

1 **Supplementary information**

2 **Screening assay**

3 Two drug libraries were screened at a fixed concentration of 16 μ M against one strain of *C. difficile*
4 (*C. difficile* NAP07) to identify active compounds. Briefly, bacteria were streaked on
5 supplemented brain heart infusion (BHIS) agar plates and incubated anaerobically at 37 °C for 48
6 hours. Colonies were scraped off the agar plates, suspended in PBS and diluted in BHIS broth at
7 a concentration of about 5×10^5 CFU/mL. Compounds, at a concentration of 16 μ M, were mixed
8 with the bacterial suspension in 96-well plates and incubated anaerobically for 48 hours at 37 °C.
9 Drugs that inhibited bacterial growth visually were considered as hits.

10 **Supplementary Table 1: Active drugs from Pharmakon library**

	Compound name	Indication
1	Amoxicillin	Antibacterial
2	Bacitracin	Antibacterial
3	Carbenicillin disodium	Antibacterial
4	Demeclocycline hydrochloride	Antibacterial
5	Furazolidone	Antibacterial
6	Fusidic acid	Antibacterial
7	Gramicidin	Antibacterial
8	Hetacillin potassium	Antibacterial
9	Minocycline hydrochloride	Antibacterial
10	Nafcillin sodium	Antibacterial
11	Oxytetracycline	Antibacterial
12	Penicillin g potassium	Antibacterial
13	Penicillin v potassium	Antibacterial
14	Piperacillin sodium	Antibacterial
15	Vancomycin hydrochloride	Antibacterial
16	Flumequine	Antibacterial
17	Methacycline hydrochloride	Antibacterial
18	Meclocycline sulfosalicylate	Antibacterial
19	Monensin sodium	Antibacterial
20	Marbofloxacin	Antibacterial
21	Linezolid	Antibacterial
22	Clinafoxacin hydrochloride	Antibacterial
23	Azlocillin sodium	Antibacterial
24	Bacampicillin hydrochloride	Antibacterial
25	Thiamphenicol	Antibacterial
26	Lasalocid sodium	Antibacterial

27	Cefpiramide	Antibacterial
28	Levofloxacin	Antibacterial
29	Moxifloxacin hydrochloride	Antibacterial
30	Enrofloxacin	Antibacterial
31	Thiostrepton	Antibacterial
32	Gatifloxacin	Antibacterial
33	Sarafloxacin hydrochloride	Antibacterial
34	Nithiamide	Antibacterial
35	Florfenicol	Antibacterial
36	Teicoplanin [a(2-1) shown]	Antibacterial
37	Gemifloxacin mesylate	Antibacterial
38	Nadifloxacin	Antibacterial
39	Pazufloxacin mesylate	Antibacterial
40	Clofoctol	Antibacterial
41	Salinomycin, sodium	Antibacterial
42	Alexidine hydrochloride	Antibacterial
43	Nitroxoline	Antibacterial
44	Nifuroxazide	Antibacterial
45	Chlorhexidine dihydrochloride	Antibacterial (topical), disinfectant
46	Rifampin	Antibacterial (tuberculostatic)
47	Streptomycin sulfate	Antibacterial (tuberculostatic)
48	Gentian violet	Antibacterial, anthelmintic
49	Tetracycline hydrochloride	Antibacterial, antiamebic, antirickettsial
50	Chlortetracycline hydrochloride	Antibacterial, antiamebic, Calcium chelator,
51	Pyrithione zinc	Antibacterial, antifungal, antiseborrheic
52	Clofazimine	Antibacterial, antilepreptic, antituberculosis
53	Chloramphenicol palmitate	Antibacterial, antirickettsial
54	Chloramphenicol sodium succinate	Antibacterial, antirickettsial
55	Chloramphenicol	Antibacterial, antirickettsial
56	Narasin	Antibacterial, antiviral
57	Nitromide	Antibacterial, coccidiostat
58	Difloxacin hydrochloride	Antibacterial, dna gyrase inhibitor
59	Rifaximin	Antibacterial, rna synthesis inhibitor
60	Dihydrostreptomycin	Antibacterial, tuberculostatic
61	Doxycycline hydrochloride	Antibacterial
62	Methicillin sodium	Antibacterial
63	Cetrimonium bromide	Antiinfective
64	Methylbenzethonium chloride	Antiinfective
65	Triclosan	Antiinfective
66	Benzethonium chloride	Antiinfective (topical)
67	Cetylpyridinium chloride	Antiinfective (topical)
68	Hexachlorophene	Antiinfective (topical)
69	Nitrofurazone	Antiinfective (topical)
70	Thonzonium bromide	Mucolytic, antibacterial, surface active agent
71	Sanguinarium chloride	Antineoplastic, antiplaque agent
72	Bithionate sodium	Anthelmintic, antiseptic
73	Norgestimate	Progestin
74	Clotrimazole	Antifungal

75	Diethylstilbestrol	Estrogen
76	Estradiol valerate	Estrogen
77	Methylprednisolone	Antiinflammatory, glucocorticoid
78	Methylthiouracil	Antithyroid agent
79	Metoclopramide hydrochloride	Antiemetic
80	Metoprolol tartrate	Antihypertensive, antianginal
81	Metronidazole	Anthelmintic
82	Miconazole nitrate	Antifungal (topical)
83	Chloroquine diphosphate	Anthelmintic, antirheumatic, intercalating agent
84	Phenylmercuric acetate	Antifungal, antimicrobial
85	Proglumide	Anticholinergic
86	Sulconazole nitrate	Antifungal
87	Ronidazole	Anthelmintic
88	Econazole nitrate	Antifungal
89	Tretinoin	Keratolytic, antiacne, antineoplastic
90	Tinidazole	Anthelmintic
91	Nifursol	Anthelmintic
92	Diclazuril	Coccidiostat
93	Chloroxine	Chelating agent, antiseborrheic
94	Niclosamide	Anthelmintic, teniacide
95	Benzalkonium chloride	Preservative
96	Nitazoxanide	Anthelmintic
97	Prednicarbate	Antiinflammatory, glucocorticoid
98	Teniposide	Antineoplastic
99	Butoconazole	Antifungal
100	Bifonazole	Antifungal, calmodulin antagonist
101	Oxiconazole nitrate	Antifungal
102	Tioconazole	Antifungal
103	Benzbromarone	Uricosuric
104	Ornidazole	Anthelmintic
105	Toremifene citrate	Antineoplastic, anti-estrogen
106	Dichlorophen	Anthelmintic
107	Broxaldine	Anthelmintic, antifungal
108	Secnidazole	Anthelmintic, antitrichomonas
109	Oxyclozanide	Anthelmintic
110	Triclabendazole	Anthelmintic
111	Dimetridazole	Anthelmintic

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12 **Supplementary Table 2: Active drugs from Johns Hopkins library**

	Name	Indication
1	Potash, sulfurated	Antibacterial
2	Pyrrithione zinc	Antibacterial
3	Acriflavine hydrochloride	Antibacterial
4	Bismuth tribromophenate	Antibacterial
5	Chloramine-t hydrate	Antibacterial
6	Chlorquinaldol	Antibacterial

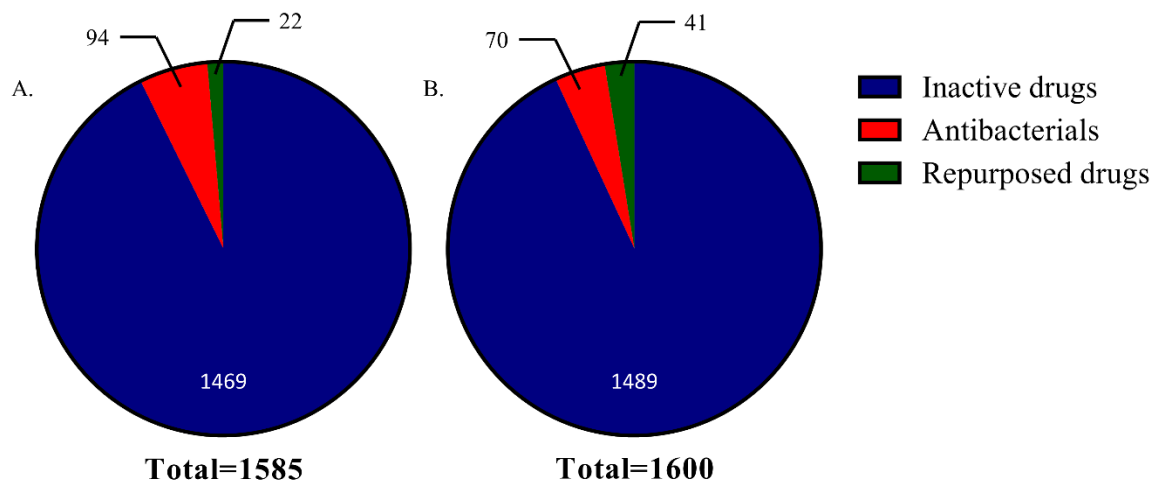
7	Dapsone	Antibacterial
8	Fenticlor	Antibacterial
9	Diclazuril	Antibacterial
10	Carbadox	Antibacterial
11	Cloxyquin	Antibacterial
12	Dihydrostreptomycin, dihydrostreptomycin sulfate	Antibacterial
13	Ornidazole	Antibacterial
14	Nebramycin	Antibacterial
15	Domiphen bromide	Antibacterial (topical)
16	Cetalkonium chloride	Antibacterial (topical)
17	Clioquinol	Antibacterial (topical)
18	Nifuroxime	Antibacterial (topical)
19	Rifampicin (rifampin)	Antibacterial (tuberculostatic)
20	3-formyl rifamycin	Antibacterial (tuberculostatic)
21	Clofazimine	Antibacterial (tuberculostatic, leprostatic)
22	Amikacin	Antibiotic
23	Benzoylpenicillin calcium	Antibiotic
24	Carbenicillin	Antibiotic
25	Cefpiramide	Antibiotic
26	Daptomycin	Antibiotic
27	Chloramphenicol	Antibiotic
28	Gatifloxacin	Antibiotic
29	Doxycycline	Antibiotic
30	Doxycycline hyclate	Antibiotic
31	Meclocycline sulfosalicylate salt	Antibiotic
32	Mezlocillin sodium	Antibiotic
33	Kanamycin	Antibiotic
34	Levofloxacin hcl	Antibiotic
35	Linezolid	Antibiotic
36	Meclocycline	Antibiotic
37	Nitrofurazone	Antibiotic
38	Minocycline	Antibiotic
39	Minocycline hydrochloride salt	Antibiotic
40	Moxifloxacin hcl	Antibiotic
41	Nafcillin sodium salt monohydrate	Antibiotic
42	Nitrofurantoin	Antibiotic
43	Penicillin g	Antibiotic
44	Penicillin g (procaine salt)	Antibiotic
45	Penicillin g potassium	Antibiotic
46	Penicillin v potassium	Antibiotic
47	Piperacillin-tazobactam	Antibiotic
48	Cefamandole	Antibiotic
49	Cefoxitin	Antibiotic
50	Cefepime hydrochloride	Antibiotic

51	Cefixime	Antibiotic
52	Cefoperazone sodium salt	Antibiotic
53	Ceforanide	Antibiotic
54	Tetracycline	Antibiotic
55	Vancomycin hcl hydrate	Antibiotic
56	Florfenicol	Antibiotic
57	Clinafloxacin hcl	Antibiotic
58	Clofoctol	Antibiotic
59	Dibekacin	Antibiotic
60	Rifamycin sv	Antibiotic
61	Rifaximin	Antibiotic
62	Metampicillin	Antibiotic
63	Nitroxoline	Antibiotic
64	Pazufloxacin	Antibiotic
65	Penimepicycline	Antibiotic
66	Pipemidic acid	Antibiotic
67	Tosufloxacin	Antibiotic
68	Sarafloxacin hcl	Antibiotic
69	Thiamphenicol	Antibiotic
70	Thiostrepton	Antibiotic
71	Amoxicillin	Antibiotic
72	Chlortetracycline, chlortetracycline hydrochloride	Antibiotic
73	Dicloxacillin, dicloxacillin sodium, dicloxacillin sodium salt	Antibiotic
74	Monensin, monensin sodium salt	Antibiotic
75	Brilliant green	Antiseptic
76	5-chlorosalicylanilide	Antiseptic
77	Ammonium citrate (ammonium hydrogencitrate)	Antiseptic
78	Castile soap	Antiseptic
79	Cetylpyridinium	Antiseptic
80	Cetylpyridinium bromide monohydrate	Antiseptic
81	Chlorhexidine gluconate	Antiseptic
82	Methylbenzethonium chloride	Antiseptic
83	Methyl Dopate hydrochloride	Antiseptic
84	Sodium iodate	Antiseptic
85	Tetradecyl sulfate, sodium salt	Antiseptic
86	Thonzonium bromide	Antiseptic
87	Triclosan	Antiseptic
88	Benzododecinium chloride	Antiseptic
89	Pidolic acid	Antiseptic
90	Trilocarban (3,4,4'-trichlorocarbanilide, 99%)	Antiseptic
91	Bithionol	Antiseptic
92	Gentian violet	Antiseptic
93	Benzethonium chloride	Antiseptic
94	Benzalkonium chloride	Antiseptic

95	Bithionoloxide	Anthelmintic bithionol
96	Dichlorophene	Anthelmintic (cestodes)
97	Dithiazanine iodide	Anthelmintic (nematodes)
98	Closantel	Anthelmintic.
99	Tinidazole	Antiprotozoal
100	Ronidazole	Antiprotozoal
101	Nifursol	Antiprotozoal (poultry)
102	Metronidazole	Antiprotozoal (trichomonas)
103	Nithiamide	Antiprotozoal (trichomonas).
104	Benznidazole	Antiprotozoal (trypanosoma)
105	Secnidazole	Antiamebic
106	Oxiconazole nitrate	Antifungal
107	Clotrimazole	Antifungal
108	Econazole	Antifungal
109	Sulconazole	Antifungal
110	Butoconazole nitrate	Antifungal (topical)
111	Miconazole	Antifungal (topical)
112	Tioconazole	Antifungal (topical)
113	Alprenolol, alprenolol hydrochloride	Antihypertensive
114	Quinaldine blue (pinacyanol chloride)	Antineoplastic
115	Puromycin	Antineoplastic
116	Rose bengal	Diagnostic aid (corneal trauma indicator).

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 16 **Supplementary Figure 1: Anticlostridial activity of drugs in Johns Hopkins and**
 17 **Pharmakon drug libraries.** Both Johns Hopkins (A) and Pharmakon (B) Libraries were
 18 screened against a single strain of *C. difficile* (Strain NAP07) at a concentration of 16 μ M.
 19 Active drugs were divided into two classes; drugs with known antibacterial activity (red) and
 20 repurposed drugs (green). Repurposed drugs from both libraries were combined for further
 21 testing.
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