ABSTRACT

**Aim:** To achieve, at least, 95% clearance of loads of treated infections so that pathogens do not develop resistance against drugs used for treatment.

**Materials and Methodology:** Lower doses of Aluminum-magnesium silicate (AMS)-stabilized antimicrobials (Piparazine, Ampicillin, Chloroquine and Sulphadimidine), supported with immune stimulants, were used to treat respective sensitive and resistant infections.

**Results:** Recommended doses of Piparazine, Ampicillin and Chloroquine cleared only 82.94%, 80.68% and 20% of *Helignosomoides bakeri*, *Salmonella gallinarum* and *Plasmodium berghei* infections, respectively. Lower doses (75%) of the AMS-stabilized Piparazine and Ampicillin cleared 96.82% and 97.84% of the treated infections. Supporting the lower doses of AMS-stabilized Chloroquine and AMS-stabilized Ampicillin with immune stimulants led to 100% clearance of *P. berghei* infection and 95.80% clearance of Ampicillin-resistant *Escherichia coli* infection, respectively.

*Corresponding author: Email: mijejoy@yahoo.com;
Conclusion: Prolonging bioavailability of drugs with AMS, minimizing side effects of drugs by using their lower doses for treatment, and enhancing immune responses of treated patients, help treatments to prevent and cure resistant infections.

Keywords: Drug-resistant infections; prolonging bioavailability; minimizing side effects; enhancing immune responses.

1. INTRODUCTION

In recent times, development of resistance against drugs used for treatment by microorganisms that cause diseases in animals and in human-beings has been on the increase [1]. This is a major challenge to medical practice, all over the world [2]. To prevent development of resistance by infections, against drugs, treatments should reduce loads of infections by at least 95% [3]. When more than 5% of loads of infections are left in the body after treatment, body’s immune responses may not be able to complete elimination of the infections and infections that survive treatments often develop resistance against drugs used [3].

The problem of antimicrobial resistance is made worse by the practice of adding antimicrobials to feeds of animals, as growth promoters [4]. This leads to development of resistance by pathogens that infect animals and when the resistant infections find their way into the human food chain, they transfer the resistance to pathogens that infect human-beings [5]. It has been reported that over 80% cases of cystitis in human-beings were due to E. coli infections contracted from animals [6].

Also, when high doses of drugs are used to treat food animals, high concentrations of residues of the drugs could be passed to human-beings who eat meat, milk and eggs of the animals. This also leads to development of resistance by microorganisms that infect the human-beings, because of constant exposure to sub-therapeutic doses of the drugs. Need therefore exists for search for drugs to combine with antimicrobial drugs, to improve their efficacy, both for veterinary treatments and for treatment of human-beings [7].

When in solution, molecules of AMS form three dimensional colloidal structures around molecules of any drug with which it is combined. By that mechanism, AMS stabilizes other drugs [8]. When stabilized, drugs remain protected from destruction. AMS thus protects drugs from being rapidly degraded by metabolic processes. Bioavailability of the drugs is thus prolonged.

When bioavailability of drugs is prolonged, their actions improve [9]. Also, AMS is made of platelets that are only 0.96 nm thick [8]. So, it is made of Nanoparticles [10]. Nanoparticles enhance delivery of drugs to targets and across blood-brain barrier [11]. So, the AMS may in addition to prolonging bioavailability of drugs, enhance their delivery to targets and across the blood-brain barrier.

Every drug has both its desired effects and its side effects. Most antimicrobial drugs cause immune-suppression when used at high doses [1,12,13]. To protect immune systems of treated animals against side effects of drugs and to enhance their responses against infections, immune stimulants which include the B-vitamins and high levels of Vitamins A, C, E and of Selenium in feed of animals are recommended [14].

Aluminum-magnesium silicate is not found in Nigeria as mineral deposits but Aluminum silicate and Magnesium silicate are abundant in the country. The two minerals are already purified and are being used as medicines. So, they were reacted [15] to get a synthetic form of AMS \( \text{Al}_4 (\text{SiO}_2)_3 + 3\text{Mg}_2\text{SiO}_4 \rightarrow 2\text{Al}_2\text{Mg}_3 (\text{SiO}_4)_3 \). A simple sugar was incorporated into the Medicinal synthetic AMS, to carry its molecules, by active transport, across mucous membranes of stomach and intestines of treated patients, into blood circulation [16].

Formulations of Ampicillin trihydrate, Piparazine citrate, Sulphadimidine and of Chloroquine phosphate were made in the medicinal synthetic AMS [17,18] and used for experimental treatments of both drug-sensitive and drug-resistant infections.

2. MATERIALS AND METHODS

Formulations of 2.5% Ampicillin trihydrate, 20% Piparazine citrate, 20% Sulphadimidine and of 20% Chloroquine phosphate in the Medicinal synthetic AMS were made and used for the experiments. The Chloroquine phosphate was sourced from IECA laboratories limited, India while the Ampicillin trihydrate was got from West Bengal pharmaceuticals, India.
Five groups of randomly selected mice, infected with *Heligmosomoides bakeri* were treated with 110 mg/kg (piparazine), 110 mg/kg (Piparazine in AMS), 82.5 mg/kg (Piparazine) and 82.5 mg/kg (Piparazine in AMS), respectively. The fifth group served as control. Numbers of *H. bakeri* Eggs Per Gramm (EPG) of faeces of each mouse in the five groups were counted. Mean EPG of the groups were compared for statistical differences, by Analysis of variance (ANOVA).

In the second experiment, four groups, each of 10 randomly selected chicks, infected with *Salmonella gallinarum* were treated with Ampicillin trihydrate (AT) for 5 days. Two groups were treated at dose rates of 10 mg and 7.5 mg of AT per Kg body weight respectively, with 100% Ampicillin. Two other groups were similarly treated with the Ampicillin-AMS drug. The fifth group served as control. Bile of 5 chicks from each group was harvested. Then 0.1 ml of bile from each chick was added to 0.9 ml of normal saline to make a 1:10 dilution. Again 0.1 ml of the 1:10 bile dilution was transferred to 0.9 ml of normal saline to make a 1:100 dilution. Finally, 0.05 ml of each diluted bile was plated on Mac Conkey agar and incubated at 37°C for 24 hours. The *S. gallinarum* colonies (X) were counted and calculated as colony forming units per ml (CFU/ml) by the formula: CFU/ml = \( \frac{X}{5} \times 10,000 \). Means of the CFU/ml of the five groups were compared for statistical differences, by ANOVA.

For the third experiment, 5 groups of randomly selected mice, infected with *Plasmodium berghei* were used. Three groups were treated at Chloroquine (CQ) dose of 7 mg/kg with CQ alone, with the CQ-AMS drug formulation and with the CQ-AMS drug + B-vitamins, respectively. The fourth group was treated at 5.25 mg/kg with CQ-AMS + B-vitamins while the fifth group served as control. For the treated groups, treatment was initiated 10 days post infection (PI) and lasted for 7 days. Parasitaemia for the five groups of mice was assessed on days 1,7,14 and 21 post treatment (PT). Means of the parasitaemia for the 5 groups were tested for statistical differences, by ANOVA.

In the fourth experiment, 5 groups of randomly selected mice, infected with Chloroquine-resistant *P. berghei* isolate were used. Ten days PI, two subgroups, each, were treated for 7 days at CQ doses of 7 mg/kg and 5 mg/kg, with 100% CQ powder and with a CQ-AMS drug formulation respectively. One subgroup served as control. Parasitaemia was assessed from each mouse in 4 replicates, post treatment (1 day, 7 days, 14 days, and 21 days). Means of parasitaemia of the 5 subgroups were compared for statistical differences, by ANOVA.

Five groups of randomly selected chicks, infected with Sulphadimidine-resistant *Escherichia coli* were used for the fifth experiment. Two groups were treated at Sulphadimidine’s dose rate of 1 g/liter of drinking water with a 100% Sulphadimidine powder and with the Sulphadimidine-AMS drug formulation, respectively. Two other groups were treated with the 100% Sulphadimidine and with the AMS-Sulphadimidine drug formulation at Sulphadimidine’s dose rate of 0.75 g/liter. The fifth group served as control. After 5 days of treatment, the chicks were sacrificed and dilutions of their bile plated on Mac Conkey agar and incubated at 37°C for 24 hours. *E. coli* colonies in each culture were counted and expressed as CFU/ml. means of the *E. coli* CFU/ml of bile of the different treatment groups were compared for statistical differences, by ANOVA.

In the sixth experiment 5 subgroups each of 5 randomly selected chicks, infected with Ampicillin-resistant *E. coli* were used. Two days before infecting the chicks, 2 subgroups were placed on poultry feed, fortified with additional 375 mg of Vitamin A, 10 mg of Vitamin C, 75 mg of Vitamin E and 12.5 mg of Selenium per 25 kg bag. Three subgroups were left on ordinary poultry feed. The 2 subgroups on the fortified feed were treated with Ampicillin and with the Ampicillin-AMS drug formulation respectively, at dose of 7.5 mg/kg for 7 days. Two of the subgroups on ordinary feed were treated at dose of 10 mg/kg with 100% Ampicillin and with the Ampicillin-AMS drug formulation respectively, for seven days while the third subgroup on ordinary feed served as control. Means of *E. coli* CFU/ml of bile of the subgroups of chicks were compared for statistical differences, by ANOVA.

3. RESULTS AND DISCUSSION

3.1 Results

Normal dose of Piparazine (110 mg/kg) led to only 82.94% reduction (P<0.05) of EPG of faeces of *H. bakeri*-infected mice. When the drug was stabilized with AMS the rate of reduction improved (P<0.05) to 92.04%. Reducing the dose to 75% of the recommended dose (82.5 mg/kg) and stabilizing it with AMS improved rate
of reduction of the EPG (P<0.05) to 96.82%.

Normal dose of Ampicillin (10 mg/kg) led to only 80.68% reduction (P<0.05) of CFU/ml of bile of S. gallinarum-infected chicks. When the drug was stabilized with AMS the reduction improved (P<0.05) to 86.36%. Reducing the dose to 75% of the recommended dose (7.5 mg/kg) and stabilizing it with AMS improved the rate of reduction of the infection load (P<0.05) to 97.84% (Table 1).

Normal dose of Chloroquine (7 mg/kg) led to only 20% reduction (P<0.05) of P. berghei parasitaemia. When the drug was stabilized with AMS, the rate of reduction of the infection load improved (P<0.05) to 29.10%. Use of 75% of normal dose of Chloroquine (5.25 mg/kg) stabilized in AMS and supported with B-vitamins, cleared (P<0.01) 100% of the parasitaemia (Table 2).

Normal dose of Chloroquine (7 mg/kg) led to increase (P<0.05) of parasitaemia of Chloroquine-resistant P. berghei infection by 15.27%. When the drug was stabilized with AMS, the 7 mg/kg dose led to death of 80% of treated mice. Reducing the dose to 5 mg/kg and stabilizing it with AMS reduced (P<0.05) the parasitaemia by 56.38% (Table 3).

Normal dose of Sulphadimidine (1 g/liter of drinking water) led to increase (P<0.05) of load of Sulphadimidine-resistant E. coli infection by 259%. When the drug was stabilized with AMS, load of the resistant infection increased further (P<0.05) by 789.10%. Reducing the dose to 5 mg/kg and stabilizing it with AMS reduced load of the resistant infection (P<0.05) by 84.34% (Table 4).

Normal dose of Ampicillin (10 mg/kg) led to reduction (P<0.05) of load of Ampicillin-resistant E. coli, just by 50%. When the drug was stabilized with AMS, rate of the drug-resistant infection reduction reduced (P<0.05) from 50% to 43.91%. Use of 75% of the normal dose (7.5 mg/kg) stabilized with AMS plus immune stimulants in feed of the chicks led to reduction (P<0.05) of load of the resistant infection by 95.78% (Fig. 1).

Table 1. Salmonella gallinarum infection load in bile of chicks treated with Ampicillin trihydrate - medicinal synthetic aluminum magnesium silicate drug formulation

<table>
<thead>
<tr>
<th>Chicks</th>
<th>Colony forming units per ml (x10^5/ml) in bile</th>
<th>Amp 10 mg/kg</th>
<th>Amp-AMS</th>
<th>7.5 mg/kg</th>
<th>Amp-AMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>0.2</td>
<td>31</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>0.1</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>1</td>
<td>9</td>
<td>0.2</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>0.7</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>4</td>
<td>8</td>
<td>0.7</td>
<td>8</td>
</tr>
<tr>
<td>Mean</td>
<td>3.4±0.81</td>
<td>2.4±0.81</td>
<td>5.4±1.93</td>
<td>0.38±0.13</td>
<td>17.6±0.11</td>
</tr>
</tbody>
</table>

Table 2. Parasitaemia (%) in Plasmodium berghei-infected mice treated with chloroquine phosphate - medicinal synthetic aluminum magnesium silicate drug formulation and with B-vitamins

<table>
<thead>
<tr>
<th>CQ</th>
<th>7 mg/kg</th>
<th>CQ-AMS+E CQ-AMS+Vit</th>
<th>CQ-AMS+Vit</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>90</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>80</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>68</td>
<td>35</td>
<td>70</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>20</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>70</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Mean</td>
<td>42.00±15.74^a</td>
<td>37.22±11.88^b</td>
<td>33.57±12.62^b</td>
</tr>
</tbody>
</table>
Table 3. Parasitaemia (%) in mice, infected with chloroquine-resistant *Plasmodium berghei* isolate and treated with chloroquine-medicinal synthetic aluminum magnesium silicate drug formulation.

<table>
<thead>
<tr>
<th>Weeks PT</th>
<th>Parasitaemia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 mg/kg(CQ)</td>
</tr>
<tr>
<td>0</td>
<td>4.50</td>
</tr>
<tr>
<td>1</td>
<td>4.40</td>
</tr>
<tr>
<td>2</td>
<td>4.30</td>
</tr>
<tr>
<td>3</td>
<td>3.38</td>
</tr>
<tr>
<td>Mean</td>
<td>4.15 ± 0.2abc</td>
</tr>
</tbody>
</table>

Table 4. Loads of sulphadimidine-resistant *Escherichia coli* in bile of infected chicks treated with sulphadimidine-medicinal synthetic aluminum magnesium silicate drug formulation.

<table>
<thead>
<tr>
<th>Chick</th>
<th>E. coli colony forming units per ml of bile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 g/liter</td>
</tr>
<tr>
<td></td>
<td>Sulpha</td>
</tr>
<tr>
<td>1</td>
<td>72,000</td>
</tr>
<tr>
<td>2</td>
<td>340,000</td>
</tr>
<tr>
<td>3</td>
<td>32,000</td>
</tr>
<tr>
<td>4</td>
<td>72,000</td>
</tr>
<tr>
<td>5</td>
<td>80,000</td>
</tr>
<tr>
<td>Mean</td>
<td>119,200</td>
</tr>
<tr>
<td>±55,800</td>
<td>±10,300</td>
</tr>
</tbody>
</table>

Fig. 1. Loads (CFU/ml X 1000) of resistant *Escherichia coli* in bile of infected chicks fed immune-stimulants and treated with ampicillin trihydrate-medicinal synthetic aluminum magnesium silicate drug formulation.

3.2 Discussion

Failure of recommended doses of Ampicillin trihydrate and Piparazine citrate to achieve the required 95% reduction of the respective drug-sensitive infections may predispose the bacterial and the helminth infections to develop resistance against the drugs, because body immune responses may not be able to clear infections when more than 5% of their loads are left after
treatments [3]. This finding suggests that most treatments regarded as successful may just cause clinical signs to cease while the treated animal or man remains infected. The remaining infections may develop resistance against drugs used for the treatment.

The observation that treatments which achieve recovery from clinical signs of diseases may not lead to recovery from infections is reason for the suggestion that effort be made to find adjuvants for drugs, to reduce incidence of resistance observed in treatment of helminthosis in ruminants and in horses [7].

Normal dose of Chloroquine that was not supported with B-vitamins achieved only 20% clearance of Plasmodium infection in mice. So, it may not even lead to clinical recovery of sick animals. This result explains the observation, in Nigeria, that Chloroquine, now, often fails to treat cases of malaria, such that most hospitals no longer use it. However, when dose of the drug was reduced to 75% in order to minimize its side effects and it was stabilized with AMS to prolong its bioavailability and the mice were given supportive Vitamins-B treatment, to enhance their immune responses, the Plasmodium parasite was cleared (100%). So, by that medication strategy Chloroquine can be made effective for treatment of malaria again. The strategy would also help to prevent malaria parasites from developing resistance against the drug.

In all the experiments, stabilizing antimicrobials with the Medicinal synthetic aluminum-magnesium silicate improved rate of clearance of drug-sensitive infections but it worsened cases of drug-resistant infections.

AMS is a stabilizing agent [8]. So, it prolongs bioavailability of antimicrobials. Also, its molecules are made of Nanoparticles and Nanoparticles pass blood-brain barrier and enhance delivery of drugs to targets [11,12]. Prolonged bioavailability and enhanced delivery of drugs to targets, improve both their antimicrobial effects and their side effects. This may explain the relief got by treating drug-sensitive infections with the AMS-drug formulations at recommended doses of the drugs while they made drug-resistant infections worse.

In the resistant infections, immune suppression by the drugs may have become more prominent than their anti-microbial effects because the infections were resisting the anti-microbial effects, hence the increase in rates of the infections.

That the increase in rate of infection did not occur when Ampicillin was used to treat Ampicillin-resistant E. coli infection suggests that Ampicillin may have less side effects (less immune suppression) than the other drugs. However, since stabilizing Ampicillin with the AMS reduced its antimicrobial effects against the resistant infection (from 50% reduction to 43.91%), it is evident that prolonging bioavailability of drugs (including Ampicillin) and enhancing their delivery to targets make their side effects more prominent than their anti-microbial effects, when high doses of drugs are used to treat resistant infections.

Reducing doses of drugs should mean that their effects would reduce, but when doses of three of the drugs used in the experiments were reduced to 75% of their recommended doses, stabilizing them in AMS achieved better infection clearance than their normal doses, so stabilized. That suggests that reducing doses of Piparazine, Ampicillin and Chloroquine may have minimized their side effects while effects of AMS on the drugs (prolonging bioavailability and enhancing delivery to targets) may have enhanced antimicrobial effects of the lower doses.

When the lower dose of AMS-stabilized Chloroquine was supported with B-vitamins, 100% clearance of the Plasmodium infection occurred. So, synergy between, enhancing antimicrobial effects of drugs with AMS, minimizing side effects of drugs by using their lower doses and enhancing immune response of treated animals with immune stimulants, helps treatments to achieve better anti-microbial effects. Also, when dose of Ampicillin was reduced to minimize its side effects and it was stabilized with AMS, supporting the treatment with immune stimulants cleared 95.80% of even an Ampicilline-resistant infection.

The strategy of minimizing side effects of drugs by using 75% of their recommended doses, potentiating antimicrobial effects of the drugs with AMS and enhancing immune responses of patients, has consistently led to better clearance of infections and would therefore prevent development of drug resistance by sensitive infections while it leads to cure of drug resistant infections [19-23].

Apart from prevention and treatment of drug resistant infections, use of lower doses of drugs
for treatment in veterinary practice would reduce
cost of treatment and also reduce amount of drug
residues in human foods of animal origin (meat,
milk and eggs). This would again reduce incidences of drug resistant infections in human-
beings who eat foods of animal origin.

4. CONCLUSION

Minimizing side effects of Ampicillin trihydrate,
 Sulphadimidine, Piparazine citrate and
 Chloroquine phosphate by treating infected
 animals with 75% of their recommended doses,
 stabilizing the lower doses with the Medicinal
 synthetic Aluminum-magnesium silicate, in order
to prolong their bioavailability, and supporting
 the treatments with immune stimulants, to
 enhance immune response of the patients,
 improve outcome of treatments so that they
 clear, 95% or more, of loads of both sensitive
 and resistant infections. So, the strategy would
 help to prevent development of resistance
 against drugs and cure infections that have
 already developed resistance against existing
 drugs.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The authors hereby declare that all experiments
have been performed in accordance with the
ethical standards laid down in the 1964
Declaration of Helsinki, as, operational in Nigeria.

COMPETING INTERESTS

Authors have declared that no competing
interests exist.

REFERENCES

1. NTP. Abstract for Tr-318-Ampicillin
Trihydrate (CASRN 7177-48-2) toxicity
and carcinogenesis studies of Ampicillin
Trihydrate (CAS No. 7177-48-2) in F344/N
Rats and 36C 3F, Mice (Gavage studies).
NTP Study Reports, National Toxicological
Program, USA Government; 1987.
2. CDC. Salmonellosis: Morbidity and
Mortality. MMWR Weekly Report. 2009;
58:25-29.
3. Kaplan RM. Anthelmintic resistance in
nematods of horses. Veterinary Research.
2002;33:491-507.
4. Diarra MS, Silversides FG, Diarrassouba
F, Pritchard J, Masson L, Brouseau
R, Bonnet C, Delaquis P, Bach S, Skura
BJ, Topp E. Impact of food supple-
mentation with antimicrobial agents on
growth per- formance of broiler chickens,
Clostridium perfringes and enterococcus
counts and antibiotic resistance phenol-
lytypes and distribution of antimicrobial
resistance deter-minants in Escherichia
coli isolates. Environmental Microbiology.
2007;73:6566-6576.
5. Johnson JR, Sannes MR, Croy C, Johnson
B, Clabots C, Kustowski MA, Bender J,
Smith KE, Wi- nokur PL and Belongia EA.
Antimicrobial drug- resistant Escherichia coli
from human and poultry prod- ucts
Minnesota and Wisconsin. Emerging
Infectious Diseases. 2007;13:1576-1585.
Available: http://dx.doi.org/10.3201/eid1306
_061576
6. Ewers C, Anto EM, Diehi I, Philip HC,
Wieler LH. Molecular epidemiology of
avian pathoge- nic Escherichia coli isolated
from colisepticaemia in poultry. Veterinary
Available: http://dx.doi.org/10.1016/j.vetmic.
2004.09.008
7. Ezeibe MCO, Dire CD, Anosa GN, Chikelu
ON, Okoroafor ON, Okorie OK, Ngene AA,
Idika IK, Ogunniran TM, Ezeala IE.
Efficacy of Piparazine Citrate, stabilized
with aluminium- magnesium silicate, against
Heligmosomoides bakeri. Health.
8. Vanderbilt Report. Technical Information:
VEEGUM: The versatile ingredient for
pharmaceutical formulations. R.T. Vander-
In: www.rtvanderbilt.com. R.T. Vander-
bilt Company, Inc., Norwalk, CT (USA);
2012.
9. Brent W, Gunderson Gigi H, Ross KHI,
John CR. What do we really know about
antibiotics pharmacodynamics? Pharma-
10. Cristina E, Ivan P, Kevin R. Nanomaterials
and nanoparticles: Sources and toxicity.
Biointerphases. 2007;2:MR17-MR71
11. Silva GA. Nanotechnology approaches to
crossing the blood brain barrier and drug
delivery to CNS. BMC Neuroscience.
2008;9:54. DOI:101186/1471-2220-9-53-
54 PMC. 2604882.

© 2015 Ezeibe and Ogbonna; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciedomain.org/review-history/10006