Overcoming Resistant Infections (Salmonella Tiphymurim) by formulating Antimicrobials (Streptomycin) with Medicinal Synthetic Aluminum Magnesium Silicate {Al4 (SiO4)3 + 3Mg2SiO4 → 2Al2 Mg3 (SiO4)3}

M. C. O. Ezeibe, C. C. Ogbu, I. J. Ogbonna, F. I. Ezeibe, C. Alex-Okoroafor, O. Agbakwuru, C. A, Akpan, M. E. Sanda, E. Kalu, N. U. Njoku & M. I. Udobi

Michael Okpara University of Agriculture Umudike

ABSTRACT

Enhancing patients` immune responses and antimicrobials` efficacies could overcome Antimicrobial Resistant Infections (AMR). Aluminum-magnesium silicate (AMS) is an approved Nano-stabilizing agent. Stabilizing antimicrobials prolongs time they stay at high-bioavailability and Nano-particles enhance antimicrobials` delivery to effect-targets. Prolonging high-bioavailability time and enhancing delivery to targets improve efficacies of antimicrobials while antioxidants improve immunity of patients. With improved efficacy, antimicrobials` lower dosages achieve desired effects thus avoiding side effects (immune- suppression) from high dosages. Based on these hypotheses, Medicinal synthetic AMS (MSAMS)- Streptomycin (antimicrobial-model) formulation and Vitamin C were used to treat Streptomycin- resistant Salmonella typhimurium infections (AMR-model) in chicks (patient-models).

Keywords: antimicrobial resistant infections; 75-% dosages of MSAMS-stabilized medicines; antioxidant-supportive treatments.

Classification: NLM Code: QV 225

Language: English

Great Britain Journals Press

LJP Copyright ID: 392835

London Journal of Medical and Health Research

Volume 23 | Issue 9 | Compilation 1.0

Overcoming Resistant Infections (*Salmonella Typhimurium*) by Formulating Antimicrobials (Streptomycin) with Medicinal Synthetic Aluminum Magnesium Silicate $\{Al_4(SiO_4)_3 + 3Mg_2SiO_4 \rightarrow 2Al_2Mg_3(SiO_4)_3\}$.

M. C. O. Ezeibe[α], C. C. Ogbu[σ], I. J. Ogbonna[ρ], F. I. Ezeibe[ↀ], C, Alex-Okoroafor[¥], O. Agbakwuru[§], C. A, Akpan[χ], M. E. Sanda[ν], E. Kalu[Θ], N. U. Njoku[ζ] & M. I. Udobi[£]

ABSTRACT

Enhancing patients` immune responses and antimicrobials` efficacies could overcome Antimicrobial Resistant Infections (AMR). Aluminum-magnesium silicate (AMS) is an approved Nano-stabilizing agent. Stabilizing antimicrobials prolongs time they stay at high-bioavailability and Nano-particles enhance antimicrobials` delivery to effect-targets. Prolonging high-bioavailability time and enhancing delivery to targets improve efficacies of antimicrobials while antioxidants improve immunity of patients. With improved efficacy, antimicrobials` lower dosages achieve desired effects thus avoiding side effects (immune-suppression) from high dosages. Based on these hypotheses, Medicinal synthetic AMS (MSAMS)-Streptomycin (antimicrobial-model) formulation and Vitamin C were used to treat Streptomycin-resistant Salmonella typhimurium infections (AMR-model) in chicks (patient-models). Streptomycin`s recommended dosage (25 mg/kg) worsened the infection, from -746.86%-reduction to -782.29 %-reduction. Stabilizing Streptomycin with MSAMS at that high dosage worsened the infection further, to -855.43 %-reduction but at 75 % dosage (18.75 mg/kg), stabilizing Streptomycin with MSAMS and supporting the treatment with Vitamin C (through feed) terminated the AMR (100 %-reduction).

Keywords: antimicrobial resistant infections; 75-% dosages of MSAMS-stabilized medicines; antioxidant-supportive treatments.

Author α σ ρ ↀ ¥ § χ ν Θ ζ £: College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Nigeria.

I. INTRODUCTION

Antimicrobial resistance (AMR) is a Global health challenge. Use of antimicrobials as growth promoters in animal farming is a major cause of AMR in humans (Agyare *et al.,* 2018). In poultry, treatment failures due to AMR result in great economic losses. AMR in poultry is also a big risk to human health (Van *et al.,* 2012; Okorie-kanu *et al.,* 2016).

Antimicrobial resistance means loss of sensitivity by microorganisms to drugs which were effective (Sinyangwe *et al.,* 2004). Mechanisms for Antimicrobial resistance include loss of surface specific receptors or transporters for drugs, rapid metabolism of drugs and alteration of specific targets for drugs. There are many reports of increases in resistance involving bacteria of Veterinary and public health importance, including *Salmonella typhimurium,* against commonly used drugs which include Streptomycin (Bortolaia *et al.,* 2016, Nguyen *et al.,* 2017).

Apart from wrong use of antimicrobials in animal farming which causes development of resistance by infections in animals before they are transmitted to human beings, use of high dosages in treating animals leads to high concentrations of residues of the drugs in human foods of animal origin which leads to development of

antimicrobial resistance in humans (Reig and Toldra, 2008, Goetting *et al.*, 2011; Okorie-kanu *et al.,* 2016; Agyare *et al.,* 2018). Despite these public health concerns, antimicrobials are essential in poultry-production. So, effort should be to find treatment-strategies that would prevent and cure AMR.

Salmonella species are the most important zoonotic bacterial food-borne pathogens (Addis *et al.,* 2015) being the most frequently isolated bacteria in food-borne disease-outbreaks (Balakrishnan *et al.,* 2018), accounting for around 93.8 million food-borne illnesses and 155,000 deaths per year, worldwide (Heredia and Garcia, 2018). *Salmonella species* have also been associated, with increasing concern for emergence and spread of antimicrobial-resistance (Ejo *et al.,* 2015).

Antibiotic-resistant *Salmonella* infections of both humans and animals are universal concerns, particularly in developing countries (Ejo *et al.,* 2015). Apart from morbidity and mortality which they cause in humans and animals, restrictions to trade and discarding contaminated food due to them are important socioeconomic problems (Tadesse *and* Tessema, 2014*).* Of zoonotic *Salmonella species, S. typhimurium and S. enteritidis are more* common (Dhama *et al.,* 2013; Tadesse and Tessema, 2014; Tegegne, 2019)

Molecules of Aluminum magnesium silicate (AMS), a WHO approved medicine/stabilizing agent, consist of *Nanoparticles* with negative electrical charges on their surfaces and positive charges on their edges (Cristina *et al.*, 2007, Vanderbilt, 2012). Presence of the two charges on AMS *Nanoparticles* makes them to hydrate to form three dimensional colloidal structures in solutions. The colloidal structures stabilize other drugs that are in formulation with AMS (Vanderbilt, 2012). For this effect, AMS is an approved pharmaceutical stabilizing agent.

Meanings for stabilize include protecting a substance from being destroyed. Drug metabolism destroys drugs and renders them no longer effective. So, stabilizing drugs reduces rate at which the body metabolizes (destroys) them so that they remain at high concentrations for extended periods. When drugs remain at high concentrations in blood for longer periods, their effectiveness improves. Also, AMS consists of *Nanoparticles* and *Nanoparticles* enhance delivery of drugs to their effect-targets which also improves efficacy.

Antioxidants (Vitamins A, C and E) reduce oxidative stress and so, protect the immune system to enhance its response to challenges from bacteria, viruses and parasites. The Vitamins are required in small dosages for the immune system to function optimally. Vitamin C supplementation, in particular, has been reported to reduce duration and severity of COVID-19.

It was therefore postulated that treatment strategy of enhancing immunity of patients with antioxidants, enhancing efficacy of antimicrobials by stabilizing them with AMS and reducing side effects of the Antimicrobials by using their lower doses for treatments could terminate treated infections so that none remains to become AMR. Infections that have already become resistant could become curable by same antimicrobials they are resisting.

Nigeria does not have natural deposits of AMS $\{Al_2Mg_3\ (SiO_4)_3\}$ still there are two other solid minerals, Aluminum silicate $\{Al_4\ (SiO_4)_3\}$ and Magnesium silicate $\{Mg_2SiO_4\}$ which are also approved medicines (Galindo and Cereso 2006), found in the country and in many other countries. The two medicines were used to formulate a form of AMS, named, Medicinal synthetic Aluminum magnesium silicate {MSAMS: $Al_4\ (SiO_4)_3 + 3Mg_2SiO_4 \rightarrow 2Al_2Mg_3\ (SiO_4)_3$: Ezeibe, 2012}. To overcome the challenge of AMS, Aluminum silicate and Magnesium silicate being un-absorbable, Dextrose monohydrate was incorporated in MSAMS so that the simple sugar conveys the electrically charged *Nanoparticles* across mucous membranes into blood by the principle of active transport (Murray, 2000) so that antimicrobials stabilized with MSAMS would have systemic effects. A formulation of 10 % MSAMS and 5 % Streptomycin, supported with Vitamin C was used to treat Streptomycin-resistant *Salmonella tiphymurim* infected chicks.

II. MATERIALS AND METHODS

Eighty (80) broiler-chicks aged 3 weeks (age beyond which *S. typhimurium* may, no longer cause much mortality) which were infected with Streptomycin-resistant *Salmonella typhimurium,* were used for trial of the new drug-formulation and the suggested treatment-strategy. The treatment- groups (20 chicks each) *were:*

Treated with Streptomycin at its 100 %-dosage,
Treated with Streptomycin-MSAMS at Streptomycin`s 100 %-dosage,
Treated with Streptomycin-MSAMS at Streptomycin`s 75 %- dosage + Vitamin C,
Untreated (control).

The treatment lasted 5 days. Before the treatment and on day-3 of the treatment, day-1 post treatment (PT) & day-4 PT, any first two (2) chicks caught on entering pen of each treatment-group were randomly selected, for bile which was used to determine *Salmonella tiphymurim* colony-forming units (CFU). Of bile from each chick, 0.1 ml was added to 0.9 ml of normal saline to get a 1:10 dilution. Again, 0.1 ml of the 1:10 bile dilution was added to 0.9 ml of normal saline to make a 1:100 bile dilution. Finally, 0.05 ml of the 1: 100 bile dilution was plated on MacConkey agar and on SSA agar before incubating at 37˚C for 24 hours. *Salmonella* colonies (X) were counted and expressed as colony-forming units per ml (CFU), using the formula: CFU = x/5 × 10,000. Infection-reduction rate for each group on each day of assessment was calculated as percentage of the mean-CFU before treatment, by which infection of that group reduced (Mean-CFU before treatment – CFU of the group on the day of assessment divided by Mean-CFU before treatment, multiplied by 100). Negative results indicate increase in load of the infection while positive results show reduction in the infection-load. The infection-reduction rates were compared by plotting them as a graph.

III. RESULTS

Mean colony forming units (CFU) in bile, of the Streptomycin-resistant *Salmonella tiphymurim*-infected chicks before treatment was 8.75. For, the untreated group of chicks the infection-reduction rates were: -482.86 % (increase), -682.86 % (increase) and -716.86 % (increase) for day-3 on the treatment, day-1 PT and day-4 PT. For the group treated at recommended dosage of Streptomycin (25 mg/kg), the CFU-reduction rates for the three days were – 1.83 %(increase), -12.57 % (increase) and -782.29 % (increase) respectively but for the group treated at same recommended dosage of Streptomycin with Streptomycin-MSAMS formulation, the CFU-reduction rates were 14.29 %, 26.40 % and -855.42 % (increase) respectively while for the group treated at 75% of recommended dosage of Streptomycin (18.75mg/kg) with the Streptomycin-MSAMS formulation plus Vitamin C, the reduction rates were 100 %, 100% and 100% respectively (infection-termination). *S. typhimurium* CFUs per/ml of bile of the treated chicks are as presented on Table 1 while their infection reduction rates are as presented on Table 2 while their comparison is as on Figure 1.

Table 1: Colony-Forming Units ($\times 10^6$) of Streptomycin-Resistant *Salmonella tiphymurim* in Bile of Chicks Treated With Streptomycin-Medicinal Synthetic Aluminum Magnesium Silicate Formulation and Vitamin C

Days of treatment	Untreated		25mg/kg Strept.		25mg/kg Strept-MSAMS		18.75mg/kg Strept-MSAMS + Vit. C	
3 days on treatment	51.00		8.91		7.50		0.00	
1 day post treatment	68.50		9.85		6.44		0.00	
4 days post reatment	74.10		77.20		83.60		0.00	

Table 2: Reduction-Rates (%) of Streptomycin-Resistant *Salmonella tiphymurim* Infections, in Chicks Treated With Streptomycin-Medicinal Synthetic Aluminum Magnesium Silicate Formulation and Vitamin C

Days of treatment:	Untreated		25mg/kg Strept.		25mg/kg Strept-MSAMS		18.75 mg/kg Strept-MSAMS + Vit. C	
3 days on treatment		-482.86		-1.83		14.29		100
1 day post treatment		-682.86		-12.57		26.40		100
4 days post treatment		-716.86		-782.29		-855.42		100

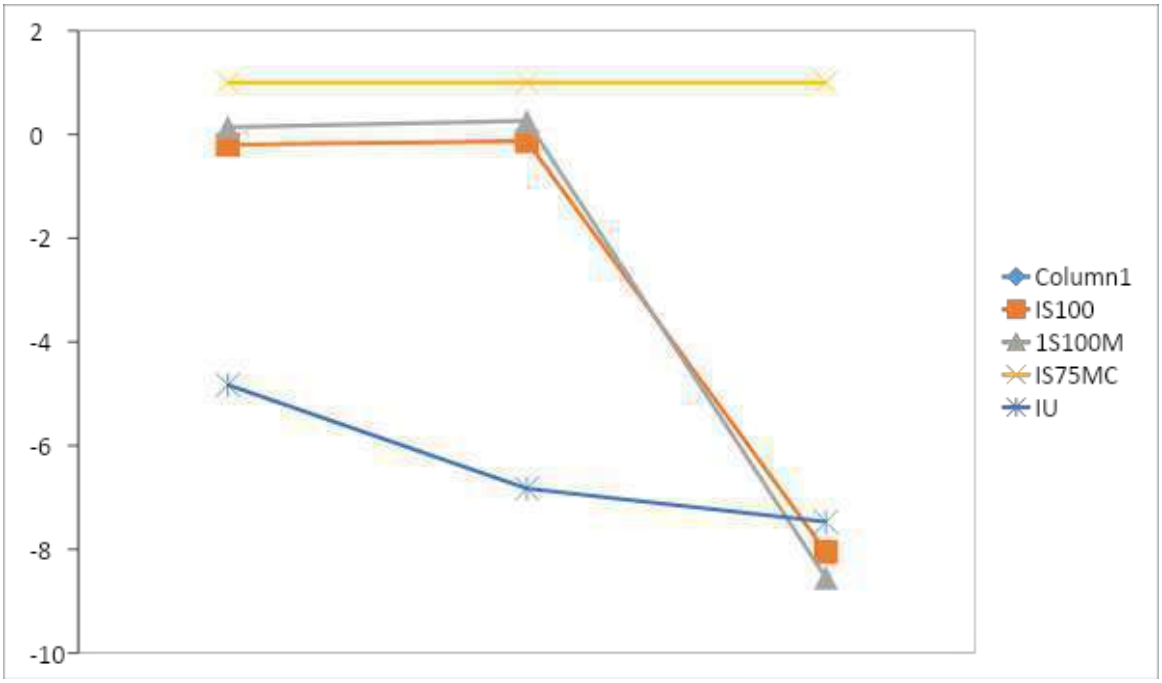

Fig. 1: Comparison of Reduction-Rates (%) of Streptomycin-Resistant *Salmonella tiphymurim* Infections, in Chicks Treated With Streptomycin-Medicinal Synthetic Aluminum Magnesium Silicate Formulation and Vitamin C

IV. DISCUSSION

Discovery of drugs that inhibit pathogenic microorganisms (antimicrobials) was a major revolution in medicine. So, development of resistance against the drugs is also a major setback to Global health. Current increase in drug-resistant pathogenic microorganisms is a big challenge to medical researchers, world over. Efforts to keep developing new drugs to replace those that disease-agents overcome is faced with many difficulties, including cost and time it takes to design new drugs. One strategy being tried to overcome AMR is development of adjuvants that could enhance efficacy of antimicrobials (Erin *et al.*, 2015).

As a stabilizing agent, AMS protects drugs against rapid degradation by metabolic processes thus prolonging time of their high bioavailability. Prolonging time of high bioavailability enhances efficacy and so, dosages of the drugs needed to achieve desired effects are reduced. When lower dosages are used for treatments, immune responses of patients enhance. Synergy between enhanced efficacy and enhanced immunity could clear ≥ 95 % of treated infections so that AMR is prevented (Brent *et al,* 2001). Even already resistant infections could be cured. Also, since AMS-units are *Nanoparticles,* they enhance delivery of drugs to targets and across

physiological barriers, including blood-brain barrier.

Drugs have both desired effects and side effects. Most antimicrobial drugs cause immune-suppression when used at high dosages. Formulating antimicrobials with the MSAMS makes it possible for their lower dosages to achieve desired effects so that their side effects which occur with high dosages could be reduced. Reducing side effects of antimicrobials improves immune responses of patients. Improving immune responses of patients help to clear infections.

Trials of the MSAMS on efficacy of different antimicrobials, so far, have shown that it improves efficacy of the antimicrobials to help them clear enough infections so that AMR is prevented. Formulating other drugs and MSAMS has led to prevention of AMR against Ampicillin trihydrate by bacteria (Ezeibe, *et al* 2012 a). It also prevented AMR by *Heligosomodes bakeri,* (helminths) against Piperazine citrate (Ezeibe *et al*, 2012 b). Formulating MSAMS with Chloroquine sulphate also led to enough clearance of Plasmodium *berghei* such that development of AMR by the malaria parasite may no longer occur (Ezeibe 2020). That strategy of formulating MSAMS with antimicrobials and supporting their treatment with antioxidants has also restored efficacy to Ampicillin trihydrate against resistant *E. coli* (Ezeibe et al 2013) and to Cotrimaxazole against resistant *Salmonella pulorum*. (Ezeibe *et al* 2019)

In current trial of MSAMS-Streptomycin formulation supported with Vitamin C against resistant *Salmonella typhimurium* infections, all that Streptomycin alone at its recommended dosage (25 mg/kg) could achieve was to slow rate at which the infection was increasing on day-3 of the treatment and on day-1 post treatment but by day-4 post treatment, the infection increased even more than that of the untreated group. That reduced effect of Streptomycin at the recommended dosage is a confirmation that the *S. typhimurium* infection was Streptomycin-resistant. The antimicrobial had lost efficacy against the infection and so could no longer cure its disease. Sudden rise in infection-rate in that group, 4 days after treatment, suggests that while Streptomycin had only negligible effect on the resistant infection, its side effects depressed immunity of the chicks which was the only defense left for the patients. So, when the treatment was withdrawn, there was nothing to hinder the infection.

In the group of chicks treated at same recommended dosage of the antimicrobial, stabilized with MSAMS, the treatment reduced (not just slowing multiplication of the infection) the resistant infection on both day-3 on the treatment and on day-1 PT but again following withdrawal of treatment, the infection flared up above any other group. That the infection actually reduced in this group while it did not reduce with Streptomycin alone, suggests that formulating Streptomycin with MSAMS improved its efficacy such that even the already resistant infection became sensitive. The sudden increase of the infection, higher than both the untreated group and the group treated with Streptomycin without MSAMS, suggests that MSAMS potentiated both desired effect of Streptomycin (efficacy) and its side effect (immune suppression) so that with withdrawal of the treatment, the infection (which treatment could not terminate), had the least resistance.

Reducing dosage of Streptomycin by 25% to use its 75%- dosage (18.75 mg/kg) for treatment with the Streptomycin-MSAMS formulation, terminated the resistant infection, after only 3-days on the treatment. The 100 % infection load reduction achieved in that group of 75% Streptomycin-dosage in MSAMS plus Vitamin C remained both on day-1 PT and on day-4 PT, confirming that the treatment terminated the resistant infection.

Apart from terminating the resistant infection which means cure for AMR, that the treatment achieved such an effect with reduction both in dosage of the drug and in course of the treatment means reduction in cost of treatment and reduction in residues of the drug in human foods of animal origin. Such reduction in drug-residues in foods will reduce incidences of AMR in humans.

When same treatment-strategy (using 75% of recommended dosages of MSAMS-stabilized antimicrobials, supported with antioxidants) was applied to Ampicillin trihydrate (Ezeibe *et al* 2013) and Cotrimoxazole (Ezeibe *et al* 2019) against resistant infections, though they achieved enough infection-reduction that could lead to cure and prevention of AMR (≥ 95 %), they could not terminate the infections. Some infections (≤ 5 %) were left which immunity could clear with time (Brent *et al*, 2001). In this study with Streptomycin, the strategy terminated the resistant infection (100 %-infection reduction). Level of resistance by isolate used in each trial and side effect of each antimicrobial being tested may be responsible for differences in rates (%) of clearance that result with that strategy of using 75 % of recommended dosages of MSAMS-stabilized antimicrobials supported with antioxidants to treat resistant infections. However, that the treatment-strategy restores efficacy to antimicrobials has remained consistent.

Results of these trials of antimicrobials-MSAMS (Nano-stabilizing agent) formulations and antioxidants on both sensitive and resistant infections of different bacteria, protozoa and helminths suggest that formulating antimicrobials with Nano-stabilizing agents and supporting their treatments with antioxidants may be an effective treatment-strategy for prevention and treatment of AMR. Also, since a reduced dosage of Streptomycin, stabilized with MSAMS, achieved 100 % clearance of the resistant infection after only three days, the strategy may also lead to reduction in course of treatments for Streptomycin and possibly for other antimicrobials, too.

REFERENCES

1. Addis M. and Sisay D. (2015). A review on major food borne bacterial illnesses. *Journal of Tropical Diseases*. 3 (4): 1–7. doi:10.4172/2329-891x.1000176.
2. Agyare, C., Boamah, V. E., Zumbi. N. C. and Osei, B. F. (2018). Antibiotics use in poultry production and its effects on bacterial resistance. Doi:10.5772/intechopen.79371 https://www.intechopen.com/book.
3. Balakrishnan, S., Sangeetha, A. and Dhanalakshmi, M. (2018). Prevalence of Salmonella in chicken meat and its slaughtering place from local markets in orathanadu, thanjavur district, Tamil nadu. *Journal of Entomology and Zoology Studies*. 6 (2): 2468–2471.
4. Brent, W., Gunderson, Gigi, H., Ross, K.H.I. and John, C.R. (2001) What Do We Really Know about Antibiotics Pharmaco- dynamics? *Pharmacotherapy*, 21, 28-31.
5. Bortolaia, V., Espinosa-Gongora, C. and Guardabassi, L. (2016). Human health risks associated with antimicrobial resistant *Enterococci and Staphylococcus aureus* on poultry meat. *Clinical Microbiology Infection* 22: 130-140.
6. Cristina, E., Ivan, P. and Kevin, R. (2007) Nanomaterials and Nanoparticles: Sources and Toxicity. *Biointerphases*, 2, 17-21.
7. Dhama, K., Rajagunalan, S. and Chakraborty, S. (2013). Food-borne pathogens of animal origin-diagnosis, prevention, control and their zoonotic significance: a review. *Pakistan Journal of Biological Sciences*. 16 (20): 10 76–1085. doi:10.3923/pjbs.2013.1076.1085.
8. Ejo, M., Garedew, L., Alebachew, Z. and Worku, W. (2016). Prevalence and anti-microbial resistance of Salmonella isolated from animal-origin food items in gondar, Ethiopia. *BioMed Research International*. 8. doi: 10.1155/2016/4290506.42905 06.
9. Gill, E. E., Octavio, L. F. and Hancock, R. E. W. (2015). Antibiotic Adjuvants: diverse strategies for controlling drug-resistant pathogens. Chemical biology and drug design. 85; 56-78.
10. Ezeibe, M. C. O. (2020). Potentials of The Medicinal Synthetic Aluminum-Magnesium Silicate: Al4 (Sio4)3 + 3mg2sio4 → 2al2 Mg3 (Sio4)3 on the Economy, Post Covid-19. *Journal Of International Series*. 1(2): 83-93.
11. Ezeibe, M. C. O. and Ogbonna, I. J. (2015). Enhancing Efficacy of Antimicrobials with the Medicinal Synthetic Aluminum-Magnesium Silicate, for Prevention and Treatment of Resistant Infections. *British Journal of Medicine & Medical Research*. 9(6): 1-8.

12. Ezeibe, M. C. O. and Ogbonna, I. J. (2016). Use of Medicinal Synthetic Aluminum Magnesium Silicate (MSAMS) to enhance efficacy of antimicrobials for prevention and treatment of resistant infection. 2nd International Conference and Exihibition on Pharmacology and Ethnopharmacology, May 02-04, 2016. *Journal of Clinical and Experimental Pharmacology*.
13. Ezeibe, M. C. O., Ezeja, M. I., Akpan, C. A. N., Onyeachonam, F. O., Sanda, M. E., Ogbonna, I. J., Kalu, E., Njoku, N. U. and Udobi, M. I. (2020). Regression of symptoms of trypanosomosis in Mice dosed with clotrimoxazole-Antivirt (AL4(SiO4)3+3Mg 2Si O4 = 2Al2Mg3 (SiO4)3). *ACTA Scientific Veterinary Sciences*. 2 (9): 23-28.
14. Ezeibe, M. C. O., Ezeobele, O. K., Essen, M. E., Ngene, A. A., Mbuko, I. J., Chukwudi, T. C, Onoja, V. U. and Ezeh, I. O. (2013). Synergy in antibacterial activities of ampicillin trihydrate stabilized with a synthetic Aluminum magnesium silicate and immune stimulants in resistant *E. coli* infection. *Health*. 5 (10): 1548-1552.
15. Ezeibe, M. C. O., Udom, A. E., Onyeachonam, F. O. and Ogbonna, I. J. (2019). Restoring Efficacy to Clotrimoxazole against Resistant *Salmonella pollurum* with Medicinal Synthetic Aluminum Magnesium Silicate (MSAMS). *Health*. 11: 1162-1168.
16. Galindo, L.A. and Cereso, P. (2006) Compositional, Technical and Safety Specification of Clay to Be Used as Pharmaceutical and Cosmetic Products. *Journal of Renal Nutrition*, 2, 38-40.
17. Goetting, V., Lee, K. A. and Tell, L. A. (2011). Pharmacokinetics of veterinary drugs in laying hens and residues in eggs: A review of the literature. *Journal of Veterinary pharmacology*. 34: 521-556.
18. Heredia, N. and Garcia, S. (2018). Animals as sources of food-borne pathogens: a review. *Animal Nutrition*. 4 (3): 250–255. doi: 10.1016/j.aninu.2018.04.006.
19. Murray, K.R. (2000) Harpers Biochemistry. McGraw Hill, New York.
20. Nguyen, T., Niwat, C. and Juan, J. (2017). Antimicrobial resistance in bacterial poultry pathogens: A review. Frontiers in Veterinary Science 16: 14-17.
21. Okorie-Kanu, O. J., Ezenduka, E. V., Okorie-Kanu, C. O., Ugwu, L.C. and Nnamani, U. J. (2016). Occurrence and antimicrobial resistance of pathogenic *Escherichia coli* and *Salmonella* spp. in retail raw table eggs sold for human consumption in Enugu state, Nigeria. Veterinary World. 9 (11): 1312-1319.
22. Reig, M. and Toldra, F. (2008). Veterinary drug residues in meat: concerns and rapid method for detection. *Meat science* 78: 60-69.
23. Tadesse, G. and Tessema, S. T. (2014). A meta-analysis of the prevalence of Salmonella in food animals in Ethiopia. *BMC Microbiology*. 14 (270): 1–9. doi:10.1186s12866 -014- 0270-y.
24. Tegegne, F. M. (2019). Epidemiology of *Salmonella* and its serotypes in human, food animals, foods of animal origin, animal feed and environment. *Journal of Food Nutrition and Health*. 2 (1) : 7–14.
25. Vanderbilt (2012) Report. Technical Information: "VEEGUM—The Versatile Ingredient for Pharmaceutical Formulations. R.T. Vanderbilt Company Bulletin No. 91R, 1984. R.T. Vanderbilt Company, Inc., Norwalk.
26. Van, T. T., Nguyen, H. N., Smooker, P. M. and Coloe, P. J. (2012). The antibiotics resistance characteristics of non-typhoid *Salmonella enterica* isolate from food producing animals, retail meat and humans in South East Asia. *International Journal of Food Microbiology*. 154: 98-106.