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Comment

Comment on "Photodegradation of sulfathiazole under simulated sunlight: Kinetics, photo-induced structural rearrangement, and antimicrobial activities of photoproducts" by Niu et al. [Water Research 124 2017 576–583]

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ABSTRACT

Recent efforts have employed antimicrobial susceptibility assays to describe the residual antimicrobial activity of antibiotics and their transformation products in a variety of environmental processes. Some authors have evaluated the results of these assays using the minimum inhibitory concentration (MIC); however, this approach has fundamental weaknesses. To highlight best practices, this comment describes the advantages of using dose-response curves to calculate the half maximal inhibitory concentration (IC_{50}) and the potential impacts of growth media on the antimicrobial activity of sulfonamide antibiotics.

1. Comment

The recent article by Niu et al. (2017) documented the photodegradation pathway and kinetics of sulfathiazole and investigated the residual antimicrobial activity of its photoproducts. This effort builds on earlier work from Boreen et al. (2004), who studied direct and indirect photolysis of sulfathiazole and four other sulfonamides with five-membered heterocyclic substituents. Wammer et al. (2006) investigated changes in the antibacterial activity of sulfathiazole and two other sulfonamides during photolysis, and found that photoproducts had no observable antimicrobial activity. Niu et al. (2017) made a similar conclusion. Here, we comment on the antimicrobial activity assay methodology and data analysis in Niu et al. (2017).

Clinicians and pharmacologists often use the minimum inhibitory concentration (MIC) as a metric for assessing the susceptibility of a specific bacterial strain to a particular antibiotic. The MIC is determined via bioassay, wherein bacterial inocula at approximately 1×10^6 colony forming units (CFU)/mL are exposed to serially diluted antibiotic solutions through a standardized protocol developed by the Clinical and Laboratory Standards Institute (CLSI, 2012). The inoculum is typically mixed with an equal volume of antibiotic solution and incubated for 16–20 h at 35 ± 2 °C. The MIC is defined as the lowest antibiotic concentration without visible bacterial growth (CLSI, 2012). We highlight two important points about MIC calculation: (1) the MIC is dependent on the antibiotic concentration of the stock solution and the serial dilution factor (typically, $2\times$); and, (2) the MIC is recorded through visual assessment and, therefore, subject to bias between individuals and laboratories.

Niu et al. (2017) reported an MIC of 55 mg/L for sulfathiazole; however, no methodology or data were provided to support this measurement. Presumably, the authors used an initial concentration of 55×2^n mg/L (where n is some integer), in accordance with the standard CLSI protocol (2012), which uses a serial dilution factor of $2\times$. For the sake of this discussion, let us assume that the initial sulfathiazole concentration was 220 mg/L, suggesting that the MIC was recorded after the second dilution: 220 mg/L diluted to

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110 mg/L (no *E. coli* growth observed); 110 mg/L diluted to 55 mg/L (no *E. coli* growth observed); and, 55 mg/L diluted to 27.5 mg/L (*E. coli* growth observed). During the phototransformation studies, Niu et al. (2017) employed an initial sulfathiazole concentration of 143 mg/L. By definition, this solution cannot demonstrate the same MIC as that reported above (*i.e.*, 55 mg/L). The dilution series for 143 mg/L sulfathiazole is as follows: 143 mg/L; 71.5 mg/L; 35.8 mg/L; 17.9 mg/L; and so on. For this reason, a disconnect inherently exists between the reported MIC value and the data that can be determined from the photodegradation studies. Using the data reported in Fig. 5 (Niu et al., 2017), the irradiated samples comprised 127 mg/L, 84 mg/L, and 71 mg/L sulfathiazole. None of these dilution series (*e.g.*, 127, 63.5, 31.8 mg/L; 84, 42, 21 mg/L; and, 71, 35.5, 17.8 mg/L) allows determination of a 55 mg/L MIC, precluding a fair comparison of the irradiated samples with the standard solution. If Niu et al. (2017) used another dilution scheme, the tested concentrations should be included for both sulfathiazole standards and irradiated samples to contextualize the relative resolution of the reported MIC values.

This analysis is further complicated by the evaluation of the estimated (MIC_{est}) and observed (MIC_{obs}) MIC values in Eqs. 9 and 10, respectively. In the discussion, Niu et al. (2017) assume that photoproducts have negligible antimicrobial activity. As sulfathiazole is photodegraded, Eqs. 9 and 10 suggest that MIC_{est} would increase and MIC_{obs} would decrease, respectively. These statements are not accurate. Actually, MIC_{obs} and MIC_{est} should be identical to MIC_{STZ} until the sulfathiazole concentration is lower than MIC_{STZ}, and then the MIC should increase sharply to reflect the low antimicrobial activity of the photoproducts. The MIC is a concentration threshold, and so MIC cannot be written as a function of the normalized concentration (C/C_0) without an appropriate definition of C_0. For this reason, Eqs. 9–10 are inherently incorrect and the analysis in Fig. 5 is flawed.

While the utility of the MIC parameter for pharmacological purposes is that it offers a conservative estimate of the antibiotic concentration needed to prevent bacterial growth, this parameter is not robust enough to be used as a measure of the antimicrobial activity associated with phototransformation products. For this reason, we recommend the use of dose-response curves. Fig. 1 shows experimental data for growth inhibition of *E. coli* (ATCC 25922) as a function of sulfathiazole and sulfadiazine concentration. Growth inhibition was calculated as previously reported (Snowberger et al., 2016). The data were fit using the Hill Equation, and 95% confidence bands were calculated by GraphPad Prism (La Jolla, California). The recorded dose-response curve precludes bias from visual assessment, initial concentration, and serial dilution factor (compared to MIC determination), as well as variations in environmental conditions (with appropriate use of positive/negative growth controls). For example, serially diluted 220 mg/L or 143 mg/L solutions in Iso-Sensitest broth (ISB) should both return an IC_{50} value of 4.93 ± 0.26 mg/L for sulfathiazole (see Fig. 1). As the IC_{50} value provides the highest sensitivity, this parameter should be used to compare the potency of serially-diluted antibiotic standards and experimental solutions.

According to the CLSI protocol (2012), the IC_{80} may be synonymous with the MIC. The IC_{80} for sulfathiazole in ISB (14.73 ± 1.30 mg/L, Fig. 1a) is lower than the 55 mg/L MIC reported by Niu et al. (2017), although the different bacterial strains (*i.e.*, *E. coli* ATCC 25922 in Fig. 1; *E. coli* NCTC 10418 in Niu et al., 2017) may be partially responsible for this difference. The bioassays shown in Fig. 1 were conducted using the microdilution protocol, whereas Niu et al. (2017) used the macrodilution approach. CLSI (2012) indicates that microdilution-based protocols are generally more sensitive than macrodilution and, therefore, more suitable for assessment of residual antimicrobial activity of environmental samples.

The mechanism of action for sulfonamides involves binding to dihydropteroate synthetase, which is responsible for conversion of *para*-aminobenzoate to dihydropteroate in the folate synthesis pathway (Capasso and Supuran, 2014). Folate is necessary for synthesis of nucleic acids. Many commercial growth media contain folate and may, therefore, interfere with growth inhibition assays involving sulfonamides. In this regard, previous researchers have highlighted interference from Luria-Bertani broth (Wammer et al., 2006). Niu et al. (2017) did not indicate what growth medium was used to generate the *E. coli* inoculum; however, we assume that they used Mueller-Hinton Broth (MHB). The Iso-Sensitest media was specifically designed to address shortcomings of Mueller-Hinton media with respect to certain antibiotics, including sulfonamides (Oxoid, 2017). In an inter-laboratory comparison, ISB and MHB performed similarly for a number of antibiotics, although the trimethoprim-sulfamethoxazole mixture was the only sulfonamide tested (Koeth et al., 2000). Here, ISB- and MHB-based *E. coli* inocula resulted in similar IC_{50} values for sulfathiazole (see Fig. 1a), but differences were observed for the less potent sulfadiazine (Fig. 1b). We, therefore, recommend using ISB to avoid interference during measurement of antimicrobial activity associated with sulfonamides.

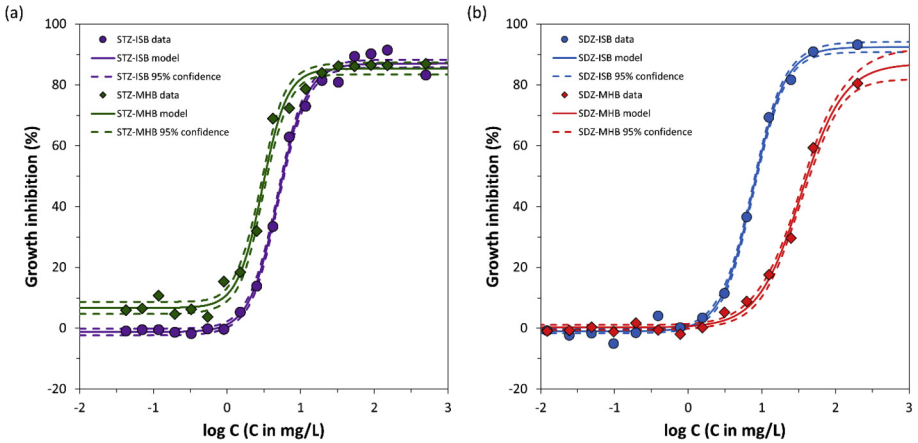

Fig. 1. Growth inhibition of *E. coli* (ATCC 25922) for (a) sulfathiazole (STZ) and (b) sulfadiazine (SDZ) in ISB and MHB growth media. The IC_{50} values for each sulfonamide and broth pair are as follows: $IC_{50,STZ,\ ISB} = 4.93 \pm 0.26$ mg/L; $IC_{50,STZ,\ MHB} = 3.02 \pm 0.27$ mg/L; $IC_{50,SDZ,ISB} = 7.62 \pm 0.40$ mg/L; $IC_{50,SDZ,MHB} = 33.80 \pm 4.13$ mg/L. Antimicrobial activity assays were run in quadruplicate following the CLSI (2012) microdilution protocol, and 95% confidence bands were generated using GraphPad Prism.

The recent article by Niu et al. (2017) contributes to the growing body of knowledge on photodegradation pathways, reaction kinetics, and residual antimicrobial activity of antibiotics and their photoproducts. With this comment, we hope to highlight best practices for analyzing the antimicrobial activity associated with sulfonamides and their transformation products.

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