Comparative In Vitro Activities of SMT19969, a New Antimicrobial Agent, against 162 Strains from 35 Less Frequently Recovered Intestinal Clostridium Species: Implications for Clostridium difficile Recurrence

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We determined the comparative activity of SMT19969 (SMT) against 162 strains representing 35 well-characterized Clostridium species in clusters I to XIX and 13 Clostridium species that had no 16S rRNA match. SMT MICs ranged from 0.06 to >512 μg/ml and were not species related. SMT might have less impact on normal gut microbiota than other Clostridium difficile infection (CDI) antimicrobials.

Clostridium difficile infections (CDI) have increased in frequency and severity over the past decade and are a leading cause of hospital-acquired infections, contributing to increased hospital length of stay and costs, as well as associated increased mortality, especially among the elderly (1, 2). Standard therapy has been associated with 20 to 30% relapse rates (3, 4). Consequently, new CDI therapeutic approaches have emerged.

Recurrences of CDI are associated with disruption in the patient microbiome, with changes in richness, evenness, and diversity (5). This antibiotic-induced depletion of normal microbiota allows C. difficile to proliferate, produce toxin, and cause disease. Several investigators have suggested that a reduced impact by antimicrobials on normal flora might lower the risk of recurrent disease, especially on the Bacteroides fragilis group species and Clostridium species cluster XIVa and, to a lesser extent, cluster IV, which contain a large number of butyrate-producing anaerobes (6–8).

SMT19969 (SMT) is a novel, narrow-spectrum, nonabsorbable agent with previously shown activity against C. difficile but with poor activity against B. fragilis (9). Information about its effect on other gut organisms is limited, including data about its activity against the other Clostridium species/clusters. Consequently, we studied the comparative in vitro activity of SMT19969 against 162 strains of Clostridium representing 35 well-characterized species and 13 strains with no PCR species match within 8 different Clostridium clusters, especially those of cluster XIVa.

Isolates were recovered from clinical specimens from 1985 to 2013. They were identified by standard methods (10, 11) and by 16S RNA gene sequencing as previously published (12) and stored in 20% skim milk at −70°C. They were taken from the freezer and transferred at least twice on supplemented brucella broth to ensure purity and good growth. Inocula were prepared by direct suspensions of cells into brucella broth to achieve the turbidity of the 0.5 McFarland standard. The final inoculum was ~10^5 CFU/spot. Susceptibility to SMT19969, fidaxomycin, vancomycin, and metronidazole was determined using the agar dilution method according to the CLSI approved standard for anaerobes (M11-A8) (13).

The results of this study are shown in Table 1. SMT MICs were variable (range, 0.06 to >512 μg/ml). Resistance (MIC > 32 μg/ml) was not cluster or species related and occurred in Clostridium ramosum (10 of 10 samples), Clostridium cadaveris (2 of 6), Clostridium colinoides (1 of 2), Clostridium glycolicum (2 of 5), Clostridium paraputrificum (6 of 8), Clostridium perfingens (9 of 11), Clostridium rectum (3 of 3), Clostridium sardiniense (1 of 1), Clostridium scindens (1 of 5), Clostridium sordellii (1 of 6), Clostridium sporogenes (3 of 5), and 8 of 13 Clostridium species with no species match by 16S RNA gene sequencing. MICs of ≥32 μg/ml also occurred with fidaxomycin (MIC range of ≤0.03 to >128 μg/ml), but these species were different from the SMT-resistant species. The MIC range for vancomycin was 0.5 to >32 μg/ml and for metronidazole was ≤0.06 to 16 μg/ml, with one or more strains of the unidentifiable Clostridium species showing decreased susceptibility or resistance.

Louie et al. (7) suggested that poor in vitro activity against aerobic and facultative Gram-negative bacteria, Bacteroides species, and other Gram-negative anaerobes would result in a “reduced ecological impact.” They performed fecal quantitative counts of Bacteroides species on patients receiving either vancomycin or fidaxomycin in a phase II trial and noted that fidaxomycin’s reduced activity was less suppressive. Tannock et al. (5) extended these observations on the fecal microbiota using temporal temperature gradient electrophoresis (TTGE) and quantification of phylogenetic groups using fluorescent in situ hybridization and flow cytometry (FISH/FC). In contrast to vancomycin, clostridial cluster XIVa and IV populations increased during and after fidaxomycin treatment. They postulated that this effect of these clusters and Bifidobacterium spp. might explain the reduced relapse rate of fidaxomycin in clinical trials.

Antharam et al. (6) studied the distal fecal flora of 39 patients with CDI and compared them to those of 36 C. difficile-colonized patients and 40 healthy controls. They found that there was a
| RMA no. | Species                        | Clostridial cluster | MIC (µg/ml) | SMT19969 | Fidaxomicin | Metronidazole | Vancomycin |
|---------|-------------------------------|---------------------|-------------|-----------|-------------|---------------|-------------|
| 18328   | Clostridium baratii           | I                   | >512        | ≤0.03     | 1           | 2             |             |
| 6392    | C. baratii-like               | I                   | 0.5         | ≤0.03     | 1           | 2             |             |
| 19025   | Clostridium butyricum         | I                   | 0.25        | 0.06      | 0.5         | 0.5           |             |
| 19848   | C. butyricum                 | I                   | 0.25        | ≤0.03     | 0.5         | 0.5           |             |
| 21418   | C. butyricum                 | I                   | 0.25        | 0.06      | 1           | 0.5           |             |
| 22044   | C. butyricum                 | I                   | 0.25        | 0.06      | 1           | 0.5           |             |
| 22081   | C. butyricum                 | I                   | 0.5         | 0.125     | 1           | 0.5           |             |
| 14198   | Clostridium cadaveris         | I                   | 1           | ≤0.03     | 0.125       | 2             |             |
| 16516   | C. cadaveris                 | I                   | 2           | ≤0.03     | 0.125       | 2             |             |
| 16863   | C. cadaveris                 | I                   | 1           | 0.06      | 0.125       | 4             |             |
| 18944   | C. cadaveris                 | I                   | 32          | ≤0.03     | 0.125       | 2             |             |
| 19962   | C. cadaveris                 | I                   | 256         | 0.06      | 0.125       | 2             |             |
| 20805   | C. cadaveris                 | I                   | 0.25        | ≤0.03     | 0.06         | 2             |             |
| 6433    | Clostridum colicani          | I                   | 0.5         | ≤0.03     | 2           | >32           |             |
| 6786    | C. colicani                  | I                   | 64          | ≤0.03     | 2           | 2             |             |
| 15999   | Clostridium disporicum       | I                   | 0.06        | ≤0.03     | 1           | 0.5           |             |
| 21544   | C. disporicum               | I                   | 0.25        | ≤0.03     | 0.25        | 0.25          |             |
| 12757   | Clostridium fallax           | I                   | 0.125       | ≤0.03     | 0.5         | 0.5           |             |
| 21095   | C. fallax                   | I                   | 0.06        | ≤0.03     | 1           | 1             |             |
| 12522   | Clostridium novyi A          | I                   | 0.25        | ≤0.03     | 1           | 0.5           |             |
| 15199   | Clostridium paraputrflicum   | I                   | 1           | 0.06      | 2           | 1             |             |
| 16518   | C. paraputrflicum            | I                   | 64          | ≤0.03     | 2           | 2             |             |
| 18947   | C. paraputrflicum            | I                   | 64          | ≤0.03     | 1           | 1             |             |
| 21627   | C. paraputrflicum            | I                   | 64          | ≤0.03     | 0.5         | 2             |             |
| 21630   | C. paraputrflicum            | I                   | 64          | ≤0.03     | 2           | 1             |             |
| 22852   | C. paraputrflicum            | I                   | 0.5         | ≤0.03     | 2           | 1             |             |
| 16521B  | C. paraputrflicum            | I                   | 64          | ≤0.03     | 1           | 1             |             |
| 16397A  | C. paraputrflicum            | I                   | 64          | ≤0.03     | 2           | 1             |             |
| 21966   | Clostridium perfringens       | I                   | 256         | ≤0.03     | 1           | 1             |             |
| 22113   | C. perfringens               | I                   | >512        | ≤0.03     | 2           | 1             |             |
| 22244   | C. perfringens               | I                   | >512        | ≤0.03     | 0.5         | 1             |             |
| 22245   | C. perfringens               | I                   | >512        | ≤0.03     | 1           | 1             |             |
| 22509   | C. perfringens               | I                   | >512        | 0.06      | 4           | 1             |             |
| 22671   | C. perfringens               | I                   | >512        | 0.06      | 4           | 1             |             |
| 22722   | C. perfringens               | I                   | >512        | ≤0.03     | 2           | 1             |             |
| 22810   | C. perfringens               | I                   | 64          | ≤0.03     | 1           | 1             |             |
| 22842   | C. perfringens               | I                   | 256         | ≤0.03     | 2           | 1             |             |
| 22885   | C. perfringens               | I                   | 1           | ≤0.03     | 0.5         | 1             |             |
| 23087   | C. perfringens               | I                   | 8           | ≤0.03     | 4           | 1             |             |
| 21091   | Clostridium sardinense       | I                   | >512        | ≤0.03     | 4           | 32            |             |
| 9638    | Clostridium sporogenes       | I                   | 0.5         | 0.06      | 0.25        | 4             |             |
| 10379   | C. sporogenes                | I                   | 64          | 0.06      | 0.25        | 2             |             |
| 10900   | C. sporogenes                | I                   | 64          | 0.06      | 0.25        | 4             |             |
| 15061   | C. sporogenes                | I                   | 64          | 0.06      | 0.25        | 2             |             |
| 16077   | C. sporogenes                | I                   | 4           | ≤0.03     | ≤0.06       | 2             |             |
| 15329   | Clostridium subterminale group| I                   | 0.125       | ≤0.03     | 0.25        | 1             |             |
| 18693   | C. subterminale group        | I                   | 2           | ≤0.03     | 0.5         | 1             |             |
| 19908   | C. subterminale group        | I                   | 0.125       | ≤0.03     | 0.5         | 0.5           |             |
| 20775   | C. subterminale group        | I                   | 0.5         | ≤0.03     | 0.25        | 1             |             |
| 8622B   | C. subterminale group        | I                   | ≤0.03       | ≤0.03     | 0.5         | 1             |             |
| 14609   | Clostridium tertium          | I                   | 0.5         | ≤0.03     | 1           | 2             |             |
| 16273   | C. tertium                  | I                   | 1           | ≤0.03     | 1           | 2             |             |
| 18836   | C. tertium                  | I                   | 4           | ≤0.03     | 2           | 2             |             |
| 19847   | C. tertium                  | I                   | 4           | ≤0.03     | 1           | 2             |             |
| 22841   | C. tertium                  | I                   | 0.5         | 0.06      | 2           | 2             |             |
| 18623   | Clostridium bartlettii       | XI                  | 1           | ≤0.03     | 1           | 2             |             |
| 5262    | Clostridium bifermentans     | XI                  | 0.125       | ≤0.03     | 0.5         | 0.5           |             |
| 5324    | C. bifermentans              | XI                  | 0.5         | ≤0.03     | 0.5         | 0.5           |             |
| 9640    | C. bifermentans              | XI                  | 0.5         | ≤0.03     | 0.5         | 0.5           |             |
| 9897    | C. bifermentans              | XI                  | 0.5         | ≤0.03     | 0.5         | 0.5           |             |

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| RMA no. | Species                  | Clostridial cluster | SMT19969 | Fidaxomicin | Metronidazole | Vancomycin |
|---------|-------------------------|---------------------|-----------|-------------|---------------|------------|
| 9948    | C. bifermantans         | XI                  | 0.5       | ≤0.03       | 0.5           | 1          |
| 21658   | C. bifermantans         | XI                  | 0.25      | ≤0.03       | 1             | 0.5        |
| 9388B   | C. bifermantans         | XI                  | 0.25      | ≤0.03       | 2             | 0.5        |
| 8910    | Clostridium glycolicum  | XI                  | 32        | ≤0.03       | 0.125         | 0.5        |
| 14467   | C. glycolicum           | XI                  | 0.5       | 0.5         | 0.25          | 0.5        |
| 15023   | C. glycolicum           | XI                  | 0.5       | 0.5         | 0.125         | 0.5        |
| 16312   | C. glycolicum           | XI                  | 0.5       | 0.25        | 0.25          | 2          |
| 7121    | C. glycolicum-like      | XI                  | 32        | 1           | 0.25          | 0.5        |
| 22811   | Clostridium mayomebi-like | XI            | 0.5      | 0.5         | 1             | 0.25       |
| 16782   | Clostridium sordellii   | XI                  | 1         | ≤0.03       | 2             | 1          |
| 18788   | C. sordellii            | XI                  | 64        | ≤0.03       | 4             | 1          |
| 21861   | C. sordellii            | XI                  | 16        | ≤0.03       | 4             | 0.5        |
| 21976   | C. sordellii            | XI                  | 1         | ≤0.03       | 8             | 1          |
| 22672   | C. sordellii            | XI                  | 8         | 0.125       | 4             | 1          |
| 4634    | C. sordellii-like       | XI                  | 2         | ≤0.03       | 1             | 1          |
| 16057   | Clostridium aldeneense  | XIVa                | 0.5       | 64          | ≤0.06         | 1          |
| 18348   | C. aldeneense           | XIVa                | 0.5       | 64          | ≤0.06         | 1          |
| 18939   | C. aldeneense           | XIVa                | 0.5       | 64          | ≤0.06         | 1          |
| 23550   | C. aldeneense           | XIVa                | 0.125     | 64          | ≤0.06         | 2          |
| 20918A  | Clostridium aminovalericum | XIVa              | 0.25      | 2           | 0.25          | 8          |
| 10036   | Clostridium bolteae     | XIVa                | 0.25      | 128         | ≤0.06         | 1          |
| 18941   | C. bolteae              | XIVa                | 0.5       | 128         | 0.125         | 2          |
| 21972   | C. bolteae              | XIVa                | 0.125     | 64          | 0.125         | 1          |
| 22131   | C. bolteae              | XIVa                | 0.5       | 64          | ≤0.06         | 1          |
| 12934   | Clostridium celercrencs | XIVa                | 0.125     | 8           | 0.5           | 1          |
| 19024   | C. celercrencs          | XIVa                | 0.25      | 32          | 0.5           | 1          |
| 19963   | C. celercrencs          | XIVa                | 0.5       | 32          | 0.5           | 1          |
| 15980   | Clostridium citroniae   | XIVa                | 0.125     | 64          | 0.25          | 1          |
| 21971   | C. citroniae            | XIVa                | 0.125     | 128         | 0.125         | 1          |
| 23088   | C. citroniae            | XIVa                | 0.125     | 64          | 0.125         | 1          |
| 16102A  | C. citroniae            | XIVa                | 0.06      | 64          | ≤0.06         | 1          |
| 16521A  | C. citroniae            | XIVa                | 0.25      | 64          | ≤0.06         | 1          |
| 20713   | Clostridium clostridioforme | XIVa             | 0.125     | 64          | 0.25          | 1          |
| 21282   | C. clostridioforme      | XIVa                | 0.25      | 128         | ≤0.06         | 1          |
| 21626   | C. clostridioforme      | XIVa                | 0.25      | >128        | ≤0.06         | 2          |
| 22060   | C. clostridioforme      | XIVa                | 0.125     | 128         | ≤0.06         | 2          |
| 22084   | C. clostridioforme      | XIVa                | 0.25      | 128         | ≤0.06         | 2          |
| 18723   | Clostridium hathewayi   | XIVa                | 0.125     | 32          | 0.25          | 0.5        |
| 20145   | C. hathewayi            | XIVa                | 0.125     | 16          | 0.125         | 0.5        |
| 20647   | C. hathewayi            | XIVa                | 0.5       | 16          | 0.5           | 0.5        |
| 21975   | C. hathewayi            | XIVa                | 0.125     | 2           | 0.125         | 0.5        |
| 2489    | Clostridium hylemonae   | XIVa                | 0.06      | ≤0.03       | 0.5           | 1          |
| 13503   | C. hylemonae            | XIVa                | 0.5       | 0.25        | 0.25          | 2          |
| 15944   | C. hylemonae            | XIVa                | 0.5       | 0.25        | 0.125         | 2          |
| 16423   | C. hylemonae            | XIVa                | 0.5       | 0.25        | 0.125         | 2          |
| 16895   | C. hylemonae            | XIVa                | 0.5       | 0.25        | 0.125         | 2          |
| 18591   | C. hylemonae            | XIVa                | 0.5       | 0.25        | 0.25          | 2          |
| 22200   | C. hylemonae            | XIVa                | 0.5       | 0.25        | 0.25          | 2          |
| 15073A  | C. hylemonae            | XIVa                | 0.5       | 0.25        | 0.125         | 2          |
| 10628   | Clostridium lavalense   | XIVa                | 0.5       | 0.06        | 0.125         | 1          |
| 12736   | Clostridium sciendens   | XIVa                | 0.125     | 0.02        | 0.125         | 0.5        |
| 21863   | C. sciendens            | XIVa                | 0.06      | 0.06        | 0.125         | 0.5        |
| 21878   | C. sciendens            | XIVa                | 0.66      | 1           | 0.5           | 0.5        |
| 22045   | C. sciendens            | XIVa                | 0.125     | 0.06        | 0.25          | 0.5        |
| 22624   | C. sciendens            | XIVa                | 0.25      | 1           | 0.25          | 0.5        |
| 20753   | Clostridium symbiosum   | XIVa                | 0.25      | 4           | 0.125         | 1          |
| 21214   | C. symbiosum            | XIVa                | 0.25      | 2           | 0.125         | 0.5        |
| 21868   | C. symbiosum            | XIVa                | 0.5       | 2           | 0.125         | 1          |
| 22082   | C. symbiosum            | XIVa                | 0.25      | 2           | 0.25          | 1          |
| 22366   | C. symbiosum            | XIVa                | 0.125     | 2           | 0.125         | 1          |

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“paucity of Firmicutes sequences in the aggregate gut microbiota” in the CDI and *C. difficile*-colonized patients compared to in controls. The majority (68.4%) of Firmicutes were clostridia, and “strikingly members of Clostridium cluster XIVa” and to a lesser extent cluster IV” were depleted in those CDI and colonized patients. They suggested that “mechanistic studies focusing on the functional roles of these organisms in diarrheal diseases and *C. difficile* colonization resistance” be performed.

Previously, Goldstein et al. (9) studied the comparative in vitro activity of SMT19969 against 174 Gram-positive and 136 Gram-negative intestinal anaerobes and 40 Gram-positive aerobes. SMT19969 was generally less active against Gram-negative anaerobes, especially the *Bacteroides fragilis* group species, than vancomycin and metronidazole, suggesting a lesser impact on the normal intestinal microbiota that maintain colonization resistance. SMT19969 showed limited activity against other Gram-positive anaerobes, including *Bifidobacterium* species, *Eggerthella lenta*, *Fusobacteria magnus*, and *Peptostreptococcus anaerobius*, with MIC values of >512, >512, 64, and 64 μg/ml, respectively. This suggested that SMT19969’s selective activity makes it an excellent candidate for therapy of CDI.

Our current study extends these observations to 162 *Clostridium* strains representing 35 species within 8 clusters. *Clostridium* species showed varied susceptibility to SMT19969. *Clostridium innocuum* (cluster XVII) was susceptible (MIC<sub>90</sub> of 1 μg/ml) and *C. ramosum* (cluster XVI) and *C. perfringens* (cluster I) were non-susceptible (MIC<sub>90</sub> of >512 μg/ml) to SMT19969. Against *Clostridium* cluster XIVa, the MICs ranged from 0.125 to 64 μg/ml.

### TABLE 1 (Continued)

| RMA no. | Species | Clostridial cluster | MIC (μg/ml) |
|---------|---------|---------------------|-------------|
|         |         |                     | SMT19969<sup>a</sup> | Fidaxomicin | Metronidazole | Vancomycin |
| 20132   | *Clostridium xylanolyticum* | XIVa | 0.125 | 16 | 1 | 0.5 |
| 15167   | *Clostridium lactifermentans* | XIVb | 0.06 | 0.06 | 0.25 | >32 |
| 5491    | *Clostridium innocuum* | XVI | 0.25 | >128 | 2 | 8 |
| 5615    | *C. innocuum* | XVI | 0.25 | >128 | 1 | 16 |
| 20638   | *C. innocuum* | XVI | 1 | 256 | 0.5 | 16 |
| 20645   | *C. innocuum* | XVI | 0.25 | 256 | 0.5 | 16 |
| 20648   | *C. innocuum* | XVI | 0.25 | 128 | 0.5 | 16 |
| 20913   | *C. innocuum* | XVI | 0.25 | 256 | 1 | 16 |
| 21213   | *C. innocuum* | XVI | 0.06 | 256 | 1 | 16 |
| 21737   | *C. innocuum* | XVI | 1 | 256 | 1 | 16 |
| 21860   | *C. innocuum* | XVI | 0.25 | 256 | 1 | 16 |
| 21903   | *C. innocuum* | XVI | 0.25 | 256 | 16 | 16 |
| 22441   | *C. innocuum* | XVI | 0.25 | 512 | 0.5 | 16 |
| 23130   | *C. innocuum* | XVI | 0.125 | 128 | 2 | 16 |
| 10072   | *Clostridium rectum*-like | XIX | >512 | >128 | 0.5 | >32 |
| 14707   | *C. rectum*-like | XIX | >512 | >128 | 1 | >32 |
| 16549   | *C. rectum*-like | XIX | 0.03 | 0.25 | 1 | 16 |
| 20917   | *Clostridium ramosum* | XVIII | >512 | >512 | 0.5 | 4 |
| 21212   | *C. ramosum* | XVIII | >512 | >512 | 0.5 | 4 |
| 21215   | *C. ramosum* | XVIII | >512 | >512 | 0.5 | 4 |
| 21414   | *C. ramosum* | XVIII | >512 | >512 | 8 | 4 |
| 21738   | *C. ramosum* | XVIII | 128 | >512 | 0.5 | 4 |
| 21862   | *C. ramosum* | XVIII | >512 | >512 | 0.5 | 4 |
| 21902   | *C. ramosum* | XVIII | 512 | >512 | 0.5 | 4 |
| 21974   | *C. ramosum* | XVIII | 512 | >512 | 1 | 4 |
| 22193   | *C. ramosum* | XVIII | >512 | >512 | 1 | 4 |
| 22623   | *C. ramosum* | XVIII | >512 | >512 | 1 | 4 |
| 705     | *Clostridium* species | 0.5 | =0.03 | 0.125 | 1 |
| 9906    | *Clostridium* species | >512 | 129 | 0.125 | 32 |
| 10271   | *Clostridium* species | 1 | =0.03 | 2 | 0.5 |
| 14137   | *Clostridium* species | 32 | >128 | 2 | >32 |
| 16187   | *Clostridium* species | >512 | >128 | 2 | >32 |
| 16338   | *Clostridium* species | >512 | >128 | 2 | >32 |
| 19909   | *Clostridium* species | 0.25 | >128 | 16 | 16 |
| 21472   | *Clostridium* species | 32 | >128 | 4 | >32 |
| 21876   | *Clostridium* species | 0.25 | =0.03 | 1 | 8 |
| 22256   | *Clostridium* species | 32 | =0.03 | 0.25 | 1 |
| 22279   | *Clostridium* species | 32 | =0.03 | 0.25 | 1 |
| 15596B  | *Clostridium* species | 0.06 | =0.03 | 0.05 | 1 |
| 18576W  | *Clostridium* species | 32 | 0.06 | 0.125 | 2 |
| 16034   | *Flavonifractor plautii* | 0.06 | =0.03 | 0.25 | 8 |
| 22112   | *Robinsoniella* species | 0.06 | 2 | 2 | 2 |

<sup>a</sup> RMA, R.M. Alden Research Laboratory number.

<sup>b</sup> SMT19969, Summit 19969.
were species specific. Comparatively, XIVa isolates, except for *Clostridium hylemonae*, *Clostridium lavalense*, and some *C. scindens* isolates, had higher MICs to fidaxomicin (0.006 to >128 μg/ml) and vancomycin (0.5 to 2 μg/ml) and lower MICs to metronidazole (0.05 to 1 μg/ml). SMT19969 had higher MICs than fidaxomicin against *C. paraputrificum* (cluster I) and *C. sordellii* (cluster XI).

These data show that SMT19969’s activity was variable according to *Clostridium* species and strains within species. Coupled with its lack of activity against *B. fragilis* and aerobic enteric flora, it might have a lesser impact than other antimicrobials used for CDI therapy on the normal gut microbiota that maintains colonization resistance. Further evaluation by clinical trials seems warranted.

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