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Synergy in antibacterial activities of Ampicillin trihydrate, stabilized with a synthetic aluminum-magnesium silicate and immune-stimulants, on resistant *Escherichia coli* infection

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ABSTRACT

To treat resistant *Escherichia coli* infection in chicks, Ampicillin was stabilized with a synthetic aluminum-magnesium silicate (AMS) to prolong its bioavailability. Its dose was also reduced to minimize adverse side effects. Vitamins A, C, E and Selenium levels in the chicks' feeds were increased to enhance immune response of the chicks. *E. coli* coloning forming units, per ml of bile of the chicks, treated with Ampicillin and with Ampicillin in AMS, were: 228800.00 ± 90103.50 and 134500.00 ± 44937.97 at 10 mg/kg, 104400.00 ± 36024.44 and 34800.00 ± 8014.97 at 7.5 mg/kg, 198400.00 ± 129301.80 and 156800.00 ± 109392.70 at 5 mg/kg. Mean bacterial loads of the untreated groups, fed normal feed and those fed the fortified feed were 824400.00 ± 322424.80 and 534800.00 ± 277832.80. At 7.5 mg/kg. Ampicillin in the AMS, effectively (P < 0.05) treated resistant *E. coli* infection in chicks, fed immune-stimulants, with the infection rate reduced by 95.8%.

Keywords: Prolonging Bioavailability of Drugs; Minimizing Side Effects of Drugs; Enhancing Immune Responses of Patients

1. INTRODUCTION

Development of resistance against drugs by bacteria and other diseases causing micro-organisms is a major concern in veterinary medicine and in human-beings [1]. *Escherichia coli* is one of the bacteria often involved in drug resistance [2] and human-beings are frequently infected by *E. coli* infections from animals [3,4]. Under adverse conditions, some of such *E. coli* infections become pathogenic [4,5]. Use of antimicrobials, to treat animals and as growth promoters, in their feeds, leads to development of resistance by pathogens and when the resistant infections find their way into the human food chain, they become a public health concern [6]. It has been reported that over 80% cases of cystitis in human-beings were caused by *E. coli* infections contracted from animals [2].

E. coli infections are also major problems of poultry. They lead to septicemia, peritonitis and salpingitis in breeders. In broilers, they cause airsacculitis, septicemia and cellulitis [7,8]. The poultry infections are also of economic importance, because they lead to condemnation of chicken at meat inspection, in addition to the mortality and decreased performance caused in affected flocks [9].

High doses of Ampicillin cause immune suppression in treated animals, but its antibacterial activity is enhanced when its bioavailability is prolonged [10]. Bioavailability of drugs is reported to be influenced by stabilizing agents used in their formulations [11]. Aluminum-magnesium silicate (AMS) is used to stabilize drugs [12,13]. Also, molecules of AMS are reported to be composed of platelets that are only 0.96 nm thick [13].

So, it is made of *Nanoparticles* which have been reported to have many medicinal potentials [14]. Toxicity tests have proved it to be safe, both for animals and for human-beings [15,16].

To stabilize means to protect against destruction, stabilizing Ampicillin trihydrate in AMS may protect the antibiotic from being rapidly degraded by metabolic processes, thus prolonging its bioavailability. To overcome the problem of impurities in the natural AMS [13], Aluminum silicate and Magnesium silicate were reacted to get a synthetic AMS, devoid of impurities [17].

The Synthetic AMS has been used for *in vivo* experiments with chicks, dogs, mice and rats [18-25] without adverse side effects on treated animals. It may therefore be possible to minimize immune suppression due to Ampicillin [1] by reducing its dose and use ***The Synthetic AMS*** to improve antibacterial activity of the lower doses. This strategy may lead to effective treatment of resistant infections.

To further protect immune systems of treated animals against suppressive effects of drugs and to enhance their responses against infections, vitamins A, C, E and selenium in feeding chicks used for the experiment were raised to the levels recommended by ***The National Research Council*** [26] for optimal immune response in poultry.

2. MATERIALS AND METHODS

Forty-five, 6 weeks old chicks were infected (Per os) with cultures of *E. coli* isolate that was resistant to Ampicillin (Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria). Feed for the chicks were fortified with additional levels of vitamins A, C, E and Selenium. To each 25 kg feed, 375 mg, 10 mg, 75 mg and 12.5 mg of vitamins A, C, E and Selenium respectively, were added [26]. Two days to the infection, 35 of the chicks were placed on the feed fortified with additional levels of the vitamins and mineral. The remaining 10 chicks were left on the feed, without additional levels of the vitamins and mineral. The infected chicks were treated by administering Ampicillin in their drinking water for 7 days, starting 6 days post infection. Five of the chicks on normal feed were treated with Ampicillin at 10 mg/kg while 5 served as untreated control. Six groups, of the chicks on the fortified feed were treated with Ampicillin at the dose rates of: 10 mg/kg (Ampicillin), 10 mg/kg (Ampicillin in AMS), 7.5 mg/kg (Ampicillin), 7.5 mg/kg (Ampicillin in AMS), 5 mg/kg (Ampicillin) and 5 mg/kg (Ampicillin in AMS), respectively. The remaining five of the chicks on the fortified feed served as untreated controls (**Table 1**).

Bile from each of the chicks was harvested for assessment of *E. coli* load in it. To 0.1 ml of bile from each chick 0.9 ml of normal saline was added to get a 1:10 dilution. Then 0.1 ml of the 1:10 bile dilution was transferred to a second 0.9 ml, normal saline to make the bile dilution 1:100. Finally, 0.05 ml of each diluted bile was plated on Mcconkey agar and incubated at 37˚C for 24 hours.

Escherichia coli colonies in the cultures were identified by their morphology. Number of *E. coli* colonies (X) that grew from bile of each chick were counted under the microscope and *E. coli* colonies per ml of bile of each chick was calculated as the infection rate, by the formula: Colony forming units per ml of bile (Infection rate) = X/5 × 10,000 CFU/ml. Means of *E. coli* infection rates of the groups were compared for statistical differences by Analysis of variance.

3. RESULTS

Chicks on ordinary feed had infection rate of 824400.00 ± 322424.80 CFU/ml, while those fed the feed fortified with higher levels of vitamins A, C, E and Selenium had mean resistant *E. coli* infection rate of 534800.00 ± 277832.80 CFU/ml. This gave 35.1% reduction in infection rate of the resistant *E. coli*. Mean infection rate in the group of chicks fed normal feed and treated with normal dose of Ampicillin (10 mg/kg) was 412400.00 ± 245471.30 CFU/ml which gave 50% reduction in infection rate.

Combination of increased levels of vitamins A, C, E and selenium in feed and different doses of Ampicillin and the Ampicillin stabilized in ***The Synthetic AMS***, led to significant ($P < 0.05$) reduction in mean loads of the resistant *E. coli* in bile of infected chicks, from 824400.00 ± 322424.80 CFU/ml to: 228800.00 ± 90103.50 CFU/ml and 134500.00 ± 44939.22 CFU/ml at 10 mg/kg, 104400.00 ± 36024.44 CFU/ml and 34800.00 ± 8014.99 CFU/ml at 7.5 mg/kg and 198400.00 ± 129301.80 CFU/ml and 156800.00 ± 104382.70 CFU/ml at 5 mg/kg (**Figure 1**). The six treatment regimens thus gave enhanced infection reduction rates of: 72.2%, 83.7%, 87.3%, 95.8%, 75.9% and 81% respectively.

Table 1. Groups of Ampicillin resistant *Escherichia coli* infected chicks, fed high levels of vitamins A, C, E and Selenium and treated with Ampicillin trihydrate stabilized with ***The synthetic Aluminum-Magnesium silicate***.

FEED FORTIFIED WITH VITAMINS A, C, E AND SELENIUM							UNFORTIFIED FEED	
10 mg/kg (Amp)	10 mg/kg (Amp In AMS)	7.5 mg/kg (Amp)	7.5 mg/kg (Amp in AMS)	5 mg/kg (Amp)	5 mg/kg (Amp in AMS)	Untreated	Untreated	10 mg/kg (Amp)

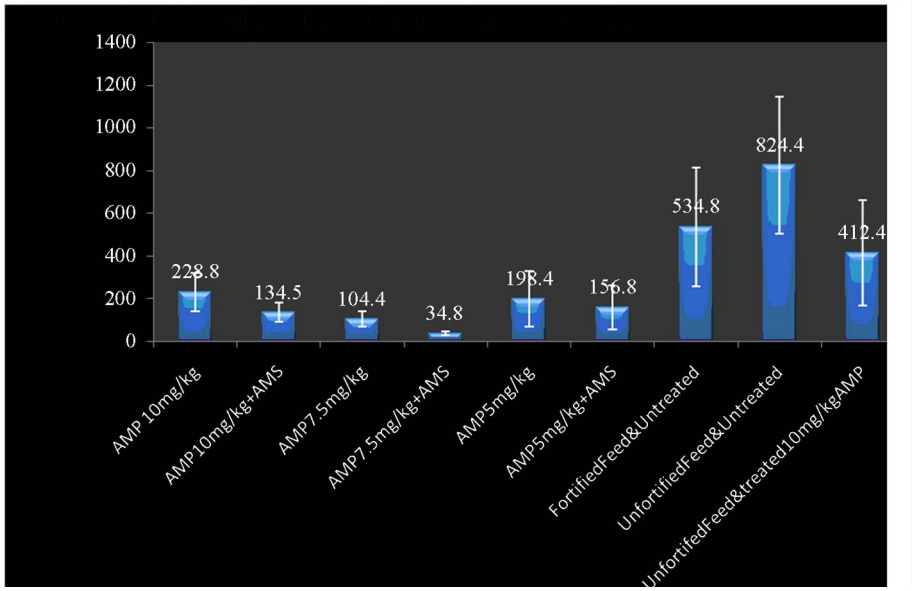

Figure 1. Loads (CFU/ml) of resistant *Escherichia coli* in bile of infected chicks fed immune-stimulants and treated with Ampicillin trihydrate stabilized with ***The synthetic Aluminum-Magnesium silicate***.

4. DISCUSSION

Resistance by a microorganism to a drug means that normal dose of the drug becomes ineffective in treating the infection. Because every drug has both the desired effects and adverse side effects, attempts to increase doses of drugs to make them have effects on resistant pathogens and make the adverse effects of the drugs become more prominent than their desired effects. So, the overall effects of the treatment become toxic.

Apart from direct interference with physiologic functions of the body, toxic effects of some drugs, including Ampicillin, lead to immune suppression [1]. When a treatment fails to inhibit causative agent of an infectious disease but rather causes immune suppression, the infection rate increases, because, the patient's immunity which was keeping it in check would have been compromised, without a corresponding level of effect on the pathogen.

Since toxic effects of Ampicillin occur only at high doses [1], an increase in activity of its lower doses would make its desired effect (inhibition of disease causing agents) more prominent than its toxic effects. Prolonging bioavailability of Ampicillin has been reported to enhance its antimicrobial activities [27]. With activity of the drug against pathogens improved, if immune response of treated animals is also enhanced, synergy between the drug and immunity may produce enough antimicrobial effects, to overcome even resistant infections.

Administration of immune stimulants to patients enhances their immune responses. The ***National Research Council*** [26] reported that when levels of vitamins A, C, E and Selenium are at least, 375 mg, 10 mg, 75 mg and 12.5 mg respectively in each 25 kg of poultry feed, immune response of the animals becomes optimal.

The group of chicks fed feeds, fortified with extra levels of vitamins A, C, E and Selenium, had only a slight reduction in load of the bacterium, which was not significant. Chicks on the fortified feed treated with normal dose of Ampicillin (10 mg/kg) had an infection rate of 228800.00 ± 90103.50 CFU/ml which was lower than both 534800.00 ± 277832.80 CFU/ml of the untreated group fed the fortified feed and 412400.00 ± 245471.30 CFU/ml of the group on ordinary feed treated with same 10 mg/kg (Ampicillin). This suggests that the lowered infection rate (228800.00 ± 90103.50 CFU/ml) resulted from synergy between enhanced immune response of the chicks due to the high levels of the vitamins and selenium in their feeds and effect of the drug, at the normal dose rate.

Reducing dose of Ampicillin to 7.5 mg/kg, in combination with the fortified feed, improved the bacterial clearance to 104400.00 ± 36024.44 CFU/ml giving a better reduction in infection rate, of 87.3% from the 72.2% got with 10 mg/kg. This suggests that reducing dose of Ampicillin may have minimized side effect of the drug [23] and so immune response of the chicks was further enhanced. When the dose was further reduced to 5 mg/kg, the infection rate (198400.00 ± 129301.80 CFU/ml) was only 75.9% reduction which is less effective than that got at 7.5 mg/kg. This may mean that maximum benefit of the dose reduction occurs at 7.5 mg/kg. The further reduction of dose reduced only antibacterial activity of the drug and had no additional contribution to

immune response. So, 7.5 mg/kg (75% of normal dose) may be the best dose to use in the regimen.

Kaplan [28] reported that for antimicrobial treatments to achieve complete cure of infections, they should clear at least 95% of the infections. So, even the 87.3% clearance of the infection got by reducing dose of Ampicillin to 7.5 mg/kg, in combination with the fortified feed, was still short of effective antimicrobial cure.

Stabilizing agents protect drugs from rapid degradation by metabolic processes and aluminum-magnesium silicate is used to stabilize drugs [13]. It has been used to stabilize Ampicillin and that enhanced the antibiotic's ability to clear *Salmonella gallinarum* infection in chicks, from 69.2% to 97.8% [23]. So, the improved clearance of *E. coli* infection rate by as much as 95.8% got in the group treated at dose of 7.5 mg/kg with Ampicillin stabilized in ***The Synthetic AMS*** in this experiment could be attributed to ability of the AMS to prolong bioavailability of Ampicillin in the treated chicks.

The Synthetic AMS improved effect of Ampicillin at all dose levels tested in this experiment, but only its effect at 7.5 mg/kg, in combination with feeds with immune-stimulating levels of vitamins A, C, E, and Selenium produced antimicrobial cure (95% infection clearance). That when antimicrobial drugs are stabilized with aluminum-magnesium silicate, best effects are got with 75% of their normal doses, has been consistent [22-25].

Results of present experiment suggest that in treating resistant infections, 75% of the normal dose of Ampicillin achieves better results than use of other doses. To enhance therapeutic effects of the lower dose so that it gives enough microbial clearance to avoid further development of resistance, the drug should be formulated with stabilizing agents, such as aluminum-magnesium silicate. This use of an AMS-stabilized lower dose to treat infected animals minimizes adverse side effects of the drug. Enhancing immune response of treated animals or human-beings with immune-stimulants improves the effects of such treatments further. So, even resistant infections could be cured.

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