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Restoring Efficacy to Cotrimoxazole, against Resistant *Salmonella pullorum*, with *Medicinal Synthetic Aluminum-Magnesium Silicate*® [MSAMS: $Al_4(SiO_4)_3 + 3Mg_2SiO_4 \rightarrow 2Al_2Mg_3(SiO_4)_3$]

Maduike Chiehiura Onwubiko Ezeibe*, Aniefiok Emerson Udom, Obiageri Favour Onyeachonam, Ijeoma Joy Ogbonna

College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria
Email: *maduikeezeibe@yahoo.com

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Abstract

To restore efficacy (≥95% infection-reduction) to drugs against resistant infections (now, posing a bigger medical challenge, globally, than even HIV/AIDS), Cotrimoxazole was formulated with a synthetic Aluminum-magnesium silicate (Medicinal mineral which molecules are made of *Nanoparticles*, that is already in use as pharmaceutical-stabilizing agent) and used with antioxidants to treat Cotrimoxazole-resistant *Salmonella pulorum* infected chicks. Chick-groups A, B and C were fed antioxidants-fortified feed while groups D, E, F, G and H were not. The treatment-groups and their Cotrimoxazole doses/formulations were: A and D (100%/MSAMS-Cotrimoxazole); B and E (75%/MSAMS-Cotrimoxazole); C and F (50%/MSAMS-Cotrimoxazole) and G (100%/Cotrimoxazole). Infection-reductions (96.23% and 94.98%) of the groups of 75%/MSAMS-Cotrimoxazole/antioxidants and 75%/MSAMS-Cotrimoxazole were significantly ($P \leq 0.05$) better than 76.99% (100%/Cotrimoxazole); 10.04% (50%/MSAMS-Cotrimoxazole/antioxidants); 1.60% (50%/MSAMS-Cotrimoxazole); −212.60% (100%/MSAMS-Cotrimoxazole/antioxidants) and −230.96% (100%/MSAMS-Cotrimoxazole).

Keywords

Cure for Resistant Infections, Salmonellosis, MSAMS

1. Introduction

Drug resistance has become a major medical challenge, not only in poultry but

in other livestock and even in human medicine. *Salmonellae* and other microorganisms exhibit resistance to many antimicrobial agents like tetracycline, ciprofloxacin, chloramphenicol, penicillin G, amoxicillin, trimethoprin, sulphamethaxazole, gentamycin [1] [2] [3]. Penicillin, ampicilin, chloramphenicol, tetracycline and nitrofurantoin have been reported to be completely resisted by *Salmonella pullorum* while gentamycin, cotrimoxazole and nalixidic acid have been moderately resisted [3]. Fluoroquinolone is said to be less resisted [4].

Development of antimicrobial resistance by avian adapted *Salmonella* strains is attributed to chromosomal mutation or genetic recombination [2]. Resistance may also result from the irrational or wrong use of antimicrobials. Bacteria may also acquire resistance by transformation, conjugation or transduction [5].

Aluminum-magnesium silicate (AMS) has been in use, both as a drug [6] [7] and as a pharmaceutical raw material for drug formulations. It is used as a stabilizing agent, carrier, adsorbent, viscosity-enhancing agent, anti-caking agent, tablet binder, tablet and capsule des-integrant because of its indefinite stable property, ability to be used both in acidic and alkaline P^H [6] [8] [9]. As a stabilizing agent, it prolongs time of high bioavailability of drugs by protecting such drugs against rapid degradation (metabolism). Also, molecules of AMS are made of particles that are only 0.96 nm thick (*Nanopaticles*) [10] and *Nanoparticles* enhance delivery of drugs to targets. Prolonging time of high bioavailability and enhancing delivery to targets improve efficacy of drugs.

When ampicilin trihydrate, chloroquin phosphate, piperazine citrate and sulphadimidine were stabilized with a synthetic AMS (*Medicinal synthetic Aluminum-magnesium silicate*: MSAMS), 75% of their doses terminated the infections by achieving ≥ 95% clearance of each [6] [11]-[16]. Stabilizing drugs with MSAMS and using them and antioxidants (vitamins A, C, E and/or selenium) to treat infected animals also made them regain effects against resistant infections [15]. This study is a test of effects of the MSAMS on Cotrimoxazole against resistant Salmonella infections.

2. Materials and Methods

Forty eight day-old cockerels were randomly assigned to eight groups (A, B, C, D, E, F, G and H) of six (6) each. The chicks were screened by the method of Roch-Silva *et al.* [17] to ensure they were free of *Salmonella species*. Each chick was infected with 1.2×10^5 CFU of a Cotrimoxazole-resistant *Salmonella pullorum* isolate. From six days post infection, they were treated for 7 days as follows: Groups A, B and C were fed with feed fortified with additional levels of Vitamin A (375 mg for every 25 Kg), Vitamin C (10 mg for every 25 Kg) and Vitamin E (75 mg for every 25 Kg) while groups D, E, F G and H were fed with normal commercial feed (without additional levels of the Vitamins). Groups A and D were treated with the MSAMS-Cotrimoxazole drug formulation at 100% of Cotrimoxazole dose; Groups B and E at 75% of Cotrimoxazole dose with MSAMS-Cotrimoxazole drug formulation; Groups C and F at 50% of Cotri-

moxazole dose with MSAMS-Cotrimoxazole drug formulation. Group G was treated at 100% of Cotrimoxazole dose (not stabilized with MSAMS) while group H was not treated at all (Control).

During the experiment, clinical signs were recorded daily. Individual cloacal swabs were collected from all the chicks on Day 4, post infection (PI) and processed as described by Wigley *et al.* [18], with some modifications as follows: Individual samples were inoculated into buffered peptone water, incubated at 37˚C for 24 hours and then sub cultured on *Salmonella-Shigella* agar, brilliant green agar and MacConkey agar respectively before incubating at 37˚C for 24 hours. The cultures were then examined to confirm growth *of Salmonella pullorum* as a confirmation of establishment of infection in the chicks. Temperature and weight of each chick were taken daily.

Two chicks from each group were euthenased on Days 1, 3 and 5, post treatment and their gall bladders were aseptically removed into sterile sample bottles. To 0.1 ml of bile, 0.9 ml of sterile normal saline was added to get 1:10 dilution of the bile. Again, 0.1 ml of the 1:10 bile dilution was diluted with 0.9 ml of sterile normal saline to get 1:100 dilution. Finally, 0.05 ml of the 1:100 diluted bile was plated on nutrient agar and incubated at 37˚C for 24 hours. *Salmonella pullorum* colonies (x) were counted (using hand lens), and calculated as colony forming units per ml of bile by the formula: $\mathrm{CFU/ml} = \frac{x}{5} \times 10,000$.

CFU per ml of bile for the 8 groups of chicks were compared for statistical differences by Analysis of variance (ANOVA).

3. Results

Mean rectal temperature (41.32˚C) of group of chicks infected with Cotrimoxazole-resistant *Salmonella pullorum* and treated at 75% dose of Cotrimoxazole stabilized with MSAMS and fed with antioxidants was significantly ($P \leq 0.05$) lower than 41.62˚C of the untreated group; 41.53˚C of the group treated with 75% dose of Cotimoxazole stabilized with MSAMS but not fed with antioxidants; 41.58˚C of the group treated with 100% dose of Cotrimoxazole; 41.49˚C of group treated at 50% dose of cotrimoxazole stabilized with MSAMS and fed antioxidants; 41.60˚C of the group treated with 50% dose of Cotrimoxazole stabilized with the MSAMS but not fed with antioxidants; 41.54˚C of group treated at 100% dose of cotrimoxazole stabilized with MSAMS and fed antioxidants and 41.61˚C of the group treated with 100% dose of cotrimoxazole stabilized with the MSAMS but not fed antioxidants, respectively. Also, mean weight-gain (520.8 g) of that group of 75% dose of Cotrimoxazole stabilized with MSAMS and antioxidants was better than that of the control while there was no difference between mean weight-gains of the other treated groups and mean weight-gain of the control.

Rate of infection-reduction (96.23%) was also significantly ($P \leq 0.05$) higher in the group treated with 75% dose of Cotrimoxazole stabilized in MSAMS and fed antioxidants and in the group treated with 75% dose of Cotrimoxazole stabi-

lized in MSAMS but without antioxidants (94.98%) than in all other groups. The group treated with 100% dose of Cotrimoxazole but not fed antioxidants had only 76.99% infection-reduction. The group treated with 50% dose of Cotrimoxazole stabilized in MSAMS and antioxidants had a reduction of only 10.04% while the group treated with 50% dose of Cotrimoxazole stabilized in MSAMS but without antioxidants, had just 1.6% reduction. The group treated with 100% dose of Cotrimoxazole stabilized in MSAMS and with antioxidants and the group treated with 100% dose of Cotrimoxazole stabilized in MSAMS but without antioxidants had −212.6% and −230.96% reduction rates respectively (infection-increases).

The rectal temperatures, body weights and rates of reduction of colony forming units of Cotimoxazole-resistant *Salmonella pullorum* isolate (in bile) in chicks following treatment with Cotrimoxazole-MSAMS drug formulation and antioxidants are as shown in Table 1.

4. Discussion

Significant reductions ($P \leq 0.05$) in CFU of Cotrimoxazole-resistant *Salmonella pullorum* in the group of chicks treated with 75% Cotrimoxazole-dose stabilized in MSAMS and high levels of antioxidants (in feed) and in the group treated with 75% Cotrimoxazole-dose in MSAMS without antioxidants, agree with earlier results [6] [19] which revealed that 75% of doses of drugs, when stabilized in MSAMS, give better clearance of infections than 100% of their doses, so stabilized.

That treatment with 75% dose of Cotrimoxazole stabilized in MSAMS and high levels of antioxidants gave better infection reduction rate of 96.23%, increased mean weight-gain of 520.8 g and reduced rectal temperature of 41.32˚C than treatment with the same 75% dose of Cotrimoxazole stabilized in MSAMS

Table 1. Rectal temperatures, body weights and infection-reduction rates in chicks infected with a Cotrimoxazole-resistant *Salmonella pullorum* isolate and treated with different doses of Cotrimoxazole stabilized in medicinal synthetic aluminum-magnesium silicate and with antioxidants.

Observations	Treatment Groups							
	A	B	C	D	E	F	G	H
Temperature (˚C)	41.54^{ab}	41.32^{c}	41.49^{b}	41.61^{ab}	41.53^{b}	41.60^{ab}	41.58^{ab}	41.62^{a}
Mean weight gain (g)	454.2^{ab}	520.8^{a}	416.7^{b}	479.2^{ab}	482.0^{ab}	454.2^{ab}	395.8^{b}	400.0^{b}
Rate of infection reduction (%)	-212.6^{e}	96.23^{a}	10.04^{c}	-230.96^{f}	94.98^{a}	1.67^{d}	76.99^{b}	
Colony forming units/ml of bile ($\times 10^4$)	24.90^{a}	0.30^{d}	7.12^{bc}	26.30^{a}	0.40^{d}	7.83^{b}	1.83^{cd}	7.97^{b}

A = 100% cotrimoxazole − medicinal synthetic aluminum-magnesium silicate (MSAMS) + fortified feed (FF); B = 75% cotrimoxazole − MSAMS + FF; C = 50% cotrimoxazole − MSAMS + FF; D = 100% cotrimoxazole − MS AMS; E = 75% cotrimoxazole − MSAMS; F = 50% cotrimoxazole − MSAMS; G = 100% cotrimoxazole only; H = infected/untreated.

but without the antioxidants (94.98% infection reduction, 482.0 g weight gain and 41.53˚C body temperature) suggests that the antioxidants stimulated immune response of the chicks and so, helped to improve their health, generally. This suggestion is supported by reports of Mubarak *et al.* [20], Sanda *et al.* [21] and Ibrahim and El-Sayed [22] who also observed that vitamins A, C and E (antioxidants) stimulate the immune system of animals. The results also agree with the report of Rajput *et al.* [23], that supplementary vitamins A, C and E in feed ensure better efficacy for treatments. Clearance of ≥95% of infections means termination for infections as immunity is able to complete elimination of ≤5% infection-load, left after treatments [24].

Treatment with 100% dose of Cotrimoxazole stabilized in MSAMS and with antioxidants in feed and treatment with 100% dose of Cotrimoxazole stabilized in MSAMS without the antioxidants caused increases in the infection loads rather than reduction (−212.55% and −230.96%). This suggests that using MSAMS to stabilize Cotrimoxazole at a normal dose of the drug makes resistant infections worse. It is possible that stabilizing Cotrimoxazole with MSAMS leads to over prolongation of time of high bioavailability and so makes it become toxic instead of being beneficial. In this study, immune suppression (side effect) may have become more prominent than the 76.99% antimicrobial effect left for Cotrimoxazole against the Salmonella infection. This may be responsible for the increase in CFU/ml in the two groups treated at 100% dose of Cotimoxazole stabilized with the MSAMS. This finding also agrees with earlier observations [15].

When the dose of Cotrimoxazole was reduced to 75% and it was stabilized in MSAMS (with antioxidants in feed) best reduction of CFU/ml, best reduction in body temperature and best weight-gain were achieved. The reduction in dose may have reduced the side effects while prolongation of high bioavailability and enhanced delivery to effect-targets improved the desired effects such that the overall result became termination of even the resistant infection (≥95% infection-reduction).

5. Conclusion

By clearing ≥95% of Cotrimoxazole-resistant *Salmonella pullorum* infection, the 75% dose of Cotrimoxazole stabilized in the MSAMS, terminated the resistant infection and cured salmonellosis (reduced temperature and improved weight gain).

Authors' Contributions

The authors collaborated for the research. Author MCOE designed the experiments and drafted the manuscript while author AEU conducted the laboratory work. Authors OFO and IJO processed the manuscript for publication. All the authors read the draft-manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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