysbiosis on the red blood cells and its indices. The mice treated with amoxicillin and septrin had reduced packed cell volume levels, hemoglobin (Hb) as well and RBC counts, which are characteristics of anemia. This is in concordance with research, which indicates that antibiotics result in dysbiosis of gut microbiota, resulting in inadequate absorption of nutrients that are crucial in the production of red blood cells, including iron and vitamin B12 (Rogers & Aronoff, 2016). This observed worsening in anemia with the combined antibiotic treatment of amoxicillin and septrin indicates that gut dysbiosis has a synergistic effect on hematopoiesis. The gut microbiota participates in the digestion and metabolism processes of nutrients required for erythrocyte synthesis (Yan *et al*., 2018; Josefsdottir *et al*., 2017). These nutrients are known to be affected due to dysbiosis, which in the long run causes anemia, according to Lin *et al*. (2018). This was evidenced by our findings of low PCV, Hb, and RBC levels, which can be attributed to antibiotic-induced dysbiosis that resulted in decreased nutrient absorption, leading to anemia.

Probiotics treatment restored the levels of Hb counts and RBC level, which signifies the prospects of the probiotics in the amelioration of gut health to enhance hematopoiesis. Recent literature has confirmed that the administration of probiotics influences the restoration of the imbalanced gut microbiota and increases nutrient absorption and hematological profiles (Kenny *et al*., 2020). This implies that probiotics could be useful in complementing the treatment measures aimed at reducing the hematological side effects of antibiotics.

Based on the levels of mean corpuscular hemoglobin (MCH), the mean corpuscular hemoglobin concentration (MCHC) and the mean corpuscular volume (MCV), the anemia type is normocytic normochromic. It is usually associated with anemia as a result of chronic diseases or acute blood loss rather than as an outcome of a lack of iron, vitamin B12, or folate that leads to microcytic or macrocytic anemia (Camaschella, 2015). This might be attributable to inflammation or antibiotic-induced dysbiosis that directly suppresses the production of red blood cells in the bone marrow (Yan *et al*., 2018).

5.1.3 Effects of Antibiotic-Induced Dysbiosis on White Blood Cell Counts and Subtypes

The study evaluated the impact of antibiotic-induced dysbiosis on the white blood cell (WBC) counts and its subtypes. Treatment with septrin alone resulted in a rise in total WBC count, which was statistically higher than the control group, amoxicillin and amoxicillin-septrin-treated group. These findings from the study are in line with previous observations suggesting that some antibiotics can elicit immune responses that result in changes in the composition of the gut microbiota, affecting systemic immune functions (Langdon *et al*., 2016). Failure to reverse leukocytosis with probiotics indicates that the use of probiotics may not provide an adequate protective shield against septrin-induced immunomodulation of the gut microbiota (Agus *et al*., 2021).

The study showed an increase in the total count of neutrophils, lymphocytes and monocytes observed after the administration of septrin. These findings are in agreement with studies suggesting that antibiotics have the potential to shift the immune cell population (He *et al*., 2020). Markedly elevated neutrophil count and lymphocyte count suggest an active immune response that may be attributed to bacterial overgrowth or translocation in the intestine due to dysbiosis (Schlechte *et al*., 2023).

The up-regulation of eosinophils when treated with septrin alone and in combination with amoxicillin, infers an allergy or parasitic infection-like response, which may be elicited through gut permeability breakdown and subsequently increased antigenicity (Foster *et al*., 2017). The gut dysbiosis causes leaky gut leading to entry of the diseases causing microbes to enter the lamina propria and into the circulation infecting other

organs thus causing recruitment of lymphocytes and macrophages (Ozaka *et al*., 2022). Antibiotic-induced dysbiosis exposes the gut epithelia to pathogenic microbes, this exposes the gut epithelia to injury and inflammatory responses and in that case causing recruitment of eosinophils to the site which may expose the host to inflammatory bowel diseases (IBDs) (Rath & Haller, 2022). The observed reduction in basophils in septrin-treated mice could be due to a trans-switch to a different type of immune response. These results support the notion of the fact that certain or distinct changes can be experienced in immune cell features in response to antibiotic treatment depending on the kind and dosage of the antibiotics used (Ruuskanen *et al*., 2021). Probiotic treatment restored the levels of white blood cells subtypes including neutrophils, lymphocytes, monocytes, eosinophils and basophils. Probiotic treatment restored the levels of white blood cell subtypes, including neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Probiotic bacteria can interact and stimulate intestinal immune cells and commensal microflora, modulating specific immune functions and homeostasis. Several studies described the positive effect of probiotics on neutrophils' function to enhance innate immunity and make the body more capable of fighting against infections (Mangrolia & Osborne, 2021; Mazziotta *et al*., 2023). Certain strains like Lactobacillus and Bifidobacterium may modulate neutrophil activity due to anti-inflammatory processes that favor phagocytic activity (Cristofori *et al*., 2021). Probiotics may also replenish lymphocyte numbers based on enhancing T-cell and B-cell responses. Study indicates that the intake of probiotics can enhance Th1 (pro-inflammatory) and Th2 (anti-inflammatory) responses, thus supporting a balanced adaptive immune system (Cristofori *et al*., 2021). Probiotics may upregulate the activity of monocytes, therefore contributing to body defense mechanisms, especially in light of dysbiosis or antibiotic treatments. Probiotics are generally associated with reducing eosinophil activities, mainly in allergic inflammatory disorders such as asthma and atopic dermatitis. Basophils are related to allergic responses, as in the case of hypersensitivity, so their levels may be modulated by probiotics, which would be favorable in the regulation of immune responses against allergens and in lessening the intensity of the allergic reactions (Lopez-Santamarina *et al*., 2021).

5.1.4 Effects of Antibiotic-Induced Dysbiosis on the Platelets Counts

The treatment with Septrin resulted in thrombocytopenia, which was characterized by lower levels of platelets in the blood. This concurs with previous findings showing that antibiotics disrupt the balance of gut bacteria and cause inflammation and abnormalities in the immune system that impact the production and longevity of platelets (Sender *et al*., 2016). Thrombocytopenia noted in the present study could be attributed to the antibiotic imbalance of the gut microbiota that alters the fundamental mechanisms of immunity and platelet regulation (Levi *et al*., 2020; Rust *et al*., 2023).

On the other hand, the administration of amoxicillin alone or in combination with septrin was seen to enhance the level of platelet relative to the control significantly. This result differs from some of the previous works but can be attributed to the variability of the impact of antibiotics on the gut microbiome and the immune system. Amoxicillin might alter the microbiota in a way that encourages platelet synthesis or discourages platelet degradation (Josefsdottir *et al*., 2017). Further research is required to detail how precisely; however, this illustrates the multifaceted nature of antibiotics' interaction with host physiology.

The normalization of platelet count after probiotic administration is in agreement with findings that show that the use of probiotics aids in the normalization of the gut microbiome and platelets profile (Zhu *et al*., 2020). Probiotics may, therefore, alleviate the negative impacts of dysbiosis through the restoration of intestinal integrity and down regulation of inflammatory responses, leading to normal platelet production.

The decline in Platelet-large cell ratio (P-LCR) and platelet distribution width (PDW) upon antibiotic therapy, while mean platelet volume (MPV) remained unchanged, confirms the selective impact on the platelets shape and production. These findings are consistent with previous results on the effects of antibiotics that affect platelet volume and distribution, which may be due to their effects on bone marrow or megakaryocyte function (Abo *et al*., 2017). The lack of change in the overall size of the MPV supports the hypothesis that only specific effects on the production and turnover of the platelets are involved.

5.1.5 Effect of Antibiotic-Induced Dysbiosis on Serum Electrolytes Levels

Various studies have linked the gut microbiota to changes in serum electrolyte balance (Lezutekong *et al*., 2018; Pan *et al*., 2021; Grüner *et al*., 2023). The gut microbiota is involved in metabolism and enhance the absorption of dietary electrolytes, a perturbation of the gut microbiota therefore can influence the serum electrolyte balance and its consequences could be fatal (Grüner *et al*., 2023). The study showed significant drop in the levels of sodium, chloride and potassium across all the test groups. The findings from this study are in line with previous studies suggesting that antibiotics disrupt the balance of gut microbiota which consequently causes fluctuations in the host's electrolyte levels (Turnbaugh *et al*., 2006; Kim, 2007; Claesson *et al*., 2012; Lew & Radhakrishnan, 2020). Probiotic treatment restored the levels of serum chloride and sodium indicating its potential in restoring serum electrolytes balance. Administration of probiotics post antibiotic treatment has beneficial impact as they were able to replenish the gut microbiota lost due to antibiotic intake and therefore, were able to bring the electrolyte balance towards the normal levels (Ray *et al*., 2014; Metchnikoff, 2007; O' Mahony *et al*., 2005).

In conclusion, this study underlines the dramatic impact of antibiotics on the gut microbiota and the physiological and biological consequences involved. It was confirmed that the administration of antibiotics at the early stages of life has the tremendous impact on the gut microbiota and can cause a range of health problems. This dysbiosis also hampers hematopoiesis, as evidenced by shifts in red and white blood cell counts and platelet levels. The supplementation of probiotics has the potential to replenish the disrupted gut bacteria and alleviation of some of these harmful side effects. Thus, it is essential to use antibiotics properly and undertake appropriate probiotic supplementation for maintaining gut health and general body health.

5.2 Effect of Antibiotic-Induced Gut Microbiota Dysbiosis on Pathological Changes

Gut microbiota plays significant role in general health, including regulation of the immune system and organ health and function (Wu & Wu, 2012). The study assessed the impact of antibiotic treatment on the gut bacterial population and subsequent pathological changes in swiss mice. The study evaluated the liver function, kidney

function and changes in immunological reaction by examining the levels of cytokines; tumor necrotic factor alpha (TNF-α), Interleukin-10 (IL-10), and Interferon gamma (IFN-γ).

5.2.1 Effects of Antibiotic-Induced Gut Dysbiosis on the Liver Function

Biochemical analysis of the serum studies was conducted to assess the negative impact of antibiotic-induced dysbiosis, on the integrity of the liver, using alanine transferase (ALT), aspartate transferase (AST), ALT: AST ratio, alkaline phosphatase (ALP) and direct bilirubin which are markers of normal liver function. There were notable elevated levels of AST, ALT, ALP and AST: ALT ratio across all the test groups in relation to control group, indicative of liver damage. These findings concurs with observations explaining that antibiotics cause the modulation of the gut microbiota, which causes systemic inflammation and hepatic stress (Yan *et al*., 2011; Xie *et al*., 2020). Increased levels of these enzymes are a sign of hepatocellular injury and cholestasis which is a sign of impaired liver function (Ferrucci *et al*., 2016). However, increase or decrease in ALT: AST ratio is indicative of liver damage since both ALT and AST are diagnostic markers of liver damage (Elgazzar *et al*., 2022).

Qualitative changes such as increased proportions of pathogenic bacteria and reduced population of beneficial bacteria, and also quantitative changes in the total amount of bacteria (overgrowth) have been associated with liver disease (Llorente & Schnabl, 2015; Zhou *et al*., 2023). Study done by Boursier *et al.*, (2016) showed that gut microbiome dysbiosis contributes to non-alcoholic fatty liver disease (NAFLD) development through the gut-liver axis by dysregulating the gut–liver axis, leading to increased intestinal permeability and unrestrained transfer of microbial metabolites into the liver.

Probiotic supplementation restored the levels of ALT and, therefore, has a protective effect on liver function. This is in line with previous findings indicating that probiotics favor the balance of the gut microbiota, decrease inflammation and have beneficial effects on the liver (Wang *et al*., 2020). The protective effects of probiotics could be due to the promoted gut barrier integrity, decreased translocation of endotoxin, and

regulation of the host's immune system, which eventually reduces hepatic inflammation and oxidative stress caused by antibiotics (Kirpich *et al*., 2008).

There were no significant changes in direct bilirubin levels in all the experimental groups compared with the blank control group. This finding implies that, although the antibiotics might have caused hepatocellular toxicity, they had no major effect on the bilirubin-conjugation and excretion process. This is particularly intriguing when compared to the increased enzyme levels, which may suggest that the antibiotics affect liver cell integrity rather than biliary function (Lazarus *et al*., 2020).

The gut-liver axis is a relatively new area of focus that describes how gut microbiota affect the performance of the liver. Disruption of the balance in the gut microbiota, can cause increased intestinal permeability, the translocation of the microbial products that cause systemic inflammation and hepatic stress (Mouzaki *et al*., 2016). In line with this notion, the present study reveals that antibiotic-induced dysbiosis results in liver damage and finds that probiotics have the potential to restore the gut microbiota balance and prevent liver injury.

5.2.2 Effect of Antibiotic-Induced Dysbiosis on the Kidney Function

This study also focused on the effect of antibiotic administration on gut microbiota and subsequently on the kidney and its functionality. Kidney is a vital organ of the body that takes part in removal of wastes and extra fluid in the body. It also removes acids produced by cells, and maintains healthy balance of water, salts and minerals in the blood. Kidney function test was done in this study by using creatinine and urea, uric acid serum levels as markers for normal kidney function or damage. Previous study has linked antibiotic administration to kidney damage. The intestinal microbiota maintains a symbiotic relationship with the host under normal conditions, but its imbalance has recently been associated with several diseases (Guldris *et al*., 2017).

The mice that were treated with amoxicillin and septrin alone or in combination developed high creatinine, urea, and uric acid levels, which are signs of impaired renal function. This is in concordance with previous findings by Patangia *et al*. (2022) showing that antibiotics administration causes detrimental effects in the gut microbiota,

which consequently alters the immune system and metabolic processes that adversely impact the kidneys. High levels of these waste products indicate low glomerular filtrate rate (GFR) and potential renal dysfunction.

The observed decrease in albumin levels in the groups that were treated with antibiotics also points towards the possibility of kidney damage. Hypoalbuminemia is a sign of kidney dysfunction and can help define impaired renal clearance (Anders *et al*., 2020). These findings are in line with some previous research showing that gut dysbiosis causes systemic inflammation and endothelial dysfunction, hence causing kidney damage (Anders *et al*., 2013).

The gut-kidney axis is a relatively new concept that attempts to make a connection between gastrointestinal microflora and renal performance. Dysbiosis can cause the generation of uremic toxins, induction of inflammation, and changes in metabolisms, all of which can cause kidney injury (Vaziri *et al*., 2013). The current study gives credence to this idea where it is shown that antibiotic-mediated disruption of the gut microbial community could result in renal dysfunction condition (Ray *et al*., 2022).

The supplementation with probiotics positively impacted the renal function since the creatinine, urea, uric acid, and albumin levels were restored to normal. This aligns with earlier studies revealing that probiotics can help in the restoration of the gut microbiota, consequently inhibiting inflammation and improving the metabolic profiles (Zhou *et al*., 2024). Probiotics may reduce organ damage by improving intestinal integrity and influencing immune regulation, thus blocking the impact of imbalanced microbiota on kidneys.

5.2.3 Effects of Antibiotic-Induced Dysbiosis on Cytokine Levels

These gut microbiome plays a fundamental role in the maturation, function, and regulation of the host-immune system from birth to old age (Bosco & Noti, 2021). Commensal microbiota can profoundly influence the development of the gut mucosal immune system and be crucial in preventing exogenous pathogen intrusion, both by direct interaction with pathogenic bacteria and by stimulation of the immune system (Purchiaroni *et al*., 2013). A cross talk between the mucosal innate immune system and

endogenous microbiota favors a mutual growth, survival and inflammatory control of the intestinal ecosystem. Gut dysbiosis, or negative alterations in gut microbial composition, can also dysregulate immune responses, causing inflammation, oxidative stress, and insulin resistance (Yoo *et al*., 2020).

The impact of antibiotic induced gut microbiota dysbiosis in the induction of inflammatory responses was assessed by evaluating the levels of cytokines in the serum. The augmented levels of TNF-α and IFN-γ upon administration of amoxicillin or septrin or, in combination, suggests active inflammation. This is in concordance with research, which shows that antibiotics alter the gut microbiota composition and promote inflammation and immune dysfunction (Willing *et al*., 2011). The higher levels of pro-inflammatory cytokines noted in the current study indicate that antibiotic imbalances in the gut microbiome have the potential to worsen the inflammatory processes, perhaps through the increased permeability of the gastrointestinal barrier and translocation of microbial components (Becattini *et al*., 2016).

The significant elevation of the pro-inflammatory cytokines TNF-α and IFN- γ, along with unchanged IL-10 levels, resulted in an imbalanced ratio of pro-inflammatory to anti-inflammatory cytokines (IFN-γ: IL-10 and TNF-α: IL-10). This imbalance is suggestive of a shift in inflammatory homeostasis, which has been proposed in other previous studies focused on the impact of gut dysbiosis on systemic inflammation (Dinarello, 1997; Hemmingsen *et al*., 2017). The sustained inflammation in the antibiotic treated groups further emphasizes the key role of gut microbiota in maintaining immune homeostasis.

All the test groups presented with comparable levels of IL-10, an anti-inflammatory cytokine. IL-10 was preferred in this study because of its ability to stimulate release of endogenous anti cytokines and able to alter the sensitivity of pro-inflammatory cytokine receptors (Zhang *et al*., 2007). This study indicates that although antibiotics increased the levels of pro-inflammatory cytokines, they had no profound effect on the secretion of IL-10. This phenomenon suggests that the changes in gut microbiota favor pro-inflammatory changes while minimally exerting suppression of anti-inflammatory processes, which has been demonstrated in other models of antibiotic-driven dysbiosis

(Vieira *et al*., 2016; Wang *et al*., 2022). Several studies have shown that gut microbiota plays important role not only in regulation of immune system responses, inflammation responses but also in regulation of oxidative stress (Shabbir *et al*., 2021). Administration of probiotics restored the levels of inflammatory cytokine. This is in line with the research done on probiotics to find out that they are capable of altering the balance of the gut microbiota, improving barrier function of the gastrointestinal tract and decreasing the overall inflammation in the body (McFarland, 2015). The anti-inflammatory effects of probiotics could be explained by the synthesis of SCFAs, regulation of functions of immune cells, and inhibition of pathogenic bacteria (Zhang *et al*., 2019).

5.2.4 Effects of Antibiotics-Induced Dysbiosis on Histopathological Changes in Organs

The histological examination of brain tissues did not reveal significant neuropathological evidence across all groups. The brain sections appeared normal, suggesting that antibiotic-induced dysbiosis did not cause pathological damage to the brain. These findings however did not appear consistent with previous studies that have shown impact on brain tissues following antibiotic treatments (Dahiya & Nigam, 2023).

Histological assessment of liver samples from mice in the amoxicillin group revealed sections of congestion. Sinusoidal congestion in the liver is characterized by stagnation of blood within the sinusoids caused by either reduced blood circulation or enhanced sinusoidal vasoconstriction. This condition can be caused by several pathophysiological processes, such as inflammation, oxidative stress or direct toxicity to hepatocytes (Lazarus *et al*., 2020). These effects were exacerbated when septrin was combined with amoxicillin; sections of the liver revealed congestion and hepatocyte swelling, and this, therefore, pointed to accumulated liver damage from these two types of antibiotics that disrupt gut microbiota and elicit subsequent systemic responses resulting in severe liver injury (Huang *et al*., 2022; Liu *et al*., 2023). The probiotic treatment alleviated these damages on the liver, however, not completely since there were notable sections of congestion, but it showed the potential of guarding the liver. These findings are in line with previous findings by Plaza-Diaz *et al*. (2019).

Histopathological changes in kidney tissues demonstrated that oral amoxicillin or septrin, singly or in combination in mice resulted in pathologic alterations in the kidneys compared to the control group. The healthy gut has tight intercellular junctions between epithelial cells to avoid the leakage of lipopolysaccharides (LPS) into the bloodstream. Dysbiosis compromises such tight junctions, increasing gut permeability by increasing bacterial endotoxins, specifically lipopolysaccharides (LPS), in circulation, which is often referred to as "leaky gut". These bacterial products can incite immune and endothelial cells in remote organs, which results in the dilation of blood vessels and increased capillary permeability, thereby causing congestion (Fernández-Ruiz *et al*., 2023; Hill *et al*., 2022). These findings are consistent with the current study results which histological examination has revealed renal sections in the amoxicillin-treated group. Histological examination of the septrin group and amoxicillin-septrin group revealed a section of interstitial hemorrhage and epithelial cell sloughing. This phenomenon is attributed to antibiotic induced gut microbiota dysbiosis, which results in bacterial products and inflammatory mediators entering the bloodstream and causing systemic inflammation and endothelial dysfunction. This impairment weakens the endothelial barrier, especially in organs such as the kidneys, making the tissues more prone to hemorrhage (Hellenthal *et al*., 2022).

The findings affirm the studies done indicating that antibiotic induced dysbiosis causes kidney damage (Feng *et al*., 2021). Dysbiosis may promote inflammation and oxidative stress and can hence cause direct injury to renal tubular epithelial cells. The damage may stem from cytokine release, increased levels of ROS, and immune cell infiltration due to dysbiosis microbial products. Desquamation of the epithelial cells reduces the hormonal receptors' efficiency and increases tissue damage in the kidney (Fernández-Ruiz *et al*., 2023). These outcomes demonstrate that dysbiosis with subsequent organ damage is systemic and proves the interdependence of the gut and systemic organ functions. Conversely, the administration of Amoxicillin+Septrin+Probiotic demonstrated some sections with renal congestion, implying blood flow impediment, yet the glomerular structure appeared normal, indicating a potential protective effect of probiotics on the kidneys (Fagundes *et al*., 2018).

Therefore, the current work proves that changes in the gut microbiota through antibiotic use have profound effects on immunological effects and produce pathological alterations to essential organs. Increased levels of AST and ALP suggested hepatocellular damage, while probiotics positively affected the liver function tests, showing their effectiveness. The study also established reduced renal function through elevated serum creatinine, urea, and uric acid levels and histological changes in the kidney tissues. Additionally, TNF-α and IFN-γ levels have been raised, whereas IL-10 remains unchanged, indicating a pro-inflammatory bias. Probiotics alleviated these effects which underscores its role in restoring the physiology of the gut and preventing damage to the systemic organs.

5.3 Effect of Antibiotic-Induced Gut Microbiota Dysbiosis on Induction of Oxidative Stress

In the current study analysis was done to determine the effect of antibiotic-induced dysbiosis in induction of oxidative stress on cells and tissues. Glutathione is perhaps the most prolific low molecular weight thiol molecule generated by organisms (Forman *et al*., 2009). It is essential for both oxidative damage protection and signaling pathways. Glutathione is oxidized to glutathione disulfide (GSSG) (Monostori *et al*., 2009).

Therefore, determining GSH and GSSG concurrently is a useful method for determining oxidative stress. (Nuhu *et al*., 2020). From the study, amoxicillin and septrin, either administered alone or in combination, resulted in a reduction in GSH levels in both the liver and brain, indicating oxidative stress. This is in agreement with another research where antibiotics alter the gut microbiota, thereby increasing Reactive Oxygen Species (ROS) production and the effects of oxidative stress in various tissues (Xie *et al*., 2020). Reduced GSH levels make cells more vulnerable to oxidative damage, which is marked by the accumulation of reactive oxygen species. GSH is the brain's primary antioxidant and protects against oxidative stress-related disorders (Dringen, 2000).

Hepatocytes, which are the main detoxifying cells of the liver, are highly vulnerable to oxidative damage, as are neuronal cells of the brain in regard to their high levels of

oxygen consumption and lipid content (Zhu *et al*., 2019). The levels of GSH were enhanced in the liver and brain tissues following probiotic supplementation, which may contribute towards the prevention of oxidative stress. This is in line with previous findings that indicate that probiotics can upregulate antioxidant mechanisms associated with gut microbiota and lower inflammation and oxidative stress levels (Colletti *et al*., 2023). The protective effects of probiotics could be related to the effects they have on the gut barrier, endotoxin-mediated inflammation and alterations in the immune response of the host.

Study has been established that the gut microbiota regulates the levels of systemic oxidative stress (Zhao *et al*., 2023). Dysbiosis is known to cause alterations in intestinal permeability, which in turn permits the movement of microbial-derived products into the systemic circulation, leading to inflammation and oxidative stress responses (Lin *et al*., 2021). This idea aligns with our findings showing that antibiotics alter the gut microbiota, resulting in oxidative stress in multiple organs, which can be addressed by the use of probiotics to rebalance the gut microbiota.

Notably, the GSH levels were increased in the spleen, kidney, lungs, and heart in all the test groups as compared to the control group. This suggests that there is an adaptive response to oxidative stress in these organs. This may actually be due to an upregulation of antioxidant enzymes such as GSH to counteract increased oxidative stress (Yuan *et al*., 2018). These observations imply that although some organs have reduced levels of GSH owing to increased oxidative stress, other organs could increase the synthesis of GSH to combat the situation (Shandilya *et al*., 2021).

Oxidative stress was assessed in organs by examining the levels of Malondialdehyde (MDA) in organs which is a marker for lipid peroxidation. Lipid peroxidation was employed as an indicator for oxidative stress in a chain reaction that produces a variety of active chemicals that cause cell injury (Singh *et al*., 2014). Malondialdehyde (MDA) is one of end products from polyunsaturated fatty acid peroxidation in cells. An elevated level of free radicals leads to excessive MDA generation (*Gawel et al*., 2004). Studies have shown that MDA can be used as a marker to measure the level of oxidative stress (Gönenç *et al*., 2001; Cherian *et al*., 2019). In the current study, administrations of

amoxicillin, septrin, or their combination led to an increase in the level of malondialdehyde (MDA) in the brain, liver, spleen, kidneys, lungs, and heart tissues when compared to the control group. The present results indicate that antibiotic-induced dysbiosis leads to oxidative damage in various organs including the brain, liver, spleen, kidneys, lungs, and the heart.

The observed increase in MDA levels is in line with earlier reports suggesting that dysbiosis affects antioxidant protection and enhances oxidative stress in tissues (Leung *et al*., 2020). Changes in gut microbiota patterns due to dysbiosis can lead to a disruption of the intestinal barrier function, permeability and translocation of bacterial products, including LPSs, into the systemic circulation. After that, LPS can induce pro-inflammatory cytokines and oxidative stress signaling resulting in lipid peroxidation and MDA production (Seki *et al*., 2012).

However, antibiotic-induced increase in MDA levels was only partially offset by the administration of probiotics. It is established that probiotics help to restore the disturbed balance of microbiota of the intestine and increase the stability of the mucosal barrier, which helps to minimize systemic oxidative stress associated with dysbiosis (Hao *et al*., 2015; Plaza-Diaz *et al*., 2019). The supplementation of probiotics restored the levels of MDA. However, the normalization was not complete, which implies the necessity of more prolonged or more directed interventions with probiotics to restore oxidative homeostasis and organ functions.

The concentrations of nitric oxide (NO) in serum were also measured to demonstrate oxidative stress. Nitric oxide (NO) is commonly regarded as one of the most significant chemicals produced by the human body, serving as a critical regulator of a wide range of vital physiological processes, including blood pressure, immunological response, and brain transmission. The capacity to generate appropriate NO levels defines a healthy endothelial cell (Pierini & Bryan, 2015). Nitric oxide is highly sensitive to the presence of free radicals, particularly superoxide, which interacts with NO to form peroxynitrite, inhibiting NO-mediated activities and increasing oxidative damage (Pacher *et al*., 2007).

The treatment of the mice with amoxicillin and septrin, either singly or in combination, increased the serum nitrites significantly. This is in line with other research showing that antibiotics alter gut microbiota, which causes increased oxidative stress and production of reactive nitrogen species (RNS) (Caparros-Martin *et al*., 2017). Gut microbiota dysbiosis leads to an alteration in the integrity of the gut barrier, consequently known as the "leaky gut." In these circumstances, microbial products such as lipopolysaccharides (LPS) cross from the gut lumen to the systemic circulation. LPS is a strong stimulus for the immune cells. It leads to the synthesis of pro-inflammatory cytokines and NO in connection with the activation of Inducible nitric oxide synthase (iNOS) in various tissues (Feng *et al*., 2018). High levels of nitrites are suggestive of a rise in nitrosative stress on cells or tissues due to the fact that nitrites are metabolites of nitric oxide (Feng *et al*., 2018; Codoñer-Franch *et al*., 2011).

Supplementation with probiotics was found to have a positive impact in decreasing the levels of nitrites but remained higher than the control. This supports the findings of previous studies where it was established that probiotics aid in replenishing the balance of gut micro-biota and decrease oxidative stress (Guo *et al*., 2016). Probiotics may help protect the lining of the gut, lower inflammation in the body and enhance the body's antioxidant defenses through the reduction of oxidative stress and nitrogen species (Hemarajata & Versalovic, 2012).

Several studies have indicated that gut microbiota is a paramount influential factor in oxidative stress. The imbalance in gut microbiota population may provoke ROS and RNS overproduction, which cause harm to cell structures and participate in disease development (Schwartz *et al*., 2020). The results of this study support the premise made in the hypothesis that antibiotic induced dysbiosis negatively alters the healthy functioning of gut microbiota, contributing to oxidation stress and increased serum nitric oxide concentration.

Therefore, present work clearly showed the effects of antibiotic-induced gut microbiota disruption in raising oxidative stress in different tissues through diminished GSH levels and higher MDA and nitric oxide concentrations. This oxidative stress leads to cellular and tissue damage, especially in the liver and kidney as evidenced by significant

elevated levels of GSH and MDA. As observed, probiotic supplementation had a protection role through increasing GSH and decreasing partly MDA and nitric oxide, which underlines the role of probiotics in the alleviating oxidative stress. These findings raise the importance of promoting balance and proportionality of the gut microbiota in avoiding oxidative stress and its negative impact on full-body organ function.

CHAPTER SIX

SUMMARY, CONCUSSION AND RECOMMENDATIONS

6.1 Summary

The study assessed the impact of antibiotic treatment on the gut microbiota population and the subsequent effect of the antibiotic-induced gut microbiota dysbiosis on the body weight, relative organ weight, biochemical, immunological, and pathological changes, and induction of oxidative stress. The study simulated a human infant during weaning with three weeks old male swiss mice model to examine the impact of antibiotic treatment at an age where milestone development of the gut microbiota occurs. The study utilized amoxicillin and septrin as representation of commonly prescribed antibiotics. The study revealed a significant reduction in the gut microbiota population, indicating that antibiotic treatment results in gut microbiota dysbiosis. The gut microbiota dysbiosis showed no substantial change in body and relative organ weights. There were significant changes in hematological components, including red blood cells, white blood cells and their subtypes, and Platelets and their indices; substantial changes in immunological responses, and histological examination revealed pathological changes in the liver and kidneys. Antibiotic-induced dysbiosis induced oxidative stress, as indicated by significant changes in glutathione, nitric oxide, and malondialdehyde levels in organs. The study shows that antibiotic treatment affects the gut microbiota population, especially in infants, producing harmful effects. Therefore, caution is necessary when administering antibiotics to individuals, especially at an early age.

6.2 Conclusion

The oral antibiotic treatment of amoxicillin and septrin singly or in conjunction in male swiss miss resulted in significant shifts in gut microbiota by reducing the gut microbiota population, disrupting the delicate balance resulting in gut microbiota dysbiosis. Gut microbiota dysbiosis resulted shift in hematological profiles including RBCs, WBCs count and its subtypes and platelets and its indices. It also resulted in serum electrolytes imbalance as there were observed significant reduction in the levels of sodium, chloride and potassium. Probiotic treatment replenished the gut microbiota and its subsequent impact was observed in restoration of the levels of RBCs and its indices, white blood cells subtypes and platelets and its indices to normal levels, it also presented positive

impact on restoring homeostatic balance by retaining the levels of electrolytes within the normal range.

The systemic repercussions of antibiotic therapy on induction of gut microbiota dysbiosis extend to pathological changes, which was observed due to changes in the levels of cytokines; elevated levels of TNF- α and INF- γ, kidney and liver function indicated by changes in levels of AST, ALT, creatinine, urea, and a drop in albumin levels. Histological examinations further revealed damage to the kidneys and liver. Probiotic treatment showed positive outcomes by blocking the induction of inflammatory responses by restoring the levels of cytokines TNF- α and INF- γ and protects the liver and kidneys from damage as shown by reversing the levels of AST, ALT, ALP, creatinine, urea, and albumin levels.

Antibiotic induced dysbiosis resulted in active oxidative stress which was evidenced by elevated levels of NO in the serum, increased levels of MDA, and GSH in the brain, heart, kidneys, spleen, lungs, and liver. Crucially, the introduction of probiotics alongside antibiotics emerges as a promising strategy, demonstrating protective effects against oxidative stress and potential in protecting organ damage suggesting a potential avenue for ameliorating complications associated with antibiotic-induced dysbiosis.

6.3 Recommendations of the Study

i. The study underscores the need for cautious antibiotic prescriptions, particularly at an early age, given the profound impact of antibiotics on inducing gut microbiota dysbiosis, and consequent impact on physiological parameters, and pathological changes in mice which can be reciprocated in human young babies. Healthcare practitioners, especially those dealing with infants and children, are urged to exercise discretion when prescribing antibiotics.
ii. The study recommends the integration of probiotics alongside antibiotics, especially in cases of anticipated prolonged or repeated use. Probiotics demonstrate protective effects, mitigating adverse outcomes associated with dysbiosis, such as restoring hematological indices, maintaining electrolyte balance, suppressing inflammatory responses, and protecting against oxidative stress.

iii. Moreover, recognizing serious adverse effects, particularly with antibiotics like Septrin, prompts exploration of alternative antibiotics with potentially fewer side effects. This multifaceted approach aligns with the study findings and contributes to the broader goal of safeguarding the health of patients, especially the vulnerable pediatric population.

6.4 Suggestions for Further Studies

i. For further studies, it is recommended to delve into microbial characterization pre- and post-treatment to identify antibiotic-resistant bacterial strains to identify potential pathogenic bacteria post antibiotic treatment in the gut microbiota.
ii. Histological examinations on the small intestine could assess antibiotic effects on intestinal structure.
iii. Study be conducted at higher dosage levels and longer treatment period to assess neuropathological changes in the brain.
iv. The study proposes similar research on older mice to ascertain conditions common in older people such as dementia. These suggestions aim to expand our understanding of antibiotic effects on gut microbiota and guide future research for more comprehensive insights.

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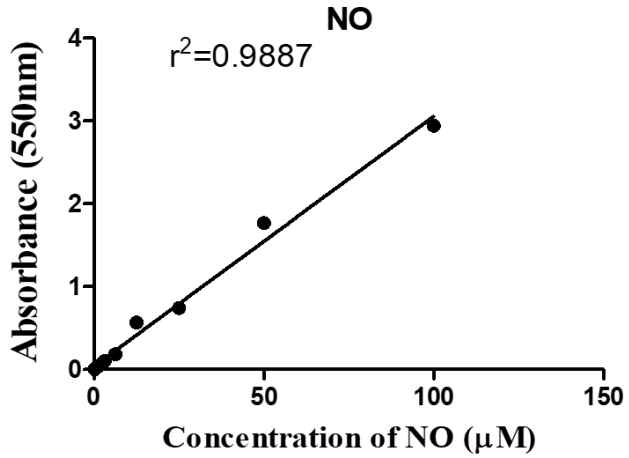
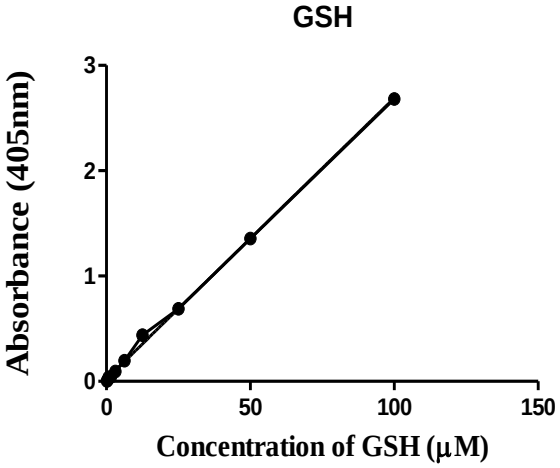
APPENDICES

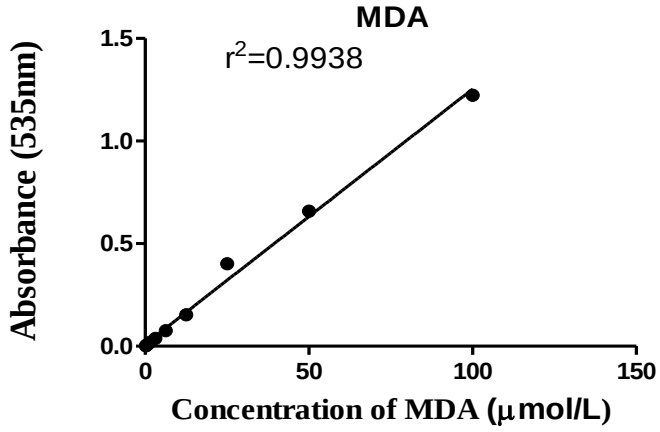
Appendix I: Standard curve for determination of Nitrites in the serum



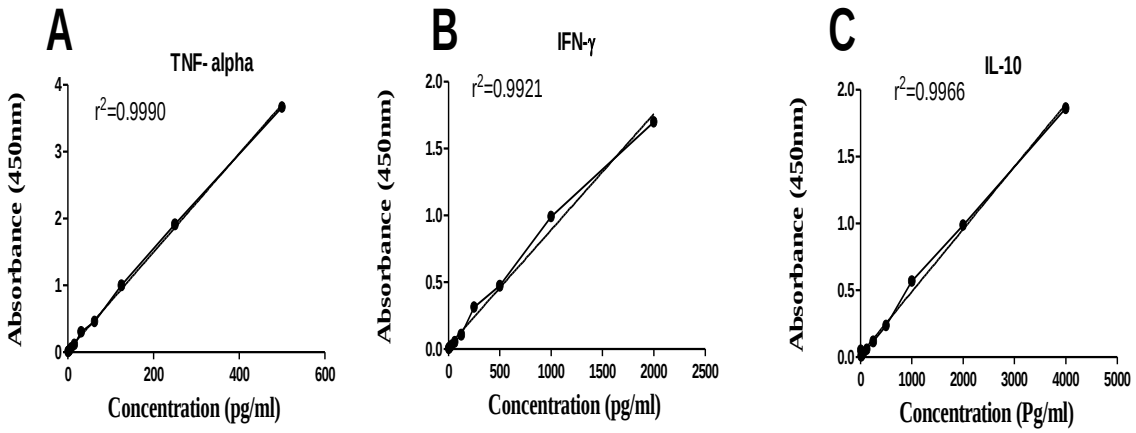
Appendix II: Standard curve for determination of Glutathione (GSH) levels in tissues.



Appendix III: Standard curve for determination of Malondealdehyde (MDA) levels in tissues.



Appendix IV: Standard curves for determination of inflammatory cytokines TNF-alpha, (A), IFN-y (B), IL-10 (C) Absorbance observed at 450nm plotted against concentration.



Appendix V: Descriptive Statistical Analysis of Physiological and Biochemical Changes

a. Effects of Antibiotic Induced Dysbiosis on Progressive Change in Body Weight

Table Analyzed	Body Weight					
One-way analysis of variance						
P value	< 0.0001					
P value summary	Ns					
Are means signif. different? (P < 0.	No					
Number of groups	5					
F	0.84					
R squared	0.10					
Bartlett's test for equal variances						
Bartlett's statistic (corrected)	22.83					
P value	0.0004					
P value summary	Ns					
Do the variances differ signif. (P < 0	No					
ANOVA Table	SS	df	MS			
Treatment (between columns)	6.5	4	1.625			
Residual (within columns)	64.12	42	1.527			
Total	70.62	46				
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant	Summary	95% CI of diff	
Wt-naive vs Septrin	0.133	0.321	No	ns	-0.820 to 1.086	
Wt-naive vs Amox-sept	0.259	0.621	No	ns	-0.694 to 1.212	
Wt-naive vs Amox-sept-Prob	0.459	1.098	No	ns	-0.494 to 1.412	
Amox vs Septrin	-0.365	0.881	No	ns	-1.318 to 0.588	
Amox vs Amox-sept	-0.239	0.549	No	ns	-1.192 to 0.714	
Amox vs Amox-sept-Prob	-0.0394	0.092	No	ns	-0.992 to 0.914	
Septrin vs Amox-sept	0.126	0.297	No	ns	-0.827 to 1.079	

Septrin vs Amox-sept-Prob	0.326	0.771	No	ns	-0.627 to 1.279
Amox-sept vs Amox-sept-Prob	0.200	0.475	No	ns	-0.753 to 1.153
Wt-naive vs Septrin	0.133	0.321	No	ns	-0.820 to 1.086
Wt-naive vs Amox-sept	0.259	0.621	No	ns	-0.694 to 1.212
Wt-naive vs Amox-sept-Prob	0.459	1.098	No	ns	-0.494 to 1.412
Amox vs Septrin	-0.365	0.881	No	ns	-1.318 to 0.588

b. Effects of Antibiotic Induced Dysbiosis on Organ Weights Brain Weight Descriptive Statistics

Table Analyzed	BRAIN					
One-way analysis of variance						
P value	0.3597					
P value summary	ns					
Are means signif. different? (P < 0.05)	No					
Number of groups	5					
F	1.127					
R squared	0.1091					
Bartlett's test for equal variances						
Bartlett's statistic (corrected)	9.097					
P value	0.1053					
P value summary	ns					
Do the variances differ signif. (P < 0.05)	No					
ANOVA Table	SS	Df	MS			
Treatment (between columns)	0.008088	4	0.002022			
Residual (within columns)	0.08255	46	0.001795			
Total	0.0833588	50				
Tukey's Multiple Comparison Test	Mean Diff.	Q	Significant	Summary	95% CI of diff	
Wt-naive vs Septrin	0.04622	2.928	No	ns	-0.02026 to 0.1127	
Wt-naive vs Amox-sept	0.0337	2.179	No	ns	-0.03144 to 0.09884	
Wt-naive vs Amox-sept-Prob	0.021	1.298	No	ns	-0.04713 to 0.08913	
Amox vs Septrin	0.0419	2.709	No	ns	-0.02324 to 0.1070	
Amox vs Amox-sept	0.026	1.647	No	ns	-0.04049 to 0.09249	
Amox vs Amox-sept-Prob	-0.01252	0.9098	No	ns	-0.07048 to 0.04544	
Septrin vs Amox-sept	-0.02522	1.733	No	ns	-0.08652 to 0.03607	
Septrin vs Amox-sept-Prob	-0.00432	0.314	No	ns	-0.06228 to 0.05364	
Amox-sept vs Amox-sept-Prob	-0.02022	1.432	No	ns	-0.07969 to 0.03924	
Wt-naive vs Septrin	-0.0127	0.8938	No	ns	-0.07254 to 0.04714	
Wt-naive vs Amox-sept	0.0082	0.6121	No	ns	-0.04821 to 0.06461	
Wt-naive vs Amox-sept-Prob	-0.0077	0.5595	No	ns	-0.06566 to 0.05026	
Amox vs Septrin	0.0209	1.471	No	ns	-0.03894 to 0.08074	
Wt-naive vs Septrin	0.005	0.3435	No	ns	-0.05630 to 0.06630	
Wt-naive vs Amox-sept	-0.0159	1.155	No	ns	-0.07386 to 0.04206	

c. Spleen Weight Descriptive Statistics

Table Analyzed	SPLEEN					
One-way analysis of variance						
P value	< 0.0001					
P value summary	Ns					
Are means signif. different? (P < 0.05)	No					
Number of groups	5					
F	0.91					
R squared	0.10					
Bartlett's test for equal variances						
Bartlett's statistic (corrected)	2.21					
P value	< 0.0001					
P value summary	Ns					
Do the variances differ signif. (P < 0.05)	No					
ANOVA Table	SS	Df	MS			
Treatment (between columns)	0.0149	4	0.003725			
Residual (within columns)	0.08011	46	0.001742			
Total	0.09501	50				
Tukey's Multiple Comparison Test	Mean Diff.	Q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	-0.01411	0.897	No	ns	-0.0496 to 0.02139	
WT-Naïve vs WT-Septrin	0.01129	0.715	No	ns	-0.02382 to 0.0464	
WT-Naïve vs WT-Amox-sept	0.01437	0.910	No	ns	-0.02019 to 0.0495	
WT-Naïve vs WT-Amox-sept-Prob	0.01711	1.07	No	ns	-0.01789 to 0.05211	
WT-Amox vs WT-Septrin	0.0254	1.345	No	ns	-0.01902 to 0.0498	
WT-Amox vs WT-Amox-sept	0.02848	1.438	No	ns	-0.01638 to 0.0536	
WT-Amox vs WT-Amox-sept-Prob	0.03122	1.59	No	ns	-0.01236 to 0.0568	
WT-Septrin vs WT-Amox-sept	0.00308	0.155	No	ns	-0.03587 to 0.08202	
WT-Septrin vs WT-Amox-sept-Prob	0.005819	0.359	No	ns	-0.05457 to 0.06620	
WT-Amox-sept vs WT-Amox-sept-Prob	0.00226	0.175	No	ns	-0.04235 to 0.02784	
WT-Naïve vs WT-Amox	-0.01411	0.897	No	ns	-0.0496 to 0.02139	
WT-Naïve vs WT-Septrin	0.01129	0.715	No	ns	-0.02382 to 0.0464	
WT-Naïve vs WT-Amox-sept	0.01437	0.910	No	ns	-0.02019 to 0.0495	
WT-Naïve vs WT-Amox-sept-Prob	0.01711	1.07	No	ns	-0.01789 to 0.05211	
WT-Amox vs WT-Septrin	0.0254	1.345	No	ns	-0.01902 to 0.0498	

d. Heart Weight Descriptive Statistics

Table Analyzed	HEART					
One-way analysis of variance						
P value	0.0095					
P value summary	Ns					
Are means signif. different? (P < 0.05)	No					
Number of groups	6					
F	3.477					
R squared	0.2743					
Bartlett's test for equal variances						
Bartlett's statistic (corrected)	4.053					
P value	0.5418					
P value summary	Ns					

Do the variances differ signif. (P < 0.05)	No					
ANOVA Table	SS	df	MS			
Treatment (between columns)	0.0039	4	0.000975			
Residual (within columns)	0.02604	45	0.000578			
Total	0.02994	49				
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	0.0075	0.895	No	ns	-0.02190 to 0.03690	
WT-Naïve vs WT-Septrin	0.0042	0.515	No	ns	-0.02744 to 0.03604	
WT-Naïve vs WT-Amox-sept	0.0057	0.712	No	ns	-0.02570 to 0.03712	
WT-Naïve vs WT-Amox-sept-Prob	0.0069	0.901	No	ns	-0.02389 to 0.03762	
WT-Amox vs WT-Septrin	-0.0033	0.410	No	ns	-0.03291 to 0.02630	
WT-Amox vs WT-Amox-sept	-0.0018	0.243	No	ns	-0.03172 to 0.02812	
WT-Amox vs WT-Amox-sept-Prob	-0.0006	0.079	No	ns	-0.03048 to 0.02932	
WT-Septrin vs WT-Amox-sept	0.0015	0.197	No	ns	-0.03122 to 0.03422	
WT-Septrin vs WT-Amox-sept-Prob	0.0027	0.371	No	ns	-0.02918 to 0.03458	
WT-Amox-sept vs WT-Amox-sept-Prob	0.0012	0.142	No	ns	-0.02892 to 0.03138	
WT-Naïve vs WT-Amox	0.0075	0.895	No	ns	-0.02190 to 0.03690	
WT-Naïve vs WT-Septrin	0.0042	0.515	No	ns	-0.02744 to 0.03604	
WT-Naïve vs WT-Amox-sept	0.0057	0.712	No	ns	-0.02570 to 0.03712	
WT-Naïve vs WT-Amox-sept-Prob	0.0069	0.901	No	ns	-0.02389 to 0.03762	
WT-Amox vs WT-Septrin	-0.0033	0.410	No	ns	-0.03291 to 0.02630	

e. Liver Weight Descriptive Statistics

Table Analyzed	LIVER					
One-way analysis of variance						
P value	0.0235					
P value summary	Ns					
Are means signif. different? (P < 0.05)	No					
Number of groups	5					
F	2.898					
R squared	0.2395					
Bartlett's test for equal variances						
Bartlett's statistic (corrected)	11.58					
P value	0.041					
P value summary	Ns					
Do the variances differ signif. (P < 0.05)	No					
ANOVA Table	SS	df	MS			

Treatment (between columns)	0.8458	4	0.1692			
Residual (within columns)	2.685	46	0.05837			
Total	3.531	51				
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	0.2933	3.257	No	ns	-0.08592 to 0.6725	
WT-Naïve vs WT-Septrin	0.3486	3.951	No	ns	-0.02293 to 0.7201	
WT-Naïve vs WT-Amox-sept	0.3176	3.443	No	ns	-0.07093 to 0.7062	
WT-Naïve vs WT-Amox-sept-Prob	0.3593	4.073	No	ns	-0.01223 to 0.7308	
WT-Amox vs WT-Septrin	0.4697	5.217	No	ns	-0.09053 to 0.5489	
WT-Amox vs WT-Amox-sept	0.05532	0.7048	No	ns	-0.2753 to 0.3859	
WT-Amox vs WT-Amox-sept-Prob	0.02435	0.2933	No	ns	-0.3253 to 0.3739	
WT-Septrin vs WT-Amox-sept	0.06602	0.8411	No	ns	-0.2646 to 0.3966	
WT-Septrin vs WT-Amox-sept-Prob	0.1764	2.191	No	ns	-0.1627 to 0.5156	
WT-Amox-sept vs WT-Amox-sept-Prob	-0.03097	0.3822	No	ns	-0.3722 to 0.3103	
WT-Naïve vs WT-Amox	0.0107	0.14	No	ns	-0.3111 to 0.3325	
WT-Naïve vs WT-Septrin	0.1211	1.543	No	ns	-0.2095 to 0.4517	
WT-Naïve vs WT-Amox-sept	0.04167	0.5143	No	ns	-0.2996 to 0.3829	
WT-Naïve vs WT-Amox-sept-Prob	0.1521	1.832	No	ns	-0.1975 to 0.5017	
WT-Amox vs WT-Septrin	0.1104	1.407	No	ns	-0.2202 to 0.4410	

f. Kidney Weight Descriptive Statistics

Table Analyzed	Kidney					
One-way analysis of variance						
P value	0.1692					
P value summary	Ns					
Are means signif. different? (P < 0.05)	No					
Number of groups	5					
F	1.637					
R squared	0.1511					
Bartlett's test for equal variances						
Bartlett's statistic (corrected)	4.855					
P value	0.4338					
P value summary	Ns					
Do the variances differ signif. (P < 0.05)	No					
ANOVA Table	SS	Df	MS			
Treatment (between columns)	0.07326	4	0.01465			
Residual (within columns)	0.4117	46	0.00895			

Total	0.485	50				
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	0.1098	3.115	No	ns	-0.03864 to 0.2583	
WT-Naïve vs WT-Septrin	0.0624	1.806	No	ns	-0.08308 to 0.2079	
WT-Naïve vs WT-Amox-sept	0.0195	0.5398	No	ns	-0.1326 to 0.1716	
WT-Naïve vs WT-Amox-sept-Prob	0.0969	2.805	No	ns	-0.04858 to 0.2424	
WT-Amox vs WT-Septrin	0.08406	2.384	No	ns	-0.06442 to 0.2325	
WT-Amox vs WT-Amox-sept	-0.04743	1.543	No	ns	-0.1769 to 0.08201	
WT-Amox vs WT-Amox-sept-Prob	-0.09033	2.779	No	ns	-0.2272 to 0.04656	
WT-Septrin vs WT-Amox-sept	-0.01293	0.4208	No	ns	-0.1424 to 0.1165	
WT-Septrin vs WT-Amox-sept-Prob	-0.02578	0.8174	No	ns	-0.1586 to 0.1070	
WT-Amox-sept vs WT-Amox-sept-Prob	-0.0429	1.352	No	ns	-0.1765 to 0.09073	
WT-Naïve vs WT-Amox	0.0345	1.153	No	ns	-0.09149 to 0.1605	
WT-Naïve vs WT-Septrin	0.02166	0.7046	No	ns	-0.1078 to 0.1511	
WT-Naïve vs WT-Amox-sept	0.0774	2.439	No	ns	-0.05623 to 0.2110	
WT-Naïve vs WT-Amox-sept-Prob	0.06456	1.986	No	ns	-0.07233 to 0.2014	
WT-Amox vs WT-Septrin	-0.01284	0.4179	No	ns	-0.1423 to 0.1166	

g. Lung Weight Descriptive Statistics

Table Analyzed	LUNGS					
One-way analysis of variance						
P value	0.1312					
P value summary	Ns					
Are means signif. different? (P < 0.05)	No					
Number of groups	5					
F	1.802					
R squared	0.1638					
Bartlett's test for equal variances						
Bartlett's statistic (corrected)	20.06					
P value	0.0012					
P value summary	**					
Do the variances differ signif. (P < 0.05)	Yes					
ANOVA Table	SS	df	MS			
Treatment (between columns)	0.03231	4	0.006463			
Residual (within columns)	0.1649	46	0.003586			
Total	0.1973	50				

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	0.06244	2.798	No	ns	-0.03154 to 0.1564	
WT-Naïve vs WT-Septrin	0.04467	2.043	No	ns	-0.04742 to 0.1368	
WT-Naïve vs WT-Amox-sept	0.04192	1.833	No	ns	-0.05439 to 0.1382	
WT-Naïve vs WT-Amox-sept-Prob	0.003367	0.154	No	ns	-0.08872 to 0.09545	
WT-Amox vs WT-Septrin	0.06322	2.833	No	ns	-0.03076 to 0.1572	
WT-Amox vs WT-Amox-sept	-0.01778	0.9138	No	ns	-0.09971 to 0.06415	
WT-Amox vs WT-Amox-sept-Prob	-0.02053	0.9977	No	ns	-0.1072 to 0.06612	
WT-Septrin vs WT-Amox-sept	-0.05908	3.037	No	ns	-0.1410 to 0.02285	
WT-Septrin vs WT-Amox-sept-Prob	0.000778	0.03897	No	ns	-0.08328 to 0.08484	
WT-Amox-sept vs WT-Amox-sept-Prob	-0.00275	0.1369	No	ns	-0.08733 to 0.08183	
WT-Naïve vs WT-Amox	-0.0413	2.181	No	ns	-0.1210 to 0.03845	
WT-Naïve vs WT-Septrin	0.01856	0.9538	No	ns	-0.06338 to 0.1005	
WT-Naïve vs WT-Amox-sept	-0.03855	1.919	No	ns	-0.1231 to 0.04603	
WT-Naïve vs WT-Amox-sept-Prob	0.02131	1.036	No	ns	-0.06534 to 0.1080	
WT-Amox vs WT-Septrin	0.05986	3.077	No	ns	-0.02208 to 0.1418	

h. Effects of Antibiotic Induced Dysbiosis on Relative Organ Weights of the Brain

Table Analyzed	Brain					
One-way analysis of variance						
P value	0.4843					
P value summary	Ns					
Are means signif. different? (P < 0.05)	No					
Number of groups	5					
F	0.9077					
R squared	0.08981					
Bartlett's test for equal variances						
Bartlett's statistic (corrected)	2.659					
P value	0.7523					
P value summary	Ns					
Do the variances differ signif. (P < 0.05)	No					
ANOVA Table	SS	df	MS			
Treatment (between columns)	0.0848	4	0.01696			
Residual (within columns)	0.8594	46	0.01868			
Total	0.9442	50				

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	-0.09078	1.782	No	ns	-0.3053 to 0.1237	
WT-Naïve vs WT-Septrin	-0.09643	1.932	No	ns	-0.3066 to 0.1138	
WT-Naïve vs WT-Amox-sept	-0.07433	1.424	No	ns	-0.2942 to 0.1455	
WT-Naïve vs WT-Amox-sept-Prob	-0.1005	2.014	No	ns	-0.3107 to 0.1097	
WT-Amox vs WT-Septrin	-0.1503	2.951	No	ns	-0.3649 to 0.06419	
WT-Amox vs WT-Amox-sept	-0.00566	0.1274	No	ns	-0.1927 to 0.1814	
WT-Amox vs WT-Amox-sept-Prob	0.01644	0.3501	No	ns	-0.1813 to 0.2142	
WT-Septrin vs WT-Amox-sept	-0.00976	0.2197	No	ns	-0.1968 to 0.1773	
WT-Septrin vs WT-Amox-sept-Prob	-0.05956	1.307	No	ns	-0.2514 to 0.1323	
WT-Amox-sept vs WT-Amox-sept-Prob	0.0221	0.482	No	ns	-0.1710 to 0.2152	
WT-Naïve vs WT-Amox	-0.0041	0.09485	No	ns	-0.1861 to 0.1779	
WT-Naïve vs WT-Septrin	-0.0539	1.214	No	ns	-0.2409 to 0.1331	
WT-Naïve vs WT-Amox-sept	-0.0262	0.5715	No	ns	-0.2193 to 0.1669	
WT-Naïve vs WT-Amox-sept-Prob	-0.076	1.618	No	ns	-0.2738 to 0.1218	
WT-Amox vs WT-Septrin	-0.0498	1.121	No	ns	-0.2368 to 0.1372	

i. Spleen ROW Descriptive Statistics

Table Analyzed	Spleen					
One-way analysis of variance						
P value	< 0.0001					
P value summary	Ns					
Are means signif. different? (P < 0.05)	No					
Number of groups	5					
F	10.89					
R squared	0.085					
Bartlett's test for equal variances						
Bartlett's statistic (corrected)	3.42					
P value	< 0.0001					
P value summary	Ns					
Do the variances differ signif. (P < 0.05)	No					
ANOVA Table	SS	df	MS			
Treatment (between columns)	0.072	4	0.018			
Residual (within columns)	0.958	45	0.021			
Total	1.03	49				

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant	Summary	95% CI of diff
WT-Naïve vs WT-Amox	0.0152	0.58	No	ns	-0.085 to 0.115
WT-Naïve vs WT-Septrin	0.0181	0.70	No	ns	-0.081 to 0.118
WT-Naïve vs WT-Amox-sept	0.0109	0.42	No	ns	-0.090 to 0.112
WT-Naïve vs WT-Amox-sept-Prob	0.0190	0.72	No	ns	-0.080 to 0.118
WT-Amox vs WT-Septrin	0.0029	0.11	No	ns	-0.097 to 0.103
WT-Amox vs WT-Amox-sept	-0.0041	0.16	No	ns	-0.104 to 0.096
WT-Amox vs WT-Amox-sept-Prob	0.0028	0.12	No	ns	-0.097 to 0.102
WT-Septrin vs WT-Amox-sept	-0.0070	0.30	No	ns	-0.107 to 0.093
WT-Septrin vs WT-Amox-sept-Prob	0.0009	0.03	No	ns	-0.099 to 0.100
WT-Amox-sept vs WT-Amox-sept-Prob	0.0079	0.30	No	ns	-0.091 to 0.107
WT-Naïve vs WT-Amox	0.0152	0.58	No	ns	-0.085 to 0.115
WT-Naïve vs WT-Septrin	0.0181	0.70	No	ns	-0.081 to 0.118
WT-Naïve vs WT-Amox-sept	0.0109	0.42	No	ns	-0.090 to 0.112
WT-Naïve vs WT-Amox-sept-Prob	0.0190	0.72	No	ns	-0.080 to 0.118
WT-Amox vs WT-Septrin	0.0029	0.11	No	ns	-0.097 to 0.103

j. Effects of Antibiotic Induced Dysbiosis on Hematological Profiles

Red Blood Cell and Hemoglobin Counts Descriptive Analysis

Table Analyzed	RBC					
One-way analysis of variance						
P value	0.0002					
P value summary	***					
Are means signif. different? (P < 0.05)	Yes					
Number of groups	5					
F	9.402					
R squared	0.7344					
ANOVA Table	SS	df	MS			
Treatment (between columns)	53.6	4	10.72			
Residual (within columns)	19.38	17	1.14			
Total	72.99	21				
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	2.196	3.808	No	ns	-0.4131 to 4.805	
WT-Naïve vs WT-Septrin	2.811	4.874	Yes	**	0.2019 to 5.420	
WT-Naïve vs WT-Amox-sept	1.858	3.222	No	***	2.7506 to 7.467	
WT-Naïve vs WT-Amox-sept-Prob	5.333	9.248	No	ns	-0.724 to 2.942	
WT-Amox vs WT-Septrin	2.208	3.829	No	ns	-0.4006 to 4.817	

WT-Amox vs WT-Amox-sept	2.615	1.152	Yes	***	2.800 to 5.030
WT-Amox vs WT-Amox-sept-Prob	-0.3375	0.6321	No	ns	-2.753 to 2.078
WT-Septrin vs WT-Amox-sept	3.138	5.876	Yes	**	0.7221 to 5.553
WT-Septrin vs WT-Amox-sept-Prob	0.0125	0.02341	No	ns	-2.403 to 2.428
WT-Amox-sept vs WT-Amox-sept-Prob	2.523	4.725	Yes	**	0.1071 to 4.938
WT-Naïve vs WT-Amox	-0.9525	1.784	No	ns	-3.368 to 1.463
WT-Naïve vs WT-Septrin	2.6025	1.128	Yes	**	2.018 to 5.813
WT-Naïve vs WT-Amox-sept	3.475	6.509	Yes	**	1.060 to 5.890
WT-Naïve vs WT-Amox-sept-Prob	0.35	0.6555	No	ns	-2.065 to 2.765
WT-Amox vs WT-Septrin	-0.125	1.853	No	ns	-4.540 to -1.7096

k. Hemoglobin (HGB) Descriptive Analysis

Table Analyzed	HGB					
One-way analysis of variance						
P value	0.0009					
P value summary	**					
Are means signif. different? (P < 0.05)	Yes					
Number of groups	5					
F	7.147					
R squared	0.6776					
ANOVA Table	SS	df	MS			
Treatment (between columns)	128.6	4	25.73			
Residual (within columns)	61.2	17	3.6			
Total	189.8	21				
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	1.375	1.342	No	ns	-3.261 to 6.011	
WT-Naïve vs WT-Septrin	1.55	1.513	No	ns	-3.086 to 6.186	
WT-Naïve vs WT-Amox-sept	7.1	6.929	Yes	**	2.464 to 11.74	
WT-Naïve vs WT-Amox-sept-Prob	0.45	0.4392	No	ns	-4.186 to 5.086	
WT-Amox vs WT-Septrin	1.55	1.513	No	ns	-3.086 to 6.186	
WT-Amox vs WT-Amox-sept	5.175	6.1845	Yes	**	1.117 to 9.467	
WT-Amox vs WT-Amox-sept-Prob	-0.925	0.9751	No	ns	-5.217 to 3.367	
WT-Septrin vs WT-Amox-sept	1.725	2.035	No	ns	-3.433 to 3.02	
WT-Septrin vs WT-Amox-sept-Prob	0.175	0.1845	No	ns	-4.117 to 4.467	
WT-Amox-sept vs WT-Amox-sept-Prob	5.55	5.85	Yes	**	1.258 to 9.842	
WT-Naïve vs WT-Amox	-1.1	1.16	No	ns	-5.392 to 3.192	
WT-Naïve vs WT-Septrin	0	0	No	ns	-4.292 to 4.292	

WT-Naïve vs WT-Amox-sept	6.65	7.01	Yes	**	2.358 to 10.94
WT-Naïve vs WT-Amox-sept-Prob	1.1	1.16	No	ns	-3.192 to 5.392
WT-Amox vs WT-Septrin	1.55	1.85	No	ns	-4.842 to 5.258

l. Effects of Antibiotic Induced Dysbiosis on Red Blood Cell Indices; Mean Cell Volume (MCV) Descriptive Statistics

Table Analyzed	MCV					
One-way analysis of variance						
P value	0.0147					
P value summary	Ns					
Are means signif. different? (P < 0.05)	No					
Number of groups	5					
F	3.947					
R squared	0.5373					
ANOVA Table	SS	df	MS			
Treatment (between columns)	245.5	4	49.1			
Residual (within columns)	211.4	17	12.44			
Total	456.9	21				
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	2	1.05	No	ns	-6.616 to 10.62	
WT-Naïve vs WT-Septrin	-0.225	0.1181	No	ns	-8.841 to 8.391	
WT-Naïve vs WT-Amox-sept	3.25	1.706	No	ns	-5.366 to 11.87	
WT-Naïve vs WT-Amox-sept-Prob	5.15	2.704	No	ns	-3.466 to 13.77	
WT-Amox vs WT-Septrin	-2.225	1.262	No	ns	-10.20 to 5.752	
WT-Amox vs WT-Amox-sept	1.25	0.7089	No	ns	-6.727 to 9.227	
WT-Amox vs WT-Amox-sept-Prob	7.275	4.126	No	ns	-0.7023 to 15.25	
WT-Septrin vs WT-Amox-sept	3.15	1.786	No	ns	-4.827 to 11.13	
WT-Septrin vs WT-Amox-sept-Prob	3.475	1.971	No	ns	-4.502 to 11.45	
WT-Amox-sept vs WT-Amox-sept-Prob	5.375	3.048	No	ns	-2.602 to 13.35	
WT-Naïve vs WT-Amox	6.025	3.417	No	ns	-1.952 to 14.00	
WT-Naïve vs WT-Septrin	1.9	1.078	No	ns	-6.077 to 9.877	
WT-Naïve vs WT-Amox-sept	-4.125	2.339	No	ns	-12.10 to 3.852	

m. Mean Cell Hemoglobin (MCH) Descriptive Statistics

Table Analyzed	MCH					
One-way analysis of variance						

P value	0.0787					
P value summary	Ns					
Are means signif. different? (P < 0.05)	No					
Number of groups	5					
F	2.419					
R squared	0.4157					
ANOVA Table	SS	df	MS			
Treatment (between columns)	1.505	4	0.301			
Residual (within columns)	2.115	17	0.1244			
Total	3.62	21				
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	0.425	2.231	No	ns	-0.4368 to 1.287	
WT-Naïve vs WT-Septrin	-0.35	1.837	No	ns	-1.212 to 0.5118	
WT-Naïve vs WT-Amox-sept	0	0	No	ns	-0.8618 to 0.8618	
WT-Naïve vs WT-Amox-sept-Prob	0.15	0.7874	No	ns	-0.7118 to 1.012	
WT-Amox vs WT-Septrin	-0.225	1.181	No	ns	-1.087 to 0.6368	
WT-Amox vs WT-Amox-sept	-0.775	4.394	No	ns	-1.573 to 0.02285	
WT-Amox vs WT-Amox-sept-Prob	-0.425	2.41	No	ns	-1.223 to 0.3729	
WT-Septrin vs WT-Amox-sept	-0.275	1.559	No	ns	-1.073 to 0.5229	
WT-Septrin vs WT-Amox-sept-Prob	-0.65	3.686	No	ns	-1.448 to 0.1479	
WT-Amox-sept vs WT-Amox-sept-Prob	0.35	1.985	No	ns	-0.4479 to 1.148	
WT-Naïve vs WT-Amox	0.5	2.835	No	ns	-0.2979 to 1.298	
WT-Naïve vs WT-Septrin	0.125	0.7088	No	ns	-0.6729 to 0.9229	
WT-Naïve vs WT-Amox-sept	0.15	0.8505	No	ns	-0.6479 to 0.9479	
WT-Naïve vs WT-Amox-sept-Prob	-0.225	1.276	No	ns	-1.023 to 0.5729	
WT-Amox vs WT-Septrin	-0.375	2.126	No	ns	-1.173 to 0.4229	

n. Mean Cell Hemoglobin Concentration (MCHC) Descriptive Statistics

Table Analyzed	MCHC					
One-way analysis of variance						
P value	0.0759					
P value summary	ns					
Are means signif. different? (P < 0.05)	No					
Number of groups	5					
F	2.45					
R squared	0.4188					
ANOVA Table	SS	df	MS			
Treatment (between columns)	26.12	4	5.223			

Residual (within columns)	36.24	17	2.132			
Total	62.36	21				
Tukey's Multiple Comparison Test	Mean Diff.	Q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	-0.225	0.2853	No	ns	-3.792 to 3.342	
WT-Naïve vs WT-Septrin	-0.6	0.7609	No	ns	-4.167 to 2.967	
WT-Naïve vs WT-Amox-sept	-1.625	2.061	No	ns	-5.192 to 1.942	
WT-Naïve vs WT-Amox-sept-Prob	-1.9	2.409	No	ns	-5.467 to 1.667	
WT-Amox vs WT-Septrin	-3.075	3.9	No	ns	-6.642 to 0.4924	
WT-Amox vs WT-Amox-sept	-0.375	0.5137	No	ns	-3.678 to 2.928	
WT-Amox vs WT-Amox-sept-Prob	-1.4	1.918	No	ns	-4.703 to 1.903	
WT-Septrin vs WT-Amox-sept	-1.675	2.294	No	ns	-4.978 to 1.628	
WT-Septrin vs WT-Amox-sept-Prob	-2.85	3.904	No	ns	-6.153 to 0.4528	
WT-Amox-sept vs WT-Amox-sept-Prob	-1.025	1.404	No	ns	-4.328 to 2.278	
WT-Naïve vs WT-Amox	-1.3	1.781	No	ns	-4.603 to 2.003	
WT-Naïve vs WT-Septrin	-2.475	3.39	No	ns	-5.778 to 0.8278	
WT-Naïve vs WT-Amox-sept	-0.275	0.3767	No	ns	-3.578 to 3.028	
WT-Naïve vs WT-Amox-sept-Prob	-1.45	1.986	No	ns	-4.753 to 1.853	
WT-Amox vs WT-Septrin	-1.175	1.609	No	ns	-4.478 to 2.128	

o. Red Blood Cell Distribution Width (RDW)

Descriptive StatisticsRDW-SD

Table Analyzed	RDW-SD					
One-way analysis of variance						
P value	0.0004					
P value summary	ns					
Are means signif. different? (P < 0.05)	No					
Number of groups	5					
F	8.145					
R squared	0.7055					
ANOVA Table	SS	Df	MS			
Treatment (between columns)	553	4	110.6			
Residual (within columns)	230.8	17	13.58			
Total	783.8	21				
Tukey's Multiple Comparison Test	Mean Diff.	Q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	-3.133	1.574	No	ns	-12.14 to 5.870	
WT-Naïve vs WT-Septrin	-5.033	2.529	No	ns	-14.04 to 3.970	
WT-Naïve vs WT-Amox-sept	-2.958	1.487	No	ns	-11.96 to 6.045	

WT-Naïve vs WT-Amox-sept-Prob	8.442	4.242	No	ns	-0.5613 to 17.44
WT-Amox vs WT-Septrin	4.892	2.458	No	ns	-4.111 to 13.89
WT-Amox vs WT-Amox-sept	-1.9	1.031	No	ns	-10.24 to 6.435
WT-Amox vs WT-Amox-sept-Prob	0.175	0.09498	No	ns	-8.160 to 8.510
WT-Septrin vs WT-Amox-sept	-3.58	1.282	No	ns	-3.240 to 14.91
WT-Septrin vs WT-Amox-sept-Prob	8.025	4.356	No	ns	-0.3102 to 16.36
WT-Amox-sept vs WT-Amox-sept-Prob	2.075	1.126	No	ns	-6.260 to 10.41
WT-Naïve vs WT-Amox	7.48	3.314	No	ns	-10.140 to 6.81
WT-Naïve vs WT-Septrin	-5.925	2.387	No	ns	-0.590 to 16.26
WT-Naïve vs WT-Amox-sept	8.4	4.187	No	ns	-0.365 to 17.74
WT-Naïve vs WT-Amox-sept-Prob	7.85	4.261	No	ns	-0.4852 to 16.19
WT-Amox vs WT-Septrin	-3.55	1.927	No	ns	-11.89 to 4.785

p. RDW-CV Descriptive Analysis

Table Analyzed	RDW-CV					
One-way analysis of variance						
P value	0.4706					
P value summary	ns					
Are means signif. different? (P < 0.05)	No					
Number of groups	5					
F	0.9572					
R squared	0.2197					
ANOVA Table	SS	df	MS			
Treatment (between columns)	28.85	4	5.77			
Residual (within columns)	102.5	17	6.028			
Total	131.3	21				
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	-2.5	1.885	No	ns	-8.499 to 3.499	
WT-Naïve vs WT-Septrin	-2.05	1.546	No	ns	-8.049 to 3.949	
WT-Naïve vs WT-Amox-sept	-2.95	2.225	No	ns	-8.949 to 3.049	
WT-Naïve vs WT-Amox-sept-Prob	-1.85	1.395	No	ns	-7.849 to 4.149	
WT-Amox vs WT-Septrin	-0.05	0.03771	No	ns	-6.049 to 5.949	
WT-Amox vs WT-Amox-sept	0.45	0.3666	No	ns	-5.104 to 6.004	
WT-Amox vs WT-Amox-sept-Prob	-0.45	0.3666	No	ns	-6.004 to 5.104	
WT-Septrin vs WT-Amox-sept	0.65	0.5295	No	ns	-4.904 to 6.204	
WT-Septrin vs WT-Amox-sept-Prob	2.45	1.996	No	ns	-3.104 to 8.004	
WT-Amox-sept vs WT-Amox-sept-Prob	-0.9	0.7331	No	ns	-6.454 to 4.654	

WT-Naïve vs WT-Amox	0.2	0.1629	No	ns	-5.354 to 5.754
WT-Naïve vs WT-Septrin	2	1.629	No	ns	-3.554 to 7.554
WT-Naïve vs WT-Amox-sept	1.1	0.896	No	ns	-4.454 to 6.654
WT-Naïve vs WT-Amox-sept-Prob	2.9	2.362	No	ns	-2.654 to 8.454
WT-Amox vs WT-Septrin	1.8	1.466	No	ns	-3.754 to 7.354

q. Effects of Antibiotic Induced Dysbiosis on White Blood Cells Count

Table Analyzed	WBC					
One-way analysis of variance						
P value	< 0.0001					
P value summary	***					
Are means signif. different? (P < 0.05)	Yes					
Number of groups	5					
F	12.9					
R squared	0.7914					
ANOVA Table	SS	df	MS			
Treatment (between columns)	105.1	4	21.02			
Residual (within columns)	27.7	17	1.629			
Total	132.8	21				
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	-4.829	7.005	Yes	**	-7.948 to -1.710	
WT-Naïve vs WT-Septrin	6.2833	10.411	Yes	**	3.835 to 8.402	
WT-Naïve vs WT-Amox-sept	0.7608	1.104	No	ns	-2.358 to 3.880	
WT-Naïve vs WT-Amox-sept-Prob	-4.773	7.572	Yes	**	-7.345 to -1.892	
WT-Amox vs WT-Septrin	-3.3767	6.5464	Yes	**	-7.495 to -1.742	
WT-Amox vs WT-Amox-sept	5.113	8.01	Yes	**	2.225 to 8.000	
WT-Amox vs WT-Amox-sept-Prob	5.59	8.758	Yes	*	2.703 to 8.477	
WT-Septrin vs WT-Amox-sept	6.603	10.34	Yes	**	3.715 to 9.490	
WT-Septrin vs WT-Amox-sept-Prob	4.453	6.976	Yes	***	1.565 to 7.340	
WT-Amox-sept vs WT-Amox-sept-Prob	-3.4775	6.7481	Yes	*	-6.410 to -1.365	
WT-Naïve vs WT-Amox	1.49	2.335	No	ns	-1.397 to 4.377	
WT-Naïve vs WT-Septrin	5.66	9.034	Yes	**	1.547 to 6.227	
WT-Naïve vs WT-Amox-sept	1.013	1.586	No	ns	-1.875 to 3.900	
WT-Naïve vs WT-Amox-sept-Prob	4.138	7.782	Yes	**	1.025 to 6.750	
WT-Amox vs WT-Septrin	-2.15	7.369	Yes	**	-5.037 to 1.7374	

Appendix VI: Effects of Antibiotic Induced Dysbiosis on Pathological Changes

a. Descriptive Statistics of Serum TNF-α Levels

Table Analyzed	TNF-alpha					
One-way analysis of variance						
P value	< 0.0001					
P value summary	***					
Are means signif. different? (P < 0.05)	Yes					
Number of groups	5					
F	11.59					
R squared	0.7732					
ANOVA Table	SS	df	MS			
Treatment (between columns)	6748	4	1350			
Residual (within columns)	1980	17	116.5			
Total	8728	21				
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	-25.51	6.378	Yes	**	-51.88 to 0.8529	
WT-Naïve vs WT-Septrin	-58.52	10.04	Yes	***	-84.88 to -32.15	
WT-Naïve vs WT-Amox-sept	-30.88	5.299	Yes	**	-57.25 to -4.515	
WT-Naïve vs WT-Amox-sept-Prob	-15.57	2.672	No	ns	-41.94 to 10.79	
WT-Amox vs WT-Septrin	-27.9	4.787	Yes	*	-54.27 to -1.533	
WT-Amox vs WT-Amox-sept	9.941	1.842	No	ns	-14.47 to 34.35	
WT-Amox vs WT-Amox-sept-Prob	-5.368	0.9948	No	ns	-29.78 to 19.04	
WT-Septrin vs WT-Amox-sept	-33	6.116	Yes	**	-57.41 to -8.592	
WT-Septrin vs WT-Amox-sept-Prob	-26.386	5.4421	Yes	**	-36.80 to 22.03	
WT-Amox-sept vs WT-Amox-sept-Prob	-25.31	6.837	Yes	**	-39.72 to 9.103	
WT-Naïve vs WT-Amox	-42.94	7.958	Yes	***	-67.35 to -18.53	
WT-Naïve vs WT-Septrin	-22.33	4.284	Yes	**	-36.74 to 12.08	
WT-Naïve vs WT-Amox-sept	-27.64	5.121	Yes	*	-52.05 to -3.224	
WT-Naïve vs WT-Amox-sept-Prob	2.982	0.5527	No	ns	-21.43 to 27.39	
WT-Amox vs WT-Septrin	30.62	5.674	Yes	**	6.206	to 55.03

b. Descriptive Statistics of Serum IL-10 Levels

Table Analyzed	IL-10					
One-way analysis of variance						
P value	0.013					
P value summary	Ns					

Are means signif. different? (P < 0.05)	No					
Number of groups	5					
F	4.073					
R squared	0.545					
ANOVA Table	SS	df	MS			
Treatment (between columns)	8406	4	1681			
Residual (within columns)	7017	17	412.7			
Total	15420	21				
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	-35.79	3.262	No	ns	-85.42 to 13.85	
WT-Naïve vs WT-Septrin	-34.97	3.922	No	ns	-83.18 to 16.10	
WT-Naïve vs WT-Amox-sept	-21.91	1.997	No	ns	-71.54 to 27.73	
WT-Naïve vs WT-Amox-sept-Prob	-33.54	3.057	No	ns	-114.6 to -13.34	
WT-Amox vs WT-Septrin	-20.27	1.848	No	ns	-69.91 to 29.36	
WT-Amox vs WT-Amox-sept	2.245	0.221	No	ns	-43.71 to 48.20	
WT-Amox vs WT-Amox-sept-Prob	13.88	1.366	No	ns	-32.08 to 59.83	
WT-Septrin vs WT-Amox-sept	-29.19	2.873	No	ns	-75.14 to 16.77	
WT-Septrin vs WT-Amox-sept-Prob	15.51	1.527	No	ns	-30.44 to 61.47	
WT-Amox-sept vs WT-Amox-sept-Prob	11.63	1.145	No	ns	-34.32 to 57.59	
WT-Naïve vs WT-Amox	-31.43	3.094	No	ns	-77.39 to 14.52	
WT-Naïve vs WT-Septrin	13.27	1.306	No	ns	-32.69 to 59.22	
WT-Naïve vs WT-Amox-sept	-43.07	4.24	No	ns	-89.02 to 2.889	
WT-Naïve vs WT-Amox-sept-Prob	1.633	0.1607	No	ns	-44.32 to 47.59	
WT-Amox vs WT-Septrin	44.7	4.4	No	ns	-1.256 to 90.65	

c. Descriptive Statistics of Serum IFN- γ Levels

Table Analyzed	IFN-γ					
One-way analysis of variance						
P value	0.0052					
P value summary	***					
Are means signif. different? (P < 0.05)	Yes					
Number of groups	5					
F	5.031					
R squared	0.5967					
ANOVA Table	SS	df	MS			
Treatment (between columns)	2672	4	534.4			

Residual (within columns)	1806	17	106.2			
Total	4478	21				
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	-1.926	0.3459	No	ns	-27.11 to 23.26	
WT-Naïve vs WT-Septrin	22.7406	4.1331	Yes	**	-24.44 to 15.92	
WT-Naïve vs WT-Amox-sept	-10.37	1.863	No	ns	-35.55 to 14.81	
WT-Naïve vs WT-Amox-sept-Prob	22.07	3.965	No	ns	-3.112 to 47.25	
WT-Amox vs WT-Septrin	20.369	1.503	Yes	***	-33.55 to 16.81	
WT-Amox vs WT-Amox-sept	-22.666	3.5174	Yes	**	-25.65 to 24.98	
WT-Amox vs WT-Amox-sept-Prob	-8.443	1.638	No	ns	-31.76 to 14.87	
WT-Septrin vs WT-Amox-sept	23.99	4.656	Yes	*	0.6817 to 47.31	
WT-Septrin vs WT-Amox-sept-Prob	-23.443	2.25	Yes	***	-29.76 to 16.87	
WT-Amox-sept vs WT-Amox-sept-Prob	-21.11	5.156	Yes	**	-34.42 to 12.20	
WT-Naïve vs WT-Amox	21.33	4.139	No	ns	-1.984 to 44.64	
WT-Naïve vs WT-Septrin	-29.109	3.768	Yes	***	-32.42 to 14.20	
WT-Naïve vs WT-Amox-sept	32.44	6.295	Yes	**	9.124 to 55.75	
WT-Naïve vs WT-Amox-sept-Prob	2	0.388	No	ns	-21.31 to 25.31	
WT-Amox vs WT-Septrin	-30.44	5.907	Yes	**	-53.75 to -7.125	

d. Effects of Antibiotic Induced Dysbiosis on Markers for Liver Function; Serum AST Levels

Table Analyzed	ALT					
One-way analysis of variance						
P value	< 0.0001					
P value summary	***					
Are means signif. different? (P < 0.05)	Yes					
Number of groups	5					
F	49.59					
R squared	0.9764					
ANOVA Table	SS	Df	MS			
Treatment (between columns)	111040	4	27760			
Residual (within columns)	3358	6	559.7			
Total	114398	10				
Tukey's Multiple Comparison Test	Mean Diff.	Q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	-236.9	14.206	Yes	***	-331.1 to 217.25	

WT-Naïve vs WT-Septrin	-315.35	16.9176	Yes	***	-409.5 to 218.80
WT-Naïve vs WT-Amox-sept	-242.55	12.543	Yes	***	-336.7 to -211.60
WT-Naïve vs WT-Amox-sept-Prob	-315.7	18.87	Yes	***	-409.8 to -221.5
WT-Amox vs WT-Septrin	-74.95	4.48	No	ns	-169.1 to 19.20
WT-Amox vs WT-Amox-sept	31.55	10.288	Yes	**	-22.60 to 70.7
WT-Amox vs WT-Amox-sept-Prob	-5.65	0.3377	No	ns	-99.80 to 88.50
WT-Septrin vs WT-Amox-sept	-278.8	26.66	Yes	**	-302.9 to -184.6
WT-Septrin vs WT-Amox-sept-Prob	-238.05	20.274	Yes	*	-332.2 to 56.10
WT-Amox-sept vs WT-Amox-sept-Prob	-327.2	18.626	Yes	***	-121.4 to 66.95
WT-Naïve vs WT-Amox	-300.3	17.95	Yes	***	-394.5 to -206.1
WT-Naïve vs WT-Septrin	-259.6	12.563	Yes	***	-323.8 to 34.55
WT-Naïve vs WT-Amox-sept	-273.1	16.32	Yes	***	-367.3 to -178.9
WT-Naïve vs WT-Amox-sept-Prob	-332.4	31.937	Yes	***	-126.6 to 61.75
WT-Amox vs WT-Septrin	-4.7	0.39	No	ns	-96.5 to 84.9

e. Descriptive Statistics of Serum ALT Levels

Table Analyzed	AST					
One-way analysis of variance						
P value	0.0038					
P value summary	**					
Are means signif. different? (P < 0.05)	Yes					
Number of groups	5					
F	12.72					
R squared	0.9138					
ANOVA Table	SS	Df	MS			
Treatment (between columns)	333640	4	83410			
Residual (within columns)	39336	6	6556			
Total	372976	10				
Tukey's Multiple Comparison Test	Mean Diff.	Q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	-418.9	9.076	Yes	*	-541.1 to 203.4	
WT-Naïve vs WT-Septrin	-489.85	10.569	Yes	**	-512.1 to 232.4	
WT-Naïve vs WT-Amox-sept	-493.4	8.124	Yes	***	-615.6 to 28.88	
WT-Naïve vs WT-Amox-sept-Prob	-144.1	2.03	No	ns	-296.3 to -21.8	
WT-Amox vs WT-Septrin	-268.3	4.686	No	ns	-590.5 to 53.93	
WT-Amox vs WT-Amox-sept	29	0.5065	No	ns	-293.2 to 351.2	
WT-Amox vs WT-Amox-sept-Prob	-174.5	3.048	No	ns	-496.7 to 147.7	
WT-Septrin vs WT-Amox-sept	-455.2	7.95	Yes	*	-777.4 to -133.0	

WT-Septrin vs WT-Amox-sept-Prob	-149.5	2.61	No	ns	-471.7 to 172.8
WT-Amox-sept vs WT-Amox-sept-Prob	-403.5	8.554	Yes	**	-525.7 to 118.7
WT-Naïve vs WT-Amox	-484.2	8.457	Yes	*	-806.4 to -162.0
WT-Naïve vs WT-Septrin	-378.5	3.117	Yes	**	-500.7 to 143.8
WT-Naïve vs WT-Amox-sept	-480.7	10.903	Yes	***	-602.9 to 41.53
WT-Naïve vs WT-Amox-sept-Prob	25.05	0.4375	No	Ns	-297.2 to 347.3
WT-Amox vs WT-Septrin	305.8	5.34	No	Ns	-16.48 to 628.0

f. Descriptive Statistics of Serum Bilirubin Levels

Table Analyzed	**BILT**					
One-way analysis of variance						
P value	0.0561					
P value summary	ns					
Are means signif. different? (P < 0.05)	No					
Number of groups	5					
F	4.155					
R squared	0.7759					
ANOVA Table	SS	Df	MS			
Treatment (between columns)	2.936	4	0.734			
Residual (within columns)	1.06	6	0.1767			
Total	3.9962	10				
Tukey's Multiple Comparison Test	Mean Diff.	Q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	0.1	0.3365	No	ns	-1.573 to 1.773	
WT-Naïve vs WT-Septrin	-0.1	0.3365	No	ns	-1.773 to 1.573	
WT-Naïve vs WT-Amox-sept	-0.5	1.682	No	ns	-2.173 to 1.173	
WT-Naïve vs WT-Amox-sept-Prob	-1.55	5.215	No	ns	-3.223 to 0.1227	
WT-Amox vs WT-Septrin	-0.35	1.178	No	ns	-2.023 to 1.323	
WT-Amox vs WT-Amox-sept	-0.2	0.6729	No	ns	-1.873 to 1.473	
WT-Amox vs WT-Amox-sept-Prob	-0.6	2.019	No	ns	-2.273 to 1.073	
WT-Septrin vs WT-Amox-sept	-1.65	5.552	No	ns	-3.323 to 0.02269	
WT-Septrin vs WT-Amox-sept-Prob	-0.45	1.514	No	ns	-2.123 to 1.223	
WT-Amox-sept vs WT-Amox-sept-Prob	-0.4	1.346	No	ns	-2.073 to 1.273	
WT-Naïve vs WT-Amox	-1.45	4.879	No	ns	-3.123 to 0.2227	
WT-Naïve vs WT-Septrin	-0.25	0.8412	No	ns	-1.923 to 1.423	
WT-Naïve vs WT-Amox-sept	-1.05	3.533	No	ns	-2.723 to 0.6227	
WT-Naïve vs WT-Amox-sept-Prob	0.15	0.5047	No	ns	-1.523 to 1.823	
WT-Amox vs WT-Septrin	1.2	4.038	No	ns	-0.4727 to 2.873	

Appendix VII: Effects of Antibiotic Induced Dysbiosis on Induction of Oxidative Stress

a. Liver GSH Concentrations Descriptive Statistics

Table Analyzed	Liver					
One-way analysis of variance						
P value	< 0.0001					
P value summary	***					
Are means signif. different? (P < 0.05)	Yes					
Number of groups	5					
F	15.87					
R squared	0.7829					
ANOVA Table	SS	df	MS			
Treatment (between columns)	106.2	4	21.24			
Residual (within columns)	29.45	22	1.339			
Total	135.7	26				
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	0.8042	1.346	No	ns	-1.830 to 3.438	
WT-Naïve vs WT-Septrin	2.961	4.956	Yes	**	0.3265 to 7.595	
WT-Naïve vs WT-Amox-sept	6.074	10.17	Yes	***	3.440 to 8.708	
WT-Naïve vs WT-Amox-sept-Prob	0.773	2.967	No	ns	-0.8614 to 4.407	
WT-Amox vs WT-Septrin	3.686	6.169	Yes	**	1.051 to 6.320	
WT-Amox vs WT-Amox-sept	2.157	7.168	Yes	***	3.1247 to 9.438	
WT-Amox vs WT-Amox-sept-Prob	0.9687	1.872	No	ns	-1.313 to 3.250	
WT-Septrin vs WT-Amox-sept	5.27	8.18	Yes	**	2.988 to 6.551	
WT-Septrin vs WT-Amox-sept-Prob	2.882	7.569	Yes	***	0.6003 to 6.163	
WT-Amox-sept vs WT-Amox-sept-Prob	-1.188	2.296	No	ns	-3.469 to 1.093	
WT-Naïve vs WT-Amox	3.113	6.016	Yes	**	0.8318 to 5.394	
WT-Naïve vs WT-Septrin	0.725	1.401	No	ns	-1.556 to 3.006	
WT-Naïve vs WT-Amox-sept	4.301	8.312	Yes	***	2.020 to 6.582	
WT-Naïve vs WT-Amox-sept-Prob	1.913	3.697	No	ns	-0.3684 to 4.194	
WT-Amox vs WT-Septrin	-2.388	5.615	Yes	**	2.669 to 6.1068	

b. Kidney GSH Concentrations Descriptive Statistics

Table Analyzed	Kidney					
One-way analysis of variance						
P value	< 0.0001					
P value summary	***					
Are means signif. different? (P < 0.05)	Yes					
Number of groups	5					
F	14.54					
R squared	0.7677					
ANOVA Table	SS	df	MS			
Treatment (between columns)	244	4	48.8			
Residual (within columns)	73.81	22	3.355			
Total	317.8	26				
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	-5.567	7.657	Yes	**	-5.738 to 4.603	
WT-Naïve vs WT-Septrin	-8.155	9.955	Yes	***	-11.77 to -4.543	
WT-Naïve vs WT-Amox-sept	-7.677	9.888	Yes	***	-7.848 to -1.4932	
WT-Naïve vs WT-Amox-sept-Prob	-9.722	10.28	Yes	***	-13.89 to -5.552	
WT-Amox vs WT-Septrin	-3.782	3.999	No	ns	-7.953 to 0.3880	
WT-Amox vs WT-Amox-sept	-1.8	2.198	No	ns	-5.412 to 1.811	
WT-Amox vs WT-Amox-sept-Prob	-2.11	2.576	No	ns	-5.722 to 1.502	
WT-Septrin vs WT-Amox-sept	-3.368	3.561	No	ns	-7.538 to 0.8026	
WT-Septrin vs WT-Amox-sept-Prob	-2.215	2.704	No	ns	-5.827 to 1.397	
WT-Amox-sept vs WT-Amox-sept-Prob	-0.3094	0.3776	No	ns	-3.921 to 3.302	
WT-Naïve vs WT-Amox	-6.354	7.757	Yes	**	-9.966 to -1.743	
WT-Naïve vs WT-Septrin	-6.4145	8.506	Yes	***	-10.026 to -3.197	
WT-Naïve vs WT-Amox-sept	-6.045	7.379	Yes	***	-9.657 to -2.433	
WT-Naïve vs WT-Amox-sept-Prob	-0.1052	0.1284	No	ns	-3.717 to 3.507	
WT-Amox vs WT-Septrin	5.94	7.251	Yes	***	2.328 to 9.551	

c. Brain GSH Concentrations Descriptive Statistics

Table Analyzed	Brain					
One-way analysis of variance						
P value	< 0.0001					
P value summary	***					
Are means signif. different? (P < 0.05)	Yes					
Number of groups	5					

F	24.39					
R squared	0.8472					
ANOVA Table	SS	Df	MS			
Treatment (between columns)	284.1	4	56.83			
Residual (within columns)	51.27	22	2.33			
Total	335.4	26				
Tukey's Multiple Comparison Test	Mean Diff.	Q	Significant	Summary	95% CI of diff	
WT-Naïve vs WT-Amox	-3.168	4.019	Yes	*	-6.644 to 0.3077	
WT-Naïve vs WT-Septrin	-5.721	7.257	Yes	***	-9.196 to -2.245	
WT-Naïve vs WT-Amox-sept	-1.407	1.785	Yes	**	-4.883 to 2.068	
WT-Naïve vs WT-Amox-sept-Prob	-2.553	3.739	No	ns	-5.563 to 0.4574	
WT-Amox vs WT-Septrin	-3.771	4.784	Yes	*	-7.247 to -0.2954	
WT-Amox vs WT-Amox-sept	-10.11	12.83	Yes	***	-13.59 to -6.637	
WT-Amox vs WT-Amox-sept-Prob	1.761	2.579	No	ns	-1.249 to 4.771	
WT-Septrin vs WT-Amox-sept	-6.945	4.17	Yes	*	-9.955 to -3.935	
WT-Septrin vs WT-Amox-sept-Prob	-1.6031	1.8834	Yes	**	-3.613 to 3.407	
WT-Amox-sept vs WT-Amox-sept-Prob	-4.313	6.318	Yes	***	1.303 to 7.323	
WT-Naïve vs WT-Amox	-4.392	6.434	Yes	**	-7.402 to -1.382	
WT-Naïve vs WT-Septrin	-2.949	3.856	Yes	**	-1.061 to 6.959	
WT-Naïve vs WT-Amox-sept	-8.706	12.75	Yes	***	-11.72 to -5.696	
WT-Naïve vs WT-Amox-sept-Prob	-2.364	3.462	No	ns	-5.374 to 0.6463	
WT-Amox vs WT-Septrin	6.342	9.29	Yes	***	3.332	to 9.352

d. Spleen GSH Levels Descriptive Statistics

Table Analyzed	Spleen					
One-way analysis of variance						
P value	< 0.0001					
P value summary	***					
Are means signif. different? (P < 0.05)	Yes					
Number of groups	5					
F	12.01					
R squared	0.7318					
ANOVA Table	SS	df	MS			
Treatment (between columns)	161.4	4	32.28			
Residual (within columns)	59.15	22	2.688			
Total	220.5	26				
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant	Summary	95% CI of diff	

WT-Naïve vs WT-Amox	-4.733	5.59	Yes	***	-8.466 to -0.9998
WT-Naïve vs WT-Septrin	-3.519	4.157	Yes	***	-7.253 to -2.2138
WT-Naïve vs WT-Amox-sept	-3.66	6.322	Yes	***	-3.393 to 2.07325
WT-Naïve vs WT-Amox-sept-Prob	-8.725	10.3	Yes	***	-12.46 to -4.992
WT-Amox vs WT-Septrin	1.308	2.269	No	ns	-2.041 to 4.575
WT-Amox vs WT-Amox-sept	1.214	1.655	No	ns	-2.019 to 4.447
WT-Amox vs WT-Amox-sept-Prob	1.073	1.463	No	ns	-2.160 to 4.306
WT-Septrin vs WT-Amox-sept	-3.992	5.444	Yes	*	-7.225 to -0.7590
WT-Septrin vs WT-Amox-sept-Prob	-1.648	1.247	No	ns	-3.808 to 0.658
WT-Amox-sept vs WT-Amox-sept-Prob	-0.1405	0.1916	No	ns	-3.374 to 3.092
WT-Naïve vs WT-Amox	-5.206	7.099	Yes	***	-8.439 to -1.973
WT-Naïve vs WT-Septrin	-1.788	5.439	Yes	***	-5.021 to 9.445
WT-Naïve vs WT-Amox-sept	-5.065	6.908	Yes	***	-8.298 to -1.832
WT-Naïve vs WT-Amox-sept-Prob	-2.5749	8.784	Yes	***	-4.881 to 9.585
WT-Amox vs WT-Septrin	-3.417	4.66	No	ns	-6.1842 to 0.0650

Appendix VIII: Ethics Review Letter

CHUKA UNIVERSITY

Knowledge is Wealth *(Sapientia divitia est)* Akili ni Mali

CHUKA UNIVERSITY INSTITUTIONAL ETHICS REVIEW COMMITTEE

Telephones: 020-2310512/18
Direct Line: 0772894438
Email: info@chuka.ac.ke,
P. O. Box 109-60400, Chuka
Website: www.chuka.ac.ke

20th September, 2023

REF: CUIERC/ NACOSTI/424
TO: **Kiptoo Kamngoror Cosmas**

RE: Effects of Co-Trimoxazole and Amoxicillin Therapy on Gut Microbiota, Biophysiological and Immunopathological Parameters in Mice.

This is to inform you that *Chuka University IERC* has reviewed and approved your above research proposal. Your application approval number is *NACOSTI/NBC/AC-0812.* The approval period is 20th September, 2023 – 20th September, 2024.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by *Chuka University IERC.*
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to *Chuka University IERC* within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to *Chuka University IERC* within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to *Chuka University IERC.*

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely

Dr. Benjamin Kanga
SECRETARY

Appendix IX: NACOSTI License

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **108240**

Date of Issue: **24/July/2024**

RESEARCH LICENSE

This is to Certify that Mr.. COSMAS KAMNGOROR KIPTOO of Chuka University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Nairobi on the topic: EFFECTS OF CO-TRIMOXAZOLE AND AMOXICILLIN THERAPY ON GUT MICROBIOTA, BIO-PHYSIOLOGICAL AND IMMUNO-PATHOLOGICAL PARAMETERS IN MICE for the period ending : 24/July/2025.

License No: **NACOSTI/P/24/38075**

108240

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

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See overleaf for conditions