EFFECTS OF ALUMINUM – MAGNESIUM SILICATE AND IMMUNE-STIMULANTS ON ANTIBACTERIAL ACTIONS OF AMPICILLIN TRihYDRATE, AGAINST RESISTANT ESCHERICHIA COLI INFECTION IN CHICKS

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE IN THE DEPARTMENT OF VETERINARY MEDICINE

SUPERVISOR: PROF. M.C.O. EZEIBE

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DECLARATION
The work presented in this Dissertation is original and was carried out by me under the supervision of Prof. M. C. O. Ezeibe. References made to the works of other investigators are duly acknowledged. No part of this Dissertation has been submitted previously or elsewhere for a degree or diploma.

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APPROVAL/CERTIFICATION

This is to certify that EZEBOBELE, Okechukwu Kingsley Friday, a post graduate student in the Department of Veterinary Medicine and with registration number PG/MSc/11/61291 has
satisfactorily completed the requirements for course and research work for the degree of Master of Science (M.Sc) in Veterinary Medicine. The work embodied in this Dissertation is original and has not been submitted in full or part for any other diploma or degree of this or any other University.

DEDICATION
This work is dedicated to my little family (wife and children) for all your dedication and troubles without which life will be meaningless and to God who has brought us together as a family in His service.
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ABSTRACT

To study effects of synergy between, additional level of vitamin A, C and E and Selenium of chick infected with resistance Escherichia coli, reduced dose of ampicillin trihydrate used for treatment and ampicillin trihydrate / Aluminium magnesium silicate drug formulation, on antimicrobial activities of ampicillin trihydrate used for treatment of resistant E. coli infections. Seventy six weeks old harco cockerel chicks were infected with Ampicillin resistant E. coli. The chicks were randomly assigned to two main treatment groups (A and B)
of 35 chicks each. Two weeks prior to the experimental infection, chicks in group A were fed commercial feed fortified with vitamins A, C and E and selenium while those in group B were given non-fortified poultry feed. Each chick was orally inoculated with 1.5 x 10^8 colony forming unit (CFU)/ml of broth of the ampicillin-resistant *E. coli* strain. Five days post-infection, chicks in the two treatment groups were further assigned to sub-groups (A1 ÷ A7 and B1 ÷ B7 respectively) of five chicks each. Sub-groups A1- A3 and B1 ÷ B3 were treated with ampicillin trihydrate at dose levels of 10, 7.5 and 5 mg/kg body weight respectively, while sub-groups A4 ÷ A6 and B4 ÷ B6 were treated with an AMS-ampicillin trihydrate at dose levels of 10, 7.5, and 5 mg/kg body weight respectively. Chicks in sub-groups A7 and B7 were not treated (control). Treatment lasted for six days in each of the treated sub-groups. Three days post treatment; all the chicks in the 14 sub-groups were sacrificed for postmortem examination and determination of *E. coli* isolation rates (*E. coli* CFU/ml of bile). One way Analysis of variance (ANOVA) was used to analyze the data generated. Significant was accepted at p < 0.05. There were no significant reductions in feed intake and body weight in all the subgroup. There was no mortality in any of the sub-groups. Moreover, no gross lesion was observed in any of the examined chicks. There was no significant reduction (P > 0.05) in the CFU/ml of bile in sub-group (B7) untreated subgroup feed unfortified feed and subgroup (A7) untreated subgroup fed fortified feed (8.24 x 10^5 ± 3.22 x 10^5 and 5.35 x 10^5 ± 2.78 x 10^5, respectively). There was no significant reduction (P > 0.05) in the CFU/ml of bile in sub-groups B1, A1 and A4 (4.12 x 10^5 ± 2.45 x 10^5, 2.29 x 10^5 ± 0.90 x 10^5 and 1.35 x 10^5 ± 0.45 x 10^5, respectively). In subgroups B1 and B4 (ampicillin dose level of 10 mg/kg), there was no significant difference (P > 0.05) in the increases in CFU/ml of bile (4.12 x 10^5 ± 2.45 x 10^5 and 4.62 x 10^5 ± 2.47 x 10^5, respectively). There was a significant increase (P < 0.05) in the CFU/ml of bile in sub groups B2 and B5 at ampicillin dose levels of 7.5 mg/kg (5.49 x 10^5 ± 3.00 x10^5 and 12.4 x 10^5 ± 7.73 x 10^5, respectively). In subgroups B3 and B6, there was a
significant increase (P < 0.05) in CFU/ml of bile at ampicillin dose level of 5 mg / kg (0.70 x 10^5 ± 0.27 x 10^5 and 9.16 x 10^5 ± 3.38 x 10^5, respectively). In the subgroups A1 and A4, there was a no significant reduction (P > 0.05) in CFU/ml of bile (2.29 x 10^5 ± 0.90 x 10^5 and 1.35 x10^5 ± 0.45 x 10^5, respectively). In subgroup A2 and A5, there was a significant difference in reduction (P < 0.05) in CFU/ml of bile (1.12 x 10^5 ± 0.38 x 10^5 and 0.35 x 10^5 ± 0.08 x 10^5, respectively). However, there was no significant difference (P > 0.05) in the CFU/ml of bile in subgroups A3 and A6 (1.98 x 10^5 ± 1.29 x 10^5 and 1.57 x 10^5 ± 1.04 x 10^5 respectively.

At 7.5 mg / kg the Ampicillin trihydrate-Aluminium magnesium silicate formulation was able to significantly (P < 0.05) clear the resistance Escherichia coli in the bile of infected chicks placed on feed fortified with additional level of vitamin A, C and E and Selenium. It can then be concluded that to treat resistant E. coli infection in chick with ampicillin trihydrate, the chicks should be placed on feed fortified with additional level of vitamin A, C and E and Selenium. And the ampicillin trihydrate should used at 7.5 mg/kg as an Ampicillin trihydrate-Aluminum magnesium silicate formulation.

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CHAPTER ONE
INTRODUCTION

1.1 General Information

*Escherichia coli* (*E. coli*) is one of the commonest microbial flora of the gastrointestinal tract of poultry and domestic animals. It is a predominant facultative anaerobe in intestines of animals and poultry but may become pathogenic (Jewetz *et al*., 1984; Levine, 1987) when immunity is suppressed. Although most isolates of *E. coli* are non-pathogenic, their presence is considered an indication of faecal contamination of food and about 10 to 15 % intestinal coli forms are opportunistic serotypes (Barnes and Gross, 1997). *E coli* cause a variety of lesions in immune-compromised hosts and in presence of other pathogens (McMullin, 2004). Avian species affected by pathogenic *E. coli* serotypes include Chickens, Turkeys, Ducks and Pheasants (Jordan, 1990). Although *E. coli* induced enteritis in poultry is reported to be rare (Watson, 1997) some reports have implicated *E. coli* in some cases of enteritis in Chickens (Schwartz, 2007). Some *E. coli* sero-groups are known to cause extra intestinal infections (Gross, 1994). Serotypes of *E. coli* that most frequently cause coli-septicemia (0₁, 0₂, 0₈, 0₁₁ and 0₇₈) are the ones that are also likely to be found in the oesophagus and upper trachea (Harry *et al*., 1965). There is enough evidence to show that coli-septicemia is primarily due to respiratory infections which develop as a result of association of the respiratory infections with infectious bronchitis virus or *Mycoplasma gallisepticum* (linam *et al*., 2007). Gross (1994) has demonstrated that infections of the respiratory tracts often produce changes that favor *E. coli* invasion. Majowicz *et al*., (2010) reported cases of acute fatal enteritis in two flocks, which occurred after the chicks were inoculated with Newcastle disease vaccine. The enteritis was believed to be a result of *E. coli* infection.
Majowicz et al. (2010) investigated a cholera-like syndrome in fowls dying during shipment and discovered that *E. coli*, under certain conditions leave the intestines to become virulent and cause septicemia in hens, especially if their resistance have been weakened by starvation, thirst, cold or poor ventilation. Kurman et al. (2010) reported that inoculation of *Mycoplasma* spp cultures into sinuses of chicks caused no symptoms or mortality but when this organism was inoculated in conjunction with *E. coli*, a mortality rate of 45% resulted in 5 days. Fibrinous pericarditis and peri-hepatitis were observed at postmortem examination of the birds that died and *E. coli* was readily isolated from the chicks. Linam et al. (2007) reported an apparently non-pathogenic *E. coli* which appeared to act synergistically with *Eimeria brunetti* infection in birds exposed to both pathogens. Mortality was 100% in chicks challenged simultaneously with *E. coli* and *E. brunetti* but groups of chicks challenged with either *E. brunetti* alone or *E. coli* alone had no mortality. Gross, (1994) reported that when *E. coli* strains isolated from cases of air sacculitis, were inoculated into air sacs of Chickens and Turkeys either alone or in combination with pleuropneumonia-like organisms, there was a condition similar to field outbreaks colibacillosis.

Fabricant and Levin (1961) reported that certain types of *pleuropneumonia-like organisms* (*PPLO*) were recognized as the aetiological agents of chronic respiratory disease (CRD). However, they noted that the field condition known as *air sac* disease or complicated CRD is not due to *PPLO* infection alone but is a result of concurrent infection with *E. coli*. Such concurrent infections produce the fibrinous or fibrino-purulent pericarditis and perihepatitis that characterize *air sac* disease along with massive air-sacculitis which are responsible for the severe mortality, weight losses and excessive condemnation rates at slaughter that make CRD a major disease problem of the American broiler industry. The situation would be worse in Nigeria with our poor hygienic conditions. The only reason, we are not aware of the problem of *E. coli* infections in Nigeria is that our case reporting and documentation system
is poor. Adene, (2004) described CRD which is a complication caused by E. coli infections as constituting a major constraint to poultry production in Nigeria. Apart from the disease, collibacilosis, caused by E. coli infection, E. coli has potential to complicate and aggravate other infections (Kurman et al. 2010). Another problem with E. coli isolates is that they often develop resistance against available first-line antibiotics like Ampicillin (Okoli et al., 2005). Ampicillin trihydrate is semi-synthetic amino penicillin, relatively acid and thermostable compared to penicillin. Ampicillin is generally regarded as one of the most inexpensive antibiotics. Recent studies have however shown increasing antimicrobial resistance against Ampicillin, especially by E. coli and other enterobacteria (Okoli et al., 2005).

Pharmacodynamic study of Ampicillin has shown that it produces time dependent killing of micro-organisms. Its pharmacokinetics show that it has 40 mcg/ml half life of 1-1.3 h. This means that it has short bioavailability (AHFS Drug Information 2004). So, if bioavailability of Ampicillins is prolonged with a stabilizing agent, its action may improve to lead to a better clearance of both susceptible and resistant infections. Aluminum-magnesium silicate (AMS) has been in use as a stabilizing agent for drug formulations used for treatment of animals and humans for many decades. Several toxicity tests have proved that AMS is safe for use as medicine and in medicines used for treatment of animals and humans (Schills, 2003). Aluminum-magnesium silicate prolongs bioavailability of drugs and because it is made of Nanoparticles. Aluminum-magnesium silicate also enhances delivery of drugs to their desired targets (Meng et al., 2011). Prolonged bioavailability and enhanced delivery of drugs to targets improve actions of the drugs (Vanderbilt, 1992).

Studies on E. coli infections have also shown that immune-compromised animals are more susceptible to collibacilosis (McMullin, 2004). So, improved immune response could reduce cases of coli-bacilosis. National Research Council of United States of America (1994) recommended that for optimal immune response in chicks, levels of Vitamins A, C & E and
Selenium in 25 kg of poultry feeds should be at least 375 mg, 10 g, 7.5 g and 12.5 mg respectively. Synergy between prolonging bioavailability of ampicillin by stabilizing it with AMS and enhancing immune response of chicks by increasing levels of Vitamin A, Vitamin C, Vitamin E and Selenium in poultry feeds may improve the antibacterial action of ampicillin trihydrate against resistant infections.

1.2 Background of the Work

Development of resistance against drugs by pathogen has become a major problem in veterinary and human medicine (Neu, 1992; Witte, 1998). Ampicillin is a broad spectrum antibiotic that produces time-dependent bactericidal effect. It has short half life of 1-1.3 h (Pharmaceutical Expedient, Aillo (1998). This short half-life and time dependent bactericidal effect limits its use in therapy especially in poultry medication where concentration dependent effect is prefered. E. coli have the capacity to complicate and aggravate most primary infections of poultry (Jordan 1990 Mullin 2004). Studies, carried out by researchers show that most E. coli isolates have developed resistance against the inexpensive broad spectrum first line antibiotics (ampicillin, nalidixic acid, cotromoxazole, nitrofurantion and chloramphenicol (Okoli et al., 2005). Perhaps a drug that can protected ampicillin from premature degradation by physical factors and metabolic processes and allow for its gradual release may potentiate its action against E.coli thereby enhancing its usage.

AMS has been shown to increase the actions of ampicillin against Salmonella typhoid (Ezeibe, 2012), anthelmintic activity of piperazine and anti-plasmodial activity of chloroquine (Ezeibe, 2012).

1.3 Statement of the Problem

1. Bioavailability: There is the need to prolong bioavailability of Ampicillin to improve its clearance of both susceptible and resistant infections since it has short bioavailability due to its short ½ life.
2. Immune Suppression: Increase in bioavailability of ampicillin may increase its side effect which is immune suppression hence the need to enhance the immunity of treated animal.

1.4 The main objective of this study

The objective of the study is to develop a strategy of treatment that may make Ampicillin trihydrate regain efficacy against resistant infections.

1.5 Specific Objectives;

1. To evaluate the effects of increased level of vitamin A, C, and E and Selenium on load of resistant *E. coli* infections and on morbidity and mortality of infected chicks.

11. To evaluate the effects of increased level of vitamin A, C, and E and Selenium plus ampicillin trihydrate, on load of resistant *E. coli* of infected chicks.

111. To evaluate the effects of Aluminum Magnesium Silicate-ampicillin trihydrate formulation, on load of resistant *E. coli* infected chicks.

1V. To evaluate synergy that may exist between additional level of vitamin A, C and E and Selenium and Aluminum Magnesium Silicate-ampicillin trihydrate formulation, on load of resistant *E. coli* infected chicks.
CHAPTER TWO

LITERATURE REVIEW

2.1 Antibacterial resistance

Antibiotic resistance; is a form of drug resistance whereby some (or less commonly, all) sub populations of a microorganism usually bacterial species are able to survive after exposure to antibiotics (Ariza et al 1995). Pathogens resistant to multiple antibiotics are considered multi drug resistant (MDR) or more colloquially, superbugs (Ariza et al., 1995). Antibiotics resistance is a serious and growing phenomenon in contemporary medicine and has emerged as one of the predominant public health concerns of the 21st century. In particular, it pertains to pathogenic organisms. A world health organization report released April 30th, 2014 states, "This serious threat is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any country." Antibiotic resistance when bacteria change so antibiotics no longer work in people who need them to treat infections is now a major threat to public health (CDC, 2009). In the simplest cases, drug-resistant organisms may have acquired resistance to first-line antibiotics, thereby necessitating the use of second-line agents. Typically, a first-line agent is selected on the basis of several factors including safety, availability and cost. A second-line agent is usually broader in spectrum, has a less favorable risk-benefit profile, and is more expensive or in dire circumstances, may be locally unavailable (CDC, 2009). Resistance may take the form of a spontaneous or induced genetic mutation or the acquisition of resistance genes from other bacterial species by horizontal gene transfer via conjugation, transduction or transformation (Donadio et al 2010). Many antibiotic resistance genes reside on transmissible plasmids, facilitating their transfer (Donadio et al 2010). Exposure to an antibiotic naturally selects for the survival of the organisms with the genes for resistance in the way, a gene for antibiotic resistance may readily spread through an ecosystem of bacteria (Donadio et al 2010).
Antibiotic resistance plasmids frequently contain genes conferring resistance to several different antibiotics (National Centre for Emerging and Zoonotic Infectious Diseases, 2012). Genes for resistance to antibiotics, like the antibiotics themselves are ancient (Bentley and Meganathan, 1982). However, the increasing prevalence of antibiotic- resistant bacterial infections seen in clinical practice stem from antibiotic use both within human medicine and veterinary medicine. Any use of antibiotic can increase selective pressure in a population of bacteria to allow the resistant bacteria to thrive and the susceptible bacteria to die off (Donadio et al 2010).

2.2 Escherichia coli

*Escherichia coli*; is a gram-negative facultative anaerobic, rod-shaped bacterium of family *Enterobacteriaceae*, and genus *Escherichia* that is commonly found in the large intestine of warm-blooded organisms (Singleton, 1999). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts and are occasionally responsible for product recalls due to food contamination (Vogt and Dippold, 2005). The harmless strains are part of the normal flora of the gut and can benefit their hosts by producing vitamin K2 (Bentley and Meganathan, 1982), and preventing colonization of the intestine by pathogenic bacteria (Hudaults et al., 2001). *Escherichia coli* and other facultative anaerobes constitute about 0.1% of gut flora, (Eckburg et al., 2005). Fecal-oral transmission is the major route through which pathogenic strains of the bacterium infect its hosts (Harry et al 1965). *Escherichia coli* can be grown easily and inexpensively in laboratory settings and has been extensively investigated for over 60 years. It is the most widely studied prokaryotic model organism and is an important species in the field of biotechnology and microbiology where it serves as host organism for majority of works with recombinant DNA. Under favorable conditions it takes only 20 minutes to reproduce (Microbiology on-line, 2014).
2.2.1 Isolation/identification of *Escherichia coli*

**Isolation of *Escherichia coli***; Growth requirements of *E. coli* are relatively simple. It can grow well on common laboratory media (CDC, 2000). All members of the Enterobacteriaceae exhibit a common coliform morphology and are therefore indistinguishable from each other on such media as nutrient agar. However, the use of MacConkey offers a convenient method for isolation of Eschericha as it enables a sharp distinction to be made between lactose non-fermenters (e.g. Salmonella, Proteus) on the one hand and lactose-fermenting organisms to which *E. coli* belongs (CDC, 2000). Since Salmonella is frequently incriminated in outbreaks of diarrhoea in animals and therefore search for these organisms should always be made, the use of MacConkey agar plates in investigation of enteric disease is further indicated. Alternatively, Edwards and Ewing (1957) suggested that instead of MacConkey agar, a less inhibitory media such as Eosine Methylene Blue medium could be used for isolation of *E. coli*. It is also desirable that, in addition to MacConkey agar, 5% sheep blood agar be employed in isolation of *E. coli*. In this medium not only *E. coli* grows profusely, majority of other bacteria also grow, thus allowing a more accurate assessment of the relative proportion of coliforms recovered in relation to other organisms (CFSPH, 2005). The use of blood agar had earlier been recommended by Edwards and Weing (1957), because it is easy to avoid contamination when picking up colonies grown on such medium. These workers also reported instances when some *E. coli* strains failed to grow on Mac Conkey medium but were easily cultivated on blood agar plates. The main practical advantage in using blood agar plates in isolation of *E. coli* is that the haemolytic ability of some strains can be tested, and also colonies grown on this medium are most suitable for K antigens determination employing the slide agglutination technique (CDC, 2000).
The technique, in attempting to isolate *E. coli* from the internal organs or intestinal contents of animals at postmortem examination is by searing a small surface of the organ or the wall of the intestine with a heated platinum spatula. A sterile loop is then introduced through this area into the parenchyma of the organ or lumen of the intestine. Inoculum thus obtained is streaked onto the surface of the MacConkey agar and 5% sheep blood agar plates, which have previously been dried in the incubator. Rectal swabs are directly plated on similar culture media. Inoculated plates are incubated aerobically at 35°C for 24 hours. The plates are examined for colonies of coliform bacteria, noting their morphological characteristics and colour, density, haemolysis, etc, which enable a tentative recognition of the type of bacteria (CDC 2000).

2.2.2 Colonial morphology of *Escherichia coli*;

On MacConkey agar *E. coli* are large, smooth, shiny, and pink. On a differential medium such as Eosin Methylene Blue (EMB), the colonies exhibit characteristic iridescent or greenish metallic sheen. On 5% sheep blood agar plates, colonies of *E. coli* are large, circular, low convex, smooth, colourless with a finely granular structure and an entire edge. Often, marked differences in consistency of colony are found with different *E. coli* types (Jawetz *et al*, 1989).

2.2.3 Microscopic identification of *Escherichia coli*;

For microscopic identification of *E. coli*, colonies are picked from the agar plates and emulsified with normal saline on clean, grease-free microscopic slides. An even spread of each emulsified colony is fixed by passing the slide three times over a Bunsen flame. The fixed smear is Gram stained following the procedures described by Cheesbrough (1984). The stained slides are examined under oil immersion objective for gram negative (pink) short rod-shaped bacteria. *E. coli* organisms as other members of the family Enterobacteriaceae are
short gram negative rods (Jawetz et al, 1989). They are flagellated rods, 2-3 mm in length and 0.6mm wide (Jordan, 1990).

2.2.4 Biochemical identification of *Escherichia coli*;

Biochemical tests for *E. coli* identification include;

i. **Urease production**

A colony of the test isolate on MacConkey agar is streaked on the slant of a urea medium and incubated at 37°C for 18-24 h. Pink colouration of medium indicates urea production. *E. coli* does not produce urease enzyme (i.e. they are urease negative) and hence the colour of the slant would remain unchanged or becomes lightly yellow (Sojka, 1965).

ii. **Hydrogen sulphide production**;

Tripple Sugar Iron (TSI) agar hydrogen sulphide production, glucose and lactose fermentation as well as gas production is also assessed for identification of *E. coli*. Using a straight inoculating wire, a small portion of the test isolate is picked and inoculated by stabbing into the butt of the agar and the slope streaked. Inoculated slant is incubated at 37°C for 24 hours and examined for blackening of the medium as indication that hydrogen sulphide has been produced. The butt and slope are also examined for colour reaction. A yellow butt indicates glucose fermentation while a red slope indicates a non-fermentation of lactose. Gas production results in the development of cracks or spaces in the butt. *E. coli* does not produce blackening but yellow colour of the butt, alkaline slope and cracks in the butt (i.e. H₂S (-), glucose (+), lactose (+) and gas (+)) (Sojka, 1965).

iii. **Methyl Red Test**;

A colony of test isolate is grown in 2 ml of glucose 6-1 phosphate (MR 1 VP) medium for 48 hours at 37°C. After the incubation period, one drop of methyl red is
added to the medium by allowing the drop to run down the side of the bottle. A bright red ring on the surface of the medium indicates a positive test while a yellow colour indicates a negative test. *Escherichia coli* is methyl red positive (Sojka, 1965).

iv. **Indole Test**

Two millilitres of peptone water are inoculated with a colony of the test isolate and incubated at 37°C for 24 h. Then a drop of Kovac’s reagent is run down the side of the bottle into the medium and observed for development of a bright red ring on the top layer of the broth culture which indicates a positive test. A negative test is indicated by the appearance of a yellow ring on the top layer of the medium. *Escherichia coli* is indole positive (Sojka, 1965).

v. **Simmon’s citrate**

A colony of the test isolate is streaked on the slope of simmon’s citrate agar and incubated at 37°C for 1–7 days. A colour change from green colour of medium to blue denotes a positive test. This implies that the organization utilizes citrate as its source of carbon. *E. coli* is citrate negative (Sojka, 1965).

vi. **Sugar fermentation tests**

In addition to glucose and lactose fermentation reactions obtained from the triple iron sugar, dulcitol and mannitol fermentation are also tested in characterizing bacterial isolates (Sojka, 1965). Five millilitre amounts of dulcitol and mannitol respectively are each inoculated with a portion of the test colony. The inoculated sugars are incubated at 37°C for 24 hours and observed for a change of colour. A positive test is indicated by the development of pink colour of the sugar broth while a negative test is indicated by no change in colour of the media. *E. coli* ferments mannitol but may or may not ferment dulcitol (Davis, 1996). Other biochemical test reactions of *E. coli* are; Adinitol negative, Inositol negative, Voges-proskauer negative, Gelatin
liquefaction negative, Phenylalanine deaminase negative and sodium malonate negative (Davis, 1996). The Eijkman test provides a useful screening procedure for identification of bacteria. An organism that produces acid and gas at 44°C in MacConkey lactose bile broth (purple MacConkey) can be tentatively regarded as *Eschericha coli* (Stenqvist, 1987).

### 2.2.5 Serological identification *Escherichia coli*

Early attempts to classify *E. coli* strains by serological tests were not completely successful, because many strains of the bacterium were inagglutinable in the living state. It was not until the discovery by Kauffmann (1954) of a previously unsuspected heat-labile surface antigen (\(\tilde{K}\tilde{O}\tilde{O}\), capsular antigen which prevents \(\tilde{O}\tilde{O}\tilde{O}\) agglutination), that it was possible to use agglutination method for this purpose. Precipitin tests were found to be of little value in differentiation of strains (Lehmann and Jusatz, 1932). Full serotyping of strains of *E. coli* involves determination of the \(\tilde{O}\tilde{O}\tilde{O}\) (somatic), \(\tilde{K}\tilde{O}\tilde{O}\) (Capsular) and \(\tilde{H}\tilde{O}\tilde{O}\) (flagellar) antigens.

There are at present over 160 \(\tilde{O}\tilde{O}\tilde{O}\) antigens, 92 \(\tilde{K}\tilde{O}\tilde{O}\) and 50 \(\tilde{H}\tilde{O}\tilde{O}\) antigens (Sojka, 1965). Somatic \(\tilde{O}\tilde{O}\tilde{O}\) antigens are located on the surface of the cell and are thermo-stable. They consist of several antigenic components and are called \(\tilde{O}\tilde{O}\tilde{O}\) groups. Different \(\tilde{O}\tilde{O}\tilde{O}\) groups may share some antigenic components. So, cross reaction between members of the O group can occur. Cross reaction between some O group of *E. coli* and other Enterobacteriacae can also occur. To identify the O antigen, it is necessary to remove the heat-labile K antigen and then carry out a tube agglutination test (Sojka, 1965). Capsular \(\tilde{K}\tilde{O}\tilde{O}\) antigens or envelope antigens inhibit agglutination of live organisms by homologous \(\tilde{O}\tilde{O}\tilde{O}\) antiserum. This effect is inhibited by heating the organism to 100°C or 124°C. Some strains possess \(\tilde{K}\tilde{O}\tilde{O}\) antigens. To identify the \(\tilde{K}\tilde{O}\tilde{O}\) antigen, a rapid agglutination test is used, and it gives an indication of which O antigen is likely to be in combination with the \(\tilde{K}\tilde{O}\tilde{O}\) antigen (Sojka, 1965). Flagellar, \(\tilde{H}\tilde{O}\tilde{O}\) antigens are thermolabile and are found in most strains. The \(\tilde{O}\tilde{O}\tilde{O}\) groups of *E. coli* usually occur in
combination with certain capsular $\text{K}$-$\text{O}$ antigens only, and therefore demonstration of the presence of a particular $\text{K}$-$\text{O}$ antigen will in most instances, indicate that such a strain belongs to the $\text{K}$-$\text{O}$ group commonly associated with this $\text{K}$-$\text{O}$ antigen. Large numbers of $\text{O}$ group of $E. \text{coli}$ are recognized in animals and in man. Serotypes identified from poultry include $\text{O}_1$, $\text{O}_2$, $\text{O}_8$ and $\text{O}_{78}$:$\text{K}_{80}$ (Harry et al., 2010) $\text{O}_{35}$ and $\text{O}_{36}$ (Frasser, 1991) and $\text{O}_{111}$ (Barr and Carman, 1957). The serotypes mostly associated with colisepticaemia are $\text{O}_1$, $\text{O}_2$, and $\text{O}_{78}$ (Mead et al, 1999). Serotypes associated with $E. \text{coli}$ infection are uncommon in other animals with the exception of $\text{OK}$ group $\text{O}_{78}$:$\text{K}_{80}$ which is frequently associated with coli septicaemia in poultry as well as systemic infections in calves and lambs (Kim et al, 2004).

2.2.6. Biochemistry of Escherichia coli;

*Escherichia coli*, is Gram-negative (bacteria which do not retain crystal violet dye), facultative anaerobic (that make ATP by aerobic respiration if oxygen is present, but is capable of switching to fermentation or anaerobic respiration if oxygen is absent) and non-sporulating (Sojka, 1965). *Escherichia coli* cells are typically rod shaped, and are about 2.0 micrometres (mm) long and 0.25 mm in diameter, with a cell volume of 0.6-0.7 mm$^3$ (Britannica online encyclopedia, 2011). It can live on a wide variety of substrates. *Escherichia coli* uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide. Since many pathways in mixed acid fermentation produce hydrogen gas, these pathways require the level of hydrogen to be low as is the case with *E. coli*. It lives together with hydrogen-consuming organisms such as methanogens or sulphate-reducing bacteria (Madigan and Martinko, 2006). Optimal growth of *E. coli* occurs at 37°C but some laboratory strains can multiply at temperature of up to 49°C (Fotadaru et al., 2005). Strains of *E. coli* that possess flagella are motile. The flagella have a peritrichous arrangement. *E. coli* and related bacteria possess ability to transfer DNA via bacterial conjugation, transduction or transformation, which allows genetic material to spread horizontally through
an existing population. This process leads to the spread of the gene encoding *Shiga* toxin from *Shigella* 70 *E. coli* O157:H7 (Ovskou *et al.*, 1977). It is however common to cite only sero-group of *E. coli* that has the O-antigen. At present about 190 sero -groups of *E. coli* are known (Stenutz *et al.*, 2006). The common laboratory strain has a mutation that prevents the formation of an O-antigen and is thus non-typable.

**2.2.7. Role of *Escherichia coli* in disease**

Although most isolates of *E. coli* strains are non-pathogenic, they are considered an indicator of faecal contamination in food and about 10 to 15% intestinal coli forms are opportunistic and pathogenic serotypes (Barnes and Gross, 1997). They cause a variety of lesions in immuno-compromised hosts and/or in the presence of other germs (McMullin, 2004). Avian species infected by *E. coli* serotypes includes Chicken, Turkey, Duck and Pheasan (Jordan, 1990). Considerable amount of evidence indicate that coli septicaemia is primarily a respiratory infection which develop as a result of an associated infection of *E. coli* with *Infectious bronchitis virus* or *Mycoplasma gallisepticum* (Linam *et al.*, 2007). Gross (1994) showed that these respiratory pathogens produce changes in the tissues of the respiratory tract which favor *E. coli* invasion. Majowicz *et al.*, (2010) reported that *E. coli* caused acute fatal enteritis in two flocks of chicks after New castle disease vaccine inoculation. Linam *et al.*, (2007) reported an apparently non-pathogenic *E. coli* that appeared to act synergically with *Eimeria brunetti* in chicks exposed to both pathogens. Mortality was 100% in chicks which received both pathogens simultaneously, though neither pathogen alone caused any mortality. Kurmar *et al.*, (2010) reported that inoculation of *Mycoplasma* cultures into sinuses of chicks caused no symptoms or mortality but when the organism was inoculated in conjunction with *E. coli*, a mortality of 45% of the chicks resulted within 5 days. Fibrinous pericarditis and perihepatitis were found in all the chicks that died at post mortem and *E. coli* was readily
isolated from their organs. Even an apparently nonpathogenic *E. coli* strain has the capacity to produce serious disease in mixed infection especially in immune-compromised hosts.

**2.2.8. Treatment and control of *Escherichia coli* infections;**

Good husbandry practices and good disease prevention programs are generally advocated in the prevention and control of colibacillosis (Bains, 1979). Also, other diseases have *E. coli* as complication (Linam *et al.*, 2007). Vaccination against *E.coli* is possible but its immunity is not well documented. Both autogenous and commercial *E. coli* vaccines are in use. Vaccination is therefore far from efficient against *E. coli* infections. Therefore antibiotic treatment is frequently necessary. However, antibiotic resistance is an ever increasing problem. Diseases caused by *E. coli* are difficult to treat (Okoli *et al* 2005). And this may be in part due to the high level of resistance (Okoli *et al* 2005). Okoli (2006) reported 72-92% resistance of *E. coli* to 10 antibiotics including Ampicillin, Nalidixic acid and Cotrimoxazole. Earlier work done in peri-urban sites spread across three senatorial zones in Imo state Nigeria to determine the antimicrobial resistance profile of commensal *E. coli* isolates from free range chickens show 100% and 78% resistance against Ampiciline and Cotrimazole respectively (Okoli *et al.*, 2005). *E. coli* isolated from five geopolitical zones of Nigeria screened for antimicrobial resistance profile shows 94.4% resistance to Ampicillin and 85.5% to Cotrimoxazole (Nsofor and Iroegbu, 2013). It is thus very worrisome that *E. coli* has developed resistance to first line antibiotics like Ampicillin. More worrisome is that ubiquitous commensal population of *E. coli* constitutes an enormous reservoir from which pathogenic strains continually emerge. The ability of *E. coli* to exist as a human/animal-adapted commensal compounded with its natural tendency for frequent genetic exchange, its ubiquitous presence and the enormous, diverse and largely uncharacterized reservoir of genetic variation found within species collective genomics all contribute to the emergence of new pathogenic strains (Dominquez *et al.*, 2002). The emergence of *E. coli* isolates with
multiple antibiotics-resistant phenotypes, involving cross resistance to four or more unrelated families of antibiotics, has been reported and is considered a serious health concern (Ariza et al, 1995).

2.3 AMPICILIN

Ampicillin has been used extensively to treat bacterial infection since 1961 (Mandell et al., 2000). Until introduction of ampicillin by the British company, Beecham, penicillin therapies had been effective against only gram-positive organisms such as staphylococci and streptococci. Ampicillin which was originally branded as Penbritin has demonstrated activity against even gram- negative organisms such as H. influenza, coli forms and proteus spp (Mandell et al, 2000). But resistance to ampicillin is increasingly common among these bacteria and this has raised a serious concern (Okoli et al 2005). Ampicillin is a beta-lactam antibiotic, a semi-synthetic penicillnase-sensitive penicillin that is part of the amino penicillin family. It is roughly equivalent to amoxicillin in terms of activity (AHFS, 2006).

2.3.1 Medical uses of Ampicillin

Ampicillin is effective against gram-negative and gram-positive bacteria and is used to treat infections of the intestinal, urinary and respiratory tracts (Mandell et al, 2000). Ampicillin is active against gram-positive bacteria including Streptococcus pneumonia, Streptococcus pyogenes, Staphylococcus aureus and some enterococci (Mandell et al, 2000). Gram negative bacteria ampicillin has activity against include Neisseria meningitides, some Haemophilus influenza and some Entero- bacteriaceae. Ampicillin is a first-line antimicrobial agent for treatment of infections caused by enterococci (Kucers et al 1997). Ampicillin is on the world health organization list of essential medicines, a list of the most important medications needed in a basic health system (WHO, 2013).
2.3.2. Mechanism of action of Ampicillin;

Belonging to the penicillin group of beta lactam antibiotic, ampicillin is able to penetrate gram-positive and some gram-negative bacteria. It differs from penicillin G. or Benzylpenicillin, only by the presence of an amino group. That amino group helps the drug penetrate the outer membrane of gram-negative bacteria (AHFS, 2006). Ampicillin acts as an irreversible inhibitor of the enzyme, transpeptidase, which is needed by bacteria to make their cell walls (AHFS, 2006). It inhibits the third and final stage of bacterial cell wall synthesis in binary fission which ultimately leads to cell lyses. Ampicillin is bacteriocidal (AHFS, 2006). The active form of ampicillin appears in the bile in higher concentration than those found in serum. Ampicillin is the least serum-bound of all the penicillins, averaging about 20% compared to approximately 60 to 90% for other penicillin (Kucers et al, 1997).

2.3.3. Interaction of Ampicillin with other drugs

With aminoglycosides, in vitro (Kucers et al, 1997), there was evidence of synergistic antibacterial effect against enterococci, used to therapeutic advantage in the treatment of endocarditis and other severe enterococcal infections potential in vitro and in vivo inactivation of aminoglycosides. With choramphenicol, there is in vitro evidence of antagonism but the clinical importance is unclear. With probenecid, there is decreased renal tubular secretion of ampicillin (Kucers et al., 1997). With sulbactam, ampicillin has synergistic bactericidal effect and against many other strains of B-lactamase-producing bacteria. With sulfonamides, in vitro, evidence of antagonism exists but its clinical importance is unclear (Anon 2001).

2.3.4. Ampicillin’s pharmacokinetics/pharmacodynamics

About 30% to 50% of an oral dose of ampicillin is absorbed from the gastrointestinal tract. In fasting adults, peak serum concentration of ampicillin is attained within 1-2 h of oral administration (Kucers et al., 1997). Following intramuscular administration of ampicillin,
peak serum concentration are generally attained more quickly and are higher than following
equivalent oral administration (Kucers et al., 1997). Following intra venous administration of
ampicillin, peak serum concentration is attained immediately and the drug may still be
detectable in serum six hours after administration (Kucers et al, 1997). Food generally
decreases rate and extent of absorption and distribution of ampicillin into ascetic, synovial
and pleural fluids. Also, distribution of the drug into liver, bile, lungs, gall bladder, prostrate,
muscle, middle ear effusions, bronchial secretions, sputum, maxillary sinus secretions,
tonsils, saliva, sweat and tears could be decreased. Highest cerebrospinal fluid concentration
of ampicillin usually occurs 3-7 h after intravenous injection. Ampicillin protein binding is
even lower in neonates. Ampicillin is also partially metabolized by hydrolysis of its B-lactam
ring to penicillin acid which is microbiologically inactive (Kucers et al., 1997). Elimination
of ampicillin is through the urine by renal tubular secretion and to a lesser extent by
glomerular filtration. Small amount the drug is also excreted in faeces and bile (Kucers et al.,
1997). In adult human beings with normal renal function, approximately 20-64% of a single
oral dose of ampicillin is excreted unchanged in urine within 6-8 hours. Approximately 60-
70% of a single intramuscular dose of the drug or 73-90% of a single intravenous dose is
excreted unchanged in urine. Half life of ampicillin is 0.7 to 1.5 hours in adult human beings
with normal renal function (Kucers et al., 1997). It is poorly bound to plasma, with 15 to25%
plasma protein binding which is the lowest of the penicillin group. Ampicillin produces time
dependent killing of susceptible micro-organisms.

2.3.5 Side effect and toxicity of Ampicillin
Among the most serious adverse effects of ampicillin on treated patients are neutropenia,
anaphylaxis, nausea, diarrhoea, fever, rashes, allergic reactions, super-infection,
thrombophlebitis, local pain and nerve degeneration at site of injection may also occur
(Jawetz, 1995). Toxicological and carcinogenic studies of ampicillin trihydrate conducted by
administering the drug formulated in corn oil shows that in male rats, ampicillin trihydrate is associated with increased incidence of mononuclear cell leukemia. Malignant lymphomas were also observed in one control male rat and two male rats that received low doses of the drug showed increased incidences of pheochromocytomas of the adrenal gland medulla. There was increased incidence of C-cell hyperplasia of the thyroid glands in male rats that received low doses of the drug and in females that received the high dose. High dose male rats showed increased incidences of hyperkeratosis and acanthosis of the fore-stomach. In male and female mice, ampicillin trihydrate administration was associated with increased incidences of fore stomach lesions, including ulcers, inflammation, hypokeratosis, acanthosis and evidence of fungal infection (NTP, 1987).

2.3.6 Abuse of Ampicillin

In 1945, in his noble lecture ‘penicillin’ Alexander Fleming warned against the use of sub-therapeutic doses of antibiotics bought by anyone in the shops without a prescription. He said ‘the time may come when penicillin can be bought by anyone in shops. Then there is the danger that the ignorant man may easily under dose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant’ (Anon 2001). Inappropriate prescribing of antibiotics has been attributed to a number of causes, including people insisting on antibiotics, physicians prescribing them as they feel they do not have time to explain why they are not necessary and physicians not knowing when to prescribe antibiotics or being overly cautious for medical and/or legal resources (Keren et al, 2006). A simple regime of ampicillin even in compliment patients leads to a greater risk of resistant organisms to that antibiotic in the person for a month to possibly a year (Costelloe, 2010).

Antibiotic resistance has been shown to increase with duration of treatment. Therefore, as long as a clinically effective lower limit is observed (that depends upon the organism and antibiotic in question), use of antibiotics by the medical community of shorter courses is
likely to decrease rates of resistance, reduce cost, and have better outcomes due to fewer complications such as *Clostridium difficile* infection and diarrhea (Li et al., 2007; Bignardit, 1998, Runyon et al; 1991; Gleisner et al., 2004; Pichichero and Cohen, 1997). In some situations a short course of an ampicillin may be inferior to a long course (Keren and Chan, 2006). A World Health Organization report incorporating data from 114 countries recommended that people help tackle drug resistance by using ampicillin only when prescribed by a doctor and to complete the full prescription, even if they feel better (WHO, 2002). Some infections require treatment long after symptoms may have ceased (Gleisner et al., 2004). Insufficient course of an ampicillin (an antibiotic) may lead to relapse of an infection that is now resistant to the ampicillin (Pechère et al, 2013). Effectiveness of treatment programs for ampicillin resistant strains of micro-organisms depends on whether or not the programs take into account the effect of structural violence (Paul et al., 2006).

Ampicillin trihydrate are used in animal production as growth promoters and for prophylactic control of infections (Paul et al., 2006). Historically, regulation of use of antibiotics in food animals has been limited to concern for drug residues in meat, egg and milk, rather than concern for development of antibiotic resistance in the animals and transfer of such resistance to human beings. Use of ampicillin trihydrate like other antibiotic in animals can be classified into different patterns according to the purpose. The most accepted purposes are, for therapeutic treatment, for prophylaxis and for growth promotion (Joint FAO/OIE/WHO Expert Workshop, 2013). All these antibiotic uses in animals lead to antibiotic resistance by bacteria. Antibiotic resistance is a natural evolutionary process, but sub-therapeutic uses of antibiotics expose larger number of animals and therefore of bacteria for more extended periods and development of resistance. They therefore greatly increase the cross section for the evolution of resistance. The World Health Organization’s conclusion is that inappropriate use of antibiotics in animal husbandry is an underlying contributor to the
emergence and spread of antibiotic-resistant genes and that the use of antibiotics as growth promoter in animal feeds should be prohibited in the absence of risk assessments. Regarding this matter, the OIE (2013) has added to the Terrestrial Animal Health Code a series of guidelines with recommendations to its members for the creation and harmonization of national antimicrobial surveillance and monitoring program for quantity of antibiotic used in animal husbandry and for recommendations to ensure the proper and prudent use of antibiotic substances. In the world, particularly developing counties like Nigeria, antibiotics (ampicillin inclusive) are wildly used on animals (Adene 2006). As in human medicine, ampicillin can often be bought for use on pets and livestock without prescription and without veterinary supervision. Bacteria that remain in such treated animals are likely to be resistant to the antibiotics used and may be passed into the environment by the excretion and secretion of materials such as milk, feces, saliva, semen, etc (Paul et al, 2006). The actual impact of these resistant germs depends on their specific type and on the animal or organism they henceforth infect (Paul et al, 2006). In recognition of the impact of veterinary use of antibiotic (ampicillin) in development of antibiotic resistance, the United States Federal Department of Agriculture (FDA) announced in December 2013 the commencement of steps to phase out use of antibiotics for the purpose of promoting livestock growth (Tavernise and Sabrina, 2013). As resistance towards antibiotics becomes more common, a greater need for alternative treatment arises. However, despite a push for new antibiotic therapies, there has been a decline in the number of newly approved drugs (Donadio et al., 2010). Antibiotic resistance therefore poses a significant problem. From the foregoing, need for solution to treatment regimen for ampicillin resistant E. coli cannot be overemphasized.
2.4 ALUMINUM MAGNESIUM SILICATE

The European Pharmacopeia defined AMS as a mixture of particles with colloidal particle size of montmorillonite and saponite free from quitz and non-swellable ore. It is a natural ore which occurs as mineral deposit in India and the United States of America. A synthetic form which is devoid of impurities has been synthesized from two other minerals found in many countries including Nigeria; aluminum silicate $\text{Al}_4\left(\text{SiO}_4\right)_3$ and magnesium silicate $\text{Mg}_2\text{SiO}_4$ (Ezeibe, 2006). Aluminum magnesium silicate has been in use for many years in pharmaceutical industries as absorbent, viscosity-enhancing agent, anticaking agent, stabilizing agent, tablet binder and capsule disintegrant (Vanderbilt, 1992). It has been described as the formulator's choice for over 50 years to stabilize suspensions, perfect emulsions and optimize flow properties (Vanderbilt, 1992).

2.4.1 Aluminum Magnesium Silicon composition and properties

Naturally, AMS is a polymeric complex of aluminum, magnesium, silicon, oxygen and water. The ore may contain elements like carbon, calcium and lithium (Smith, 1984). Aluminum magnesium silicon is described as an odorless, tasteless, small creamy white powder (Kerr, 1979). It has density of 2.41 g/cm and moisture content of 6.0 to 9.98% and is insoluble in water (Vanderbilt, 1992). The complex compound is a polymeric complex of Aluminum, Magnesium, Silicon, Oxygen and water. Its average chemical analysis is expressed as 13% Magnesium oxide, 61% Silicon oxide, 2% Calcium oxide, 0.1% Titanium oxide, 0.9% Feric oxide and 2.7% Calcium oxide. The complex is composed of three lattice layer of octahedral alumina and two tetrahedral silicate sheets. The Aluminum is often substituted to varying degrees by Magnesium, Potassium or Sodium to balance the electrical charges. Iron, Lithium, Calcium and Carbon could be present in smaller quantities (Vanderbilt 1992).
In the pharmaceutical industry, Aluminum-magnesium silicate is marketed as Veegum® or Pyropes®. Other synonyms for the chemical include Aluminosilic acid, Neusilin, Angast, Carisob, Geisor, Magnabite, etc. A microscopic clay particle of AMS is composed of thousands of sandwiched platelets with exchangeable cat ions and a layer of water between each. When AMS hydrates, water penetrates between the platelets forcing them further apart. The cations begin to diffuse away from the platelet faces and are replaced by water. As the platelets’ edges are attracted to the negatively charged platelet faces, a three dimensional colloidal structure forms. The attraction of platelet edges and faces can be on any of the sides giving it different forms and shapes hence the amorphous nature the structures (Vanderbilt, 1992). The “corridor” created by the “house of cards” of AMS platelets can be interconnected to form long wide channels which allow easy movement of resident ions and molecules in and out of the channels. Drug molecules that are in these “corridors” formed by platelets of AMS are protected from degradations by physical factors and from metabolic processes. From there they are released gradually (Vanderbilt, 1992). Hydrated AMS is said to give characteristic test for Aluminum, Magnesium and Silicates (Ezeibe et al., 2006).

2.4.2 Uses of Aluminum Magnesium Silicon;

Aluminum magnesium silicon has been used for many years in the field of cosmetics, pharmacy, and veterinary medicine, as adsorbent, stabilizing agent, tablets binder and viscosity increasing agent (Vanderbilt, 1992). It is used either alone or in combination with suspending agents, such as charcoal and tannic acid, in oral and topical formulations (Wail et
Aluminum magnesium silicon has been used for a long time in treatment of acidosis, animal bites, constipation, detoxification, dysentery, heat disorders, poisoning, skin fairness, stomach disorders, vomiting, nausea, and weight loss with no reports of allergies (Ramachandran, 2007). Dosage of AMS is 10-50 mg/kg of body weight for all animal species (Kerr, 1979).

2.4.3 Aluminum magnesium silicon Toxicity;
AMS has also been clinically assessed by the Committee for Veterinary Medical Products and entered into annex 11 of council regulation (EEC) 237790 and declared safe for use on food animals (Schills, 2003).

2.4.4 Aluminum magnesium silicon as a zeolite
Zeolites are micro porous, aluminosilicate minerals commonly used as commercial absorbents and catalysts (Grace, 2010). The term zeolite was originally coined in 1756 by Swedish mineralogist Axel Fredrik Cronstedt, who observed that when rapidly heated, the material silbite, produced large amounts of steam from water it had absorbed. Based on this, he called the material zeolite, from the Greek (zeo) meaning óo boilô (lithos), meaning ótoneô(Kerr, 1979). Zeolites occur naturally but are also produced industrially on a large scale. Like AMS zeolites have a porous structure that can accommodate a wide variety of cations, such as Na+, K+, Ca2+, Mg2+ and others. These positive ions are rather loosely held and can readily be exchanged for others in a contact solution. Zeolites are aluminosilicate members of the family of microporous solids known as ómolecular sievesô The term molecular sieve refers to a particular property of these materials, that is, the ability to selectively sort molecules based primarily on a size exclusion process. This is due to a very regular pore structure of molecular dimension. The maximum size of the molecular or ionic species that can enter the pores of a zeolite is controlled by the dimensions of the channels (Vanderbilt, 1992).
2.4.5 Aluminum magnesium silicon as a Nanomaterial

Scientists have not unanimously settled on a precise definition of a nanomaterial, but it has been agreed on size that qualifies a particle as a nanoparticle (NIEHS, 2014). A nanometer is one millionth of a millimetre, approximately 100,000 times smaller than the diameter of a human hair (NIEHS, 2014). Nano sized particles exist in nature and they can also be created from a variety of products, such as carbon or minerals like silver but nanomaterials by definition must have at least one dimension that is less than 100 nanometers. Most nano scale materials are too small to be seen with the naked eye and even with conventional microscope. Aluminum magnesium silicate (a clay particle) is one nanometer thick and some hundred nanometers across (Scenihr, 2009). As a nano particle, AMS may help in delivering drug molecules to target cells. Drug molecules in “corridors” of AMS “house of cards” are also bound by charged faces and edges of its platelets. So, they are protected from degradation by physical factors and from metabolic processes but are released gradually into blood of treated patients. So, bioavailability of such drugs would be prolonged (Scenihr, 2009). This prolonging of bioavailability is a very good advantage for chemotherapeutics especially Ampicillin trihydrate which has poor plasma binding and thus very short, half life but has time-dependent antimicrobial effect (Kucers et al, 1997).

2.4.6 Aluminum magnesium silicon as a stabilizing (potentiating) agent;

Aluminum magnesium silicon is reported to be a drug stabilizer (Vanderbilt, 1992). By stabilizing drugs, AMS may increase potency of drugs. If it is found to potentiate drugs, its use may reduce drug dosages and thus save cost, especially in veterinary practice. Potentiated drugs may also be useful in handling drug resistant micro organisms. However, some drugs are known to be tightly bound to AMS leading to low bioavailability of such drugs, example amphetamine sulfate, teobutamide, diazepan, warfarin sodium and diclofenac sodium (Pharmaceutical excipient, 2010). AMS has been shown to increase the potency of
sulphadimidine against coccidian, anti plasmodial activity of chloroquine and anthelmintic activity of piperazine (Ezeibe et al., 2011).

2.5 IMMUNE STIMULANTS

2.5.1 Vitamins

Hopskins defined vitamin as vital amines needed by the body. Vitamin is an organic compound required by an organism as a vital nutrient in limited amounts (Lieberman and Bruning, 1990). An organic chemical compound (or related set of compounds) is called a vitamin when it cannot be synthesized in sufficient quantities by an organism and must be obtained from the diet (Lieberman, 1990). Thus, the term is conditional both on the circumstances and on the particular organism. Supplementation is important for the treatment of certain health problems but there is little evidence of benefit when used by those who are otherwise healthy (Fortmann et al., 2013). By convention, the term vitamin includes neither other essential aminoacids (which are needed in larger amounts than vitamins) nor the large number of other nutrients that promote health but are otherwise required less often (Maton et al., 1993). Thirteen vitamins are universally recognized at present. Vitamins are classified by their biological and chemical activity, not their structure. Thus each ‘vitamin’ refers to a number of vitamer compounds that all show the biological activity associated with a particular vitamin. Such a set of chemicals is grouped under an alphabetized vitamin ‘generic descriptor’ title, such as ‘vitamin A’ which includes all the compounds, retinal, retinol and four known carotinoids. Vitamers by definition are convertible to the active form of the vitamin in the body and are sometimes inter-convertible to one another as well. Vitamins have diverse biochemical functions. Some such as vitamin D, have hormone-like functions are regulators of mineral metabolism or regulations of cell and tissue growth and differentiation (such as some form of vitamin A). Others function as antioxidants (e.g. vitamin E and sometimes vitamin C) (Bender and David, 2003). The largest number of
vitamin (the B complex vitamins) function as precursors for enzyme cofactors that help enzymes in their work as catalysts in metabolism. In this role, vitamins may be tightly bound to enzymes as part of prosthetic group. For example, biotin is part of enzymes involved in making fatty acids. They may also be less tightly bound to enzyme catalysts as coenzymes, detachable molecules that function to carry chemical groups or electrons between molecules. For example, folic acid may carry methyl, formyl and methylene groups in the cell. Although these roles in assisting enzyme substrate reactions are vitamin’s best known function, the other vitamin functions are equally important (Bolander, 2006). Three recognized immune stimulant vitamins in poultry are vitamin A, E and C (NRC, 1994). These three together with selenium are required in certain quantity for optimal immune response in poultry (NRC, 1994).

2.5.1.1 Vitamin A

Vitamin A is a group of unsaturated nutritional organic compounds that includes retinol, retinal, retinoic acid and provitamin A carotenoids, among which beta carotene is the most important (Fennema and Owen, 2008). Vitamin A has multiple functions, as it is important in growth and development, for the maintenance of the immune system and good vision (Tanumihardjo, 2011). Vitamin A is needed by the retina of the eye in the form of retinal, which combines with protein opsin to form rhodopsin, the light absorbing molecule (Wolf, 2001) necessary for both low light (scotopic vision) and colour vision (Wolf, 2001). Vitamin A also functions in a very different role as retinoic acid (an irreversibly oxidized form of retinol), which is an important hormone like growth factor for epithelial and other cells (Tanumihardjo, 2011) help maintain the integrity of cell membrane and integrity of white and red blood cells.
2.5.1.2 Vitamin C;

Vitamin C or L-ascorbic acid or simply ascorbic acid and its salts as well as some oxidized forms of the molecule like dehydro ascorbic acid are both naturally present in the body when either of these is introduced into cells, since the forms interconvert according to pH. Vitamin C is a cofactor in at least eight enzymatic reactions including several collagen synthesis reactions that when dysfunctional, cause the most severe symptoms of scurvy (Padayatty et al., 2003). In animals, these enzymatic reactions are especially important in wound healing and in preventing bleeding from capillaries. Ascorbates may also act as an antioxidant against oxidative stress (Padayatty et al., 2003). However, it is a fact that enantiomer D-ascorbate (not found in nature) has identical antioxidant activity to L-ascorbate, yet far less vitamin activity (Aboul-Enein et al., 1999). This underscores the fact that most of the function of L-ascorbate as a vitamin relies not on its antioxidant properties but upon enzymatic reactions that are stereo specific. "Ascorbate" without the letter \textit{D.} for the enantiomeric form is always presumed to be the chemical L-ascorbate. Ascorbate is also not synthesized by some species of birds and fish, thereby making it necessary to be added in the diet. Deficiency in this vitamin causes the diseases scurvy in humans (Higdon, 2006). Ascorbic acid is also widely used as a food additive to prevent oxidation. Vitamin C is essential element in collagen formation (strengthens blood vessels, form scar tissues, is a matrix for bone growth); an antioxidant, strengthen resistance to infection and improves absorption of iron (Biscontini, 2007).

2.5.1.3 Vitamin E

Vitamin E refers to a group of eight fat soluble compounds that include both tocopherols and tocotrienols (Brigelius-Flohé and Traber, 1999). Of the many different forms of vitamin E, Υ-tocopherol is the most common in the North American diet, (Traber, 1998). Gamma tocopherol can be found in corn oil, soya bean oil, magarine and dressings, (Bieri and Evarts, 1974), (Brigelius-Flohé and Traber, 1999). Útocopherol, the most biologically active form of
vitamin E, is the second most common form of vitamin E in the diet (Haederle, 2011). This variant can be found most abundantly in wheat germ oil, sunflower and safflower oils (Haederle, 2011). Vitamin E stops the production of reactive oxygen species formed when fat undergoes oxidation (NIH, 2009), Herrera and Barbas (2001), Packer et al. (2001). Amounts over 1,000 mg (1:500 iu) per day (Brigelius-Flohé and Traber, 1999) are called hypervitaminosis E as they may increase the risk of bleeding problems and vitamin K deficiency. Vitamin E is a fat soluble antioxidant. Help maintain cell membrane, red blood cell integrity, protects vitamin A and fatty acid from oxidation (Biscontini, 2007).

2.5.2 Selenium

Selenium is a chemical element with symbol Se, and atomic number 34. It is a non-metal with properties that are intermediate between those of its periodic table column adjacent chalcogen elements sulfur and tellurium (Greenwood et al., 1997). It rarely occurs in its elemental state in nature or as pure compounds. Selenium is found impure in metal sulphide ores, where it partially replaces the sulphur. Commercially, selenium is produced as a bye product in the refining of these ores, most often during copper production. Minerals that are pure selenide or selenate compounds are known, but are rare. Selenium salts are toxic in large amounts, but trace amounts are necessary for cellular function in many organisms, including all animals. Selenium is a component of the antioxidant enzymes glutathione peroxidase and thioredoxin reductase (which indirectly reduce certain oxidized molecules in animals and some plants). It is also found in three deiodinase enzymes, which converts one thyroid hormone to another. It helps to protect cells from damage.
CHAPTER THREE

Materials and Methods

3.1 Materials

3.1.1 Experimental Birds

Eighty Hacco cockerel chicks that were six weeks of age were used for the study. The chicks were bought from a hatchery in Ibadan, Nigeria at a day old and raised for 4 weeks in a farm at Enugu, Nigeria. They were transferred to experimental house of Department of Veterinary Medicine, University of Nigeria, Nsukka at four week of age. Earlier the house had been disinfected with Caritol® a Benzakonium chloride Sanitizer / Detergent and rested for 1 week. The birds received vaccinations against Infectious Bussal Disease at two weeks of age. They were also vaccinated against Newcastle Disease at day 1 with intraocular and at 4 week with Lassota strain. The birds were only treated with oxytetracyclin / Vitamin formulation on their first week. They were managed on deep liter and given commercial feed and pipe born water. After the transfer they were then acclimatized for two weeks before commencement of the experiment.

3.1.2 Drugs

- Ampicillin; The West Bengal pharmaceuticals (India) brand of ampicillin trihydrate was obtained from a reputable pharmacy.

- AMS; AMS and AMS-Ampicillin drug formulation was according to Ezeibe 2006. Aluminum magnesium silicate-Ampicillin trihydrate drug formulation is formulated by Sal veterinary company Nsukka and sold in the market as AdmacinR. This market brand was used in the experiment.

- Vitamins; A, C, E, and Selenium were obtained from a pharmacy in the market.
3.1.3 E. coli

An ampicillin-resistant *E. coli* isolate was obtained from Department of Veterinary Pathology and Microbiology, University of Nigeria Nsukka.

3.2 Experimental Infection

The *E. coli* isolate was sub-cultured on nutrient agar and incubated at 37°C for 24 hours. A colony of the growth was harvested with a sterile loop and was sub cultured in a nutrient broth for 24 hours at 37oC. Mc Ferland standards turbidity method (CDC, 200) was used to prepare an inoculum of 1.5x10^8 per ml. A 0.1 ml (1.5 x 10^7) of the inoculum was given (per os) to each of 35 chicks in each of group A and group B. Five chicks in each of the groups were not inoculated so that they served as controls.

3.3 Experimental design

The chicks were assigned into two main treatment groups (A and B) of 40 chicks each.

Two days before the experimental infection with the resistance *E. coli*, chicks in group A were placed on a feed fortified with additional levels of vitamins A, C and E and Selenium. To each 25 kg of the feed, 375 mg, 10 mg, 75 mg and 12.5 mg of Vitamins A, C, E and Selenium, respectively, were added as recommended by USA-NRC (1994). Group B chicks (40 chicks) were retained on unfortified poultry feed (unfortified with additional levels of the vitamins and Selenium).

**Sub-groups**

A1 and B1; 5 chicks each, treated at dose of 10 mg/kg of body weight with 100 % Ampicillin trihydrate.

A2 and B2; 5 chicks each, treated at dose of 7.5 mg/kg of body weight with 100 % Ampicillin trihydrate.

A3 and B3; 5 chicks each, treated at dose of 5 mg/kg of body weight with 100 % Ampicillin trihydrate.
A_4 and B_4; 5 chicks each, treated at dose of 10 mg/kg of body weight with AMS Ampicillin trihydrate drug formulation.

A_5 and B_5; 5 chicks each, treated at dose of 7.5 mg/kg of body weight with AMS Ampicillin trihydrate drug formulation.

A_6 and B_6; 5 chicks each, treated at dose of 5 mg/kg of body weight with AMS - Ampicillin trihydrate drug formulation.

A_7 and B_7; 5 chicks each, infected but untreated with neither ampicillin trihydrate nor ampicillin trihydrate - AMS drug formulation (positive control).

A_8 and B_8; 5 chicks each were left uninfected and untreated with neither ampicillin trihydrate nor ampicillin trihydrate - AMS drug formulation (negative control).

3.4 Methods

3.4.1 Watering;

Portable tap water was supplied to chicks in each group, *ad libitum*, and during medication adjustment was made daily based on previous consumption of water to ensure they finished the medicated water daily and that the water given was just enough for daily need of the chicks. The treatment was through drinking water and lasted for six days. The chicks were observed daily for signs of disease (morbidity) and mortality throughout the study.

3.4.2 Sample Collection of bile;

The whole chicks from each sub-group were sacrificed on day three post treatment by severing the head from the body using sharp scissors. Bile was harvested from the gall bladder of each chick at postmortem, using sterile syringe and needle.

3.4.3 Assessment of *E. coli* isolation rate in bile of the infected chicks:

0.1 ml of the harvested bile was drawn, using a sterile micropipette and pip petted into a sterile bottle containing 0.9 ml normal saline to get a 1:10 dilution. Then 0.1 ml of the 1:10
of each bile dilution was transferred to a second bottle of 0.9 ml normal saline to get a 1:100 dilution. Each time the bottle was rocked to give uniform solution.

Finally, 0.05 ml of the diluted bile was plated on MacConkey agar and incubated at 37°C for 24 hours. *Escherichia coli* colonies in the cultures were identified by their morphology. The number of *E. coli* colonies (x) that grew from bile of each chick was counted using a hand lens. *E. coli* CFU/ml of bile of each chick were calculated as the infection rate, by the formula: CFU/ml of bile (infection load) = \( x \div 5 \times 10,000 \) CFU/ml (Harold, 1999).

3.5 Statistical Analysis;

Data obtained from the study were analysed using ANOVA followed by Duncan Post Hoc test. A difference in values less than a probability of 0.05% was considered significant.
CHAPTER FOUR
RESULTS

4.1 Morbidity / Mortality

No sign of morbidity was observed in the chicks in all the groups and sub-groups. No mortality recorded. There were no significant reduction in feed intake and no significant reductions in weight among chicks in the untreated sub-group placed on ordinary feed. No mortality was observed in any of the sub-groups. No evidence of pathological lesions observed in the kidney, liver, spleen, heart lungs, intestine and serosal surface at postmortem examination. No gross lesions were observed in the chicks when sacrificed.

4.2. Escherichia coli isolation from bile;

4.2.1. Chicks on ordinary feed had E. coli isolation rate of $8.24 \times 10^5 \pm 3.22 \times 10^5$ while those fed the feed fortified with higher levels of Vitamins A, C, E and Selenium had mean resistant E. coli isolation rate of $5.35 \times 10^5 \pm 2.78 \times 10^5$CFU/ml. This gave 35.1% reduction in infection load of the resistant E. coli (Table 1). At normal dose level of 10 mg/kg of 100% ampicillin trihydrate (B1 and A1) and AMS- Ampicillin trihydrate drug formulation (A4 and B4) the isolation rate were $4.12 \times 10^5 \pm 2.45 \times 10^5$, $2.29 \times 10^5 \pm 0.90 \times 10^5$, $1.35 \times 10^5 \pm 0.45 \times 10^5$ and $4.62 \times 10^5 \pm 2.47 \times 10^5$ respectively showing 50%, 72.25%, 83.70% and 43.91% reduction in isolation rate as against the control with isolation rate of $8.24 \times 10^5 \pm 3.22 \times 10^5$ (Table 2). The E. coli isolation rate (CFU/ml of bile) of subgroups on ordinary feed treated with 100% ampicillin trihydrate and those treated with AMS Ampicillin trihydrate drug formulation at different dose levels were : $4.12 \times 10^5 \pm 2.45 \times 10^5$ and $4.62 \times 10^5 \pm 2.47 \times 10^5$ (P > 0.05) at ampicillin trihydrate dose level of 10 mg/kg, $5.49 \times 10^5 \pm 3.00 \times 10^5$ and $12.42 \times 10^5 \pm 7.73 \times 10^5$ (P < 0.05) at
ampicillin trihydrate dose level of 7.5 mg/kg; $0.70 \times 10^5 \pm 0.27 \times 10^5$ and $9.16 \times 10^5 \pm 4.36 \times 10^5$ ($P < 0.05$) at ampicillin trihydrate dose level of 5 mg/kg (Table 3). The E. coli isolation rate (CFU/ml of bile) of subgroups on vitamins-Selenium fortified feed treated with 100% ampicillin trihydrate and AMS- Ampicillin trihydrate drug formulation were: $2.29 \times 10^5 \pm 0.90 \times 10^5$ and $1.35 \times 10^5 \pm 0.45 \times 10^5$ ($P > 0.05$) at ampicillin trihydrate dose level of 10 mg/kg, $1.12 \times 10^5 \pm 0.32 \times 10^5$ and $0.35 \times 10^5 \pm 0.08 \times 10^5$ ($P < 0.05$) at ampicillin trihydrate dose level of 7.5 mg/kg, $1.98 \times 10^5 \pm 1.29 \times 10^5$ and $1.59 \times 10^5 \pm 1.04 \times 10^5$ ($P > 0.05$) at ampicillin trihydrate dose level of 5 mg/kg (Table 4). Comparing isolation rate of the resistant infection in untreated chicks fed ordinary poultry feed with the isolation rate in similarly infected chicks fed feed with the immune enhancing levels of vitamins A, C, E and Selenium and treated with different doses of ampicillin trihydrate or with the ampicillin trihydrate stabilized in the synthetic AMS, there were mean reduction ($P \leq 0.05$) in isolation rate of the resistant E. coli in bile of: 72.2% to 83.7% at ampicillin trihydrate dosage of 10 mg/kg, 86.4% to 95.8% at ampicillin trihydrate dosage of 7.5 mg/kg and 75.9% to 81% at ampicillin trihydrate dosage of 5 mg/kg (Table 4).
**Table 1: Escherichia. coli CFU/ml of bile of infected but untreated chicks**

<table>
<thead>
<tr>
<th>Birds</th>
<th>Fed ordinary poultry feed</th>
<th>Fed fortified feed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A7</td>
<td>B7</td>
</tr>
<tr>
<td></td>
<td>E. coli CFU/ ml of bile</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>62,000</td>
<td>16,000</td>
</tr>
<tr>
<td>2</td>
<td>28,000</td>
<td>126,000</td>
</tr>
<tr>
<td>3</td>
<td>25,200</td>
<td>1,040,000</td>
</tr>
<tr>
<td>4</td>
<td>736,080</td>
<td>1,300,000</td>
</tr>
<tr>
<td>5</td>
<td>152,400</td>
<td>1,640,000</td>
</tr>
<tr>
<td>Mean</td>
<td>520,400.00</td>
<td>824,400.00</td>
</tr>
<tr>
<td>SE</td>
<td>± 280,920.20</td>
<td>±322,424.81</td>
</tr>
</tbody>
</table>
Table 2: *Escherichia. coli* CFU/ml of bile of infected chick treated at 10 mg/kg with 100% Ampicillin trihydrate.

<table>
<thead>
<tr>
<th></th>
<th>Unfortifiedfeed +Ampicillin(B1)</th>
<th>Fortifiedfeed +Ampicillin(A1)</th>
<th>Fortified feed+AMS +Ampicillin (A4)</th>
<th>Untreated(Control B7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birds</td>
<td>Escherichia coli CFU/ml of Bile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4,000</td>
<td>88,000</td>
<td>126,000</td>
<td>16,000</td>
</tr>
<tr>
<td>2</td>
<td>6,000</td>
<td>178,000</td>
<td>68,000</td>
<td>126,000</td>
</tr>
<tr>
<td>3</td>
<td>884,000</td>
<td>64,000</td>
<td>--</td>
<td>104,000</td>
</tr>
<tr>
<td>4</td>
<td>112,800</td>
<td>564,000</td>
<td>264,000</td>
<td>130,0000</td>
</tr>
<tr>
<td>5</td>
<td>40,000</td>
<td>250,000</td>
<td>80,000</td>
<td>164,0000</td>
</tr>
<tr>
<td>Mean</td>
<td>412,400</td>
<td>228,800.00</td>
<td>134,500.00</td>
<td>824,400.00</td>
</tr>
<tr>
<td>SE</td>
<td>245,471.30</td>
<td>±44,979.77</td>
<td>±322,424.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>±90,103.50</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3: *Escherichia coli* CFU/ml of bile of infected chicks fed ordinary poultry feed (B group)

<table>
<thead>
<tr>
<th>Birds</th>
<th>Ampicillin (B1)</th>
<th>AMS-Ampicillin (B4)</th>
<th>10 mg/kg</th>
<th>Ampicillin (B2)</th>
<th>AMS-Ampicillin (B5)</th>
<th>7.5 mg/kg</th>
<th>Ampicillin (B3)</th>
<th>AMS-Ampicillin (B6)</th>
<th>5 mg/kg</th>
</tr>
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<tbody>
<tr>
<td>1</td>
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<td>48400</td>
</tr>
<tr>
<td>2</td>
<td>6,000</td>
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<td>68000</td>
<td>50000</td>
<td>28000</td>
<td>9800</td>
<td>112000</td>
<td>15800</td>
<td>100000</td>
</tr>
<tr>
<td>3</td>
<td>884,000</td>
<td>912,000</td>
<td>1174000</td>
<td>2280000</td>
<td>158000</td>
<td>1284000</td>
<td>2380000</td>
<td>24000</td>
<td>209600</td>
</tr>
<tr>
<td>4</td>
<td>1128,000</td>
<td>1200000</td>
<td>1284000</td>
<td>3800000</td>
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<td>1128,000</td>
<td>158000</td>
<td>100000</td>
</tr>
<tr>
<td>5</td>
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<td>110000</td>
<td>916000</td>
<td>92000</td>
<td>110000</td>
<td>916000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th><strong>Escherichia coli CFU/ml of bile</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>412,400.00 462,400.00 549,200.00 1,241,600.00 70,400.00 916,000.00</td>
</tr>
<tr>
<td><strong>SE</strong></td>
<td>±245,571.30 ±246,791.73 ±300,003.73 ±772,585.63 ±27,080.68 ±337,513.76</td>
</tr>
</tbody>
</table>
Table 4: *Escherichia coli* CFU/ml of bile of infected chicks fed feed fortified with immune enhancing levels of Vitamins A, C & E and Selenium (Agroup).

<table>
<thead>
<tr>
<th>Birds</th>
<th><em>Escherichia coli</em></th>
<th>10 mg/kg Ampicillin (A1)</th>
<th>AMS-Ampicillin (A4)</th>
<th>7.5 mg/kg Ampicillin (A2)</th>
<th>AMS-Ampicillin (A5)</th>
<th>5 mg/kg Ampicillin (A3)</th>
<th>AMS-Ampicillin (A6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>88000</td>
<td>126000</td>
<td>8000</td>
<td>12000</td>
<td>26000</td>
<td>16000</td>
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<tr>
<td>2</td>
<td></td>
<td>178000</td>
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<td>96000</td>
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<td>--</td>
<td>64000</td>
<td>52000</td>
<td>48000</td>
<td>30000</td>
</tr>
</tbody>
</table>

Means: 228,899.00 ± 90,183.50, 134,500.00 ± 44,939.77, 112,400.00 ± 38,264.34, 34800.00 ± 8,014.99, 198,400.00 ± 129,301.82, 156,800.00 ± 104,392.72
CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion;

The absence of clinical signs of colibacillosis in the *E. coli* inoculated chicks could mean that the *E. coli* isolate used was one of the non pathogenic strains of *E. coli* (Bains, 1979). It may also be that the breed of chicks used is resistant or refractory to *E. coli* infection (Oladele et al (2008). The results obtained from this study are similar to that reported by Oladele et al (2008) which showed that broilers infected with Nigerian isolates of *E. coli* had only mild disease. However, since *E. coli* was recovered from bile of all the chicks confirms that the infection established. Lack of significant difference (P >0.05) in *E. coli* isolation rate of the treated at normal dose (10 mg/kg ampicillin) B1, A1 (4.12 x 10^5 ± 2.45 x 10^5, 2.29 x 10^5 ± 0.90 x 10^5) and untreated B7, A7 (8.24 x 10^5 ± 3.22 x 10^5 and 5.35 x 10^5 ± 2.78 x 10^5) showed that the isolate was resistant to ampicillin. Resistance by amicro-organism to a drug means that normal dose of the drug becomes ineffective in treating an infection arising from the organism. Since every drug has both its desired effects and adverse side effects, increasing doses of drugs to make them have effects on resistant pathogens also increases adverse effects of the drugs which may become more prominent than their desired effects. Thus overall effects of such treatments may become toxic. Apart from direct interference with physiologic functions of the body, toxic effects of some drug, including Ampicillin lead to immune suppression (National Toxicological program USA, 1987). Ampicillin is known to cause neutropaenia (Jawetz, 1995). It thus impairs cell mediated immune responses of treated animals. When a treatment fails to inhibit causative agent of an infectious disease but rather causes immune suppression, the infection load increases, because, the patient’s immunity
which keeps infections in check would have been compromised without a corresponding level of effect on the infection itself. This may explain the reduction in bacteria load of A1 than B1 group (2.29 x 10^5 ± 0.90 x 10^5 than 4.12 x 10^5 ± 2.45 x 10^5) though not significant (P > 0.05). Since toxic effects of ampicillin trihydrate occur at high doses (above 10 mg/kg) (National Toxicological Program USA, 1987) an increase in activity of its lower doses (bellow 10 mg/kg) may make its desired effect (inhibition of disease causing agents) more prominent than its toxic effects. Ampicillin is a time dependent killer of micro-organisms and is poorly bound to plasma. That means that it has short half life (Barza and Weinstein, 1976). Prolonging bioavailability of ampicillin trihydrate has been reported to enhance its antimicrobial activities (Brent et al, 2001). With activity of the ampicillin trihydrate against E. coli improved by formulating it with aluminum magnesium silicate, if immune response of treated birds is also enhanced, synergy between the ampicillin trihydrate and improved immunity may produce enough antimicrobial effects to overcome even resistant infections. Administration of vitamin A, C and E and selenium to birds enhances their immune responses (NRC 1994). That E. coli CFU/ml in the bile of chicks placed on non fortified feed (8.24 x 10^5 ± 3.22 x 10^5) reduced to (5.35 x 10^5 ± 2.78 x 10^5) when the chicks were placed on feed fortified with the recommended levels of Vitamins A, C, E and Selenium suggests that the increased levels of the vitamins and selenium in the feed improved the chicks immune responses which then reduced the infection. Ampicillin-resistant E. coli CFU/ml in bile of inoculated chicks on non fortified poultry feed treated with ampicillin trihydrate at normal dose rate of 10mg/kg (4.12 x 10^5 ± 2.45 x 10^5) was only reduced by 50% from 8.24 x 10^5 ± 3.22 x 10^5 of the group on same unfortified feed that was not treated.. This shows that normal dose of ampicillin trihydrate (10 mg/kg) could not clear the E. coli isolate used to inoculate the chicks. Higher CFU/ml of bile of the amicillin trihydrate resistant E. coli were recorded for the sub-groups of chicks on non fortified feed and treated with the AMS-
Ampicillin trihydrate formulation at all dose levels 10 mg/kg, 7.5 mg/kg, 5 mg/kg, (4.62 x 10^5 ± 2.47 x 10^5, 12.4 x 10^5 ± 7.73 x 10^5 and 9.16 x 10^5 ± 3.38 x 10^5) than in those treated with 10mg/kg ampicillin trihydrate (4.12 x 10^5 ± 2.45 x 10^5). At 7.5 mg/kg (75% of normal dose), the number of resistant E. coli infection in chicks treated without fortifying their feed increased from 5.49 x 10^5 ± 3.00 x10^5 in the group treated with 10 mg/kg of ampicillin trihydrate alone to 12.4 x 10^5 ± 7.73 x 10^5 (P = 0.006.) when compared to the group treated with the AMS-Ampicillin trihydrate drug formulation. This suggests that treating the inoculated chicks with AMS -ampicillin trihydrate formulation without protecting their immune systems with immune stimulants aggravated the resistant infection. This may be because of immune suppressive action of the prolonged bioavailability of ampicillin trihydrate. So, chicks infected with drug resistant E. coli should be treated with immune stimulants when placed on AMS-ampicillin drug formulation. The group of chicks fed, feed fortified with Vitamins A, C and E and Selenium and treated at normal dose of ampicillin trihydrate (10 mg/kg) with 100% ampicillin trihydrate had isolation rate of 2.29 x ± 0.90 x 10^5 CFU/ml which was lower than both 5.35 x 10^5 ± 2.78 x 10^5 CFU/ml of the untreated group fed the fortified feed and 4.12 x 10^5 ± 2.45 x 10^5 CFU/ml of the group non fortified feed treated with the same 10 mg/kg (ampicillin). This suggests that the lowered isolation rate (2.29 x 10^5 ± 0.90 x 10^5 CFU/ml) which was 72.2% clearance resulted from synergy between enhanced immune response of the chicks due to the high levels of the vitamins and Selenium in their feed and effect of the drug, at the normal dose level. Reducing dose of ampicillin to 7.5 mg/kg in chicks fed feed fortified with the immune enhancing levels of vitamins and Selenium, gave the highest bacterial clearance in chicks treated with 100 % ampicillin (1.12 x 10^5 ± 0.38 x 10^5 CFU/m) than in those treated with the Ampicillin-AMS drug formulation (0.35 x 10^5 ± 0.08 x 10^5 CFU/ml). This suggests that maximizing the desired effect of ampicillin also occurred at 7.5 mg/kg. So, by reducing dose of ampicillin
trihydrate its side effects may have been minimized so that there was improved immune response in the chicks which then synergized prolongation of bioavailability of the ampicillin trihydrate (Ezeibe et al 2012). When the dose of ampicillin trihydrate was further reduced to 5 mg/kg, the isolation rate (1.98 x 10^5 ± 1.29 x 10^5 CFU/ml) was only reduced by 75.9% which is less than the isolation rate at 7.5 mg/kg. This suggests that maximum benefit of the dose reduction occurs at 7.5 mg/kg. The further reduction of dose may have reduced only antibacterial activity of the drug and had no additional contribution to immune response. So, 7.5mg/kg (75% of normal dose) may be the best dose to use in the regimen of synergizing prolonged bioavailability of Ampicillin trihydrate and immune stimulants (vitamin A, C and E and selenium) on treated birds. Feed as route of administration of the vitamins was chosen to avoid direct contact between heavy metals in the AMS and vitamins which could lead to chelating effects. Kaplan (2002) reported that for antimicrobial treatments to achieve cure of infections, and not just cure of clinical signs, they should clear at least 95% of the infections. So, even the 87.3% clearance of the infection achieved by reducing dose of ampicillin to 7.5 mg/kg in combination with the fortification of feed with immune stimulants was still short of effective antimicrobial cure. When the 7.5 mg/kg ampicillin dose was used to treat chicks on feed fortified with immune enhancing vitamins and selenium with an AMS-Ampicillin drug formulation, 95.8% of the resistant infection was cleared. Stabilizing agents protect drugs from rapid degradation by metabolic processes and Aluminum magnesium silicate is used to stabilize drugs (Vanderbilt 1992). It has been used to stabilize ampicillin against an ampicillin-sensitive infection and that enhanced clearance of the Salmonella gallinarum infection in chicks, from 69.2% to 97.8% (Ezeibe et al 2012). So, the improved clearance of the resistant infection by as much as 95.8% in the group treated at dose of 7.5 mg/kg with ampicillin trihydrate stabilized in the synthetic AMS in this experiment could be attributed to ability of the AMS to prolong bioavailability of ampicillin in treated chicks. By stabilizing
ampicillin trihydrate, AMS may have made it have longer half life. It thus acted for longer time on the resistant bacteria. So, ampicillin (a time dependent killer) had more time to act on the resistant bacteria. The result was 95.8% clearance of the resistant infection. The fact that when antimicrobial drugs are formulated in Aluminum-magnesium silicate, best effects are got with 75% of their normal doses has been consistent. This treatment strategy (using AMS to stabilize drugs) improved anticoccidial activity of suphadimidine against avian coccidia (Ezeibe et al, 2011), antibiotic efficacy of ampicillin trihydrate against susceptible Salmonella gallinarum infection (Ezeibe et al, 2012a), antiplasmodial effects of Chloroquine phosphate against Plasmodium berghei (Ezeibe et al, 2012b) and anthelminthic potency of Piperazine citrate against Helignosomoides bakeri (Ezeibe et al, 2012c).

5.2 Contribution to Knowledge; The effective clearance (95.8%) of the resistance infection suggests that an effective treatment regimen for resistant E. coli infection with Ampicillin trihydrate may have been developed for chicks.

5.3 Conclusion; It can be concluded that the best ampicillin dose level in combination with aluminum magnesium silicate for the clearance of resistance E. coli was found to be 75% of the normal dose. This treatment must go on simultaneously with increased level of vitamins A, C, E and selenium in the feed to protect the birds from immune suppression arising from prolong bioavailability of ampicillin trihydrate in the blood. It can be inferred from the deduction of the study that aluminum magnesium silicate prolongs bioavailability of ampicillin in vivo thereby increasing its action on resistant E. coli.

5.4 Recommendation; To treat ampicillin trihydrate resistant. coli infections in birds its recommended that ampicillin trihydrate be formulated in aluminum magnesium silicate. The formulation should be given at dose level of 7.5mg/kg and the birds placed on feed fortified with additional level of vitamin A, C and E and selenium.
REFERENCES


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Appendix 1

Collection of bile
Figure 1; No evidence of pathological lesions observed in the organs in all the groups (kidney, liver, heart and lungs) at post mortem examination.