

## RESEARCH ARTICLE

Screening novel antiviral compounds to treat *Clostridioides difficile* infectionsBrice J. Stoltz<sup>1,2</sup>, Ahmed A. Abouelkhair<sup>1,2</sup>, Mohamed N. Seleem<sup>1,2\*</sup>

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## Abstract

*Clostridioides difficile* is a major cause of nosocomial infections, often associated with individuals who have gut dysbiosis from previous antibiotic therapies. *C. difficile* infections (CDI) have a high recurrence rate and impose significant financial and mortality burdens on the healthcare system. Therefore, novel anti-*C. difficile* drugs are urgently needed to treat and reduce the severity and recurrence of infection. In this study, we screened a library of 618 antiviral drugs to identify a potential candidate for repurposing as novel anti-*C. difficile* therapeutics. Following our preliminary screening, we identified 9 novel compounds that inhibited *C. difficile* at a concentration of 16  $\mu$ M or lower. Among these, 4 antiviral compounds demonstrated the most potent anti-*C. difficile* activity against a panel of 15 *C. difficile* isolates, with minimum inhibitory concentrations (MICs) comparable to the drug of choice, vancomycin. These include rottlerin (MIC<sub>50</sub> = 0.25  $\mu$ g/mL),  $\alpha$ -mangostin (MIC<sub>50</sub> = 1  $\mu$ g/mL), dryocrassin ABBA (MIC<sub>50</sub> = 1  $\mu$ g/mL), and obelafazod (MIC<sub>50</sub> = 4  $\mu$ g/mL). All exhibited minimal to no activity against representative members of the human gut microbiota. Interestingly,  $\alpha$ -mangostin, a natural xanthone derived from the mangosteen fruit, exhibited strong bactericidal action, clearing a high inoculum of *C. difficile* in less than an hour. All other drugs exhibited bacteriostatic activity. Given their characteristics, these compounds show great promise as novel treatments for CDI.

## Introduction

Healthcare-associated infections are one of the greatest burdens on healthcare systems. Most bacteria responsible for a part of this burden are associated with antimicrobial resistance, with one notable exception: *C. difficile*. Despite lacking a significant antimicrobial resistance profile, *C. difficile* is still considered an urgent threat and one of the deadliest bacterial infections responsible for approximately 30,000 deaths annually, primarily affecting older populations [1–5]. The incidence of *C. difficile* infections (CDI) in the United States has increased over the last two decades and has remained relatively stable from 2021 to 2022, with the COVID-19 pandemic seeing a drop in cases due to improved strategies preventing the spread of

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