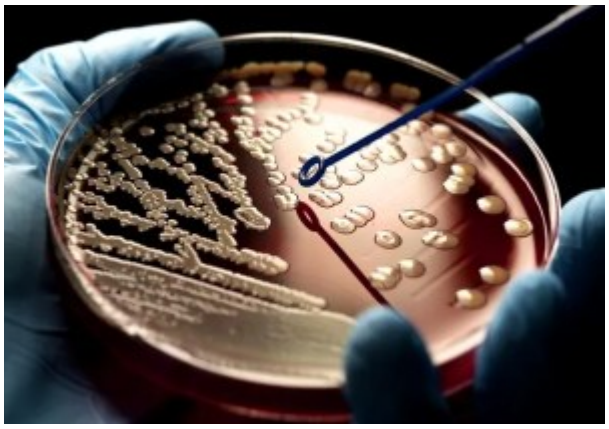
Researchers from the United States investigated the ability of different classes of antiviral drugs to induce antimicrobial resistance and contribute to cross-resistance to other antiviral and antibiotic drugs. They also performed whole genome sequencing of antiviral-resistant strains. A culture-based study, using the Gram-negative bacteria *Escherichia coli* and the Gram-positive bacteria *Bacillus cereus* showed that antiviral drugs have previously unrecognized antibacterial properties. In addition, most antiviral-resistant strains had fewer than 10 unique genetic alterations compared with wild type, with most genetic alterations occurring in genes that are not known to be involved in antibiotic resistance.

Antiviral drugs are intended to target viral replication, but, they can also have off-target effects such as the inhibition of bacterial growth. The antibacterial activities of antiviral drugs affect the presence, development, spread, and treatment of antimicrobial resistant infections.



About the study

The study evaluated antibacterial activity of fourteen antiviral drugs belonging to four distinct antiviral classes, including antiherpetic, nucleoside reverse transcriptase inhibitors, integrase inhibitors, and non-nucleoside reverse transcriptase inhibitors against *Escherichia coli* and *Bacillus cereus*.

Antivirals were tested at concentrations ranging from 0.1–100 µg/mL over a period of 0–24 hours. These concentrations covered inhibitory and sub-inhibitory concentrations that had been shown in previous *in vitro* tests with bacteria. The concentration ranges of antiviral drugs also included therapeutic or circulating concentrations, such as the plasma concentration of zidovudine (0.016–1.7 mg/L) and the therapeutic window for efavirenz (1–4 mg/L).

Results

Antiviral-resistant mutants of *Escherichia coli* and *Bacillus cereus* were successfully isolated after being repeatedly exposed to antiviral drugs with demonstrated antibacterial properties. The results show that antivirals can significantly alter the phenotypic antimicrobial resistance profiles of *Escherichia coli* and *Bacillus cereus,* which was previously unknown.

Eight of fourteen antivirals tested inhibited the growth of *E. coli*, while only three of fourteen antivirals showed antibacterial activity against *B. cereus.* The nucleoside analogs acyclovir, didanosine, lamivudine, stavudine, and zidovudine significantly inhibited *E. coli* but not *B. cereus.* Dolutegravir, efavirenz, and raltegravir significantly reduced the growth of both *E. coli* and *B. cereus*.

The bacteria exposed to the antivirals zidovudine, dolutegravir and raltegravir developed cross-resistance to the commonly used antibiotics trimethoprim, tetracycline, clarithromycin, erythromycin, and amoxicillin.

Whole genome sequencing of antiviral-resistant *E. coli* isolates revealed numerous unique single base pair mutations, as well as multi-base pair insertions and deletions, in genes known or suspected to play a role in antimicrobial resistance, such as genes encoding multidrug efflux pumps, carbohydrate transport, and cellular metabolism.

Most antiviral-resistant strains had fewer than 10 unique genetic alterations compared to wild type. Whole genome sequencing of antiviral-resistant E. coli revealed relatively few base pair mutations compared to wild type E. coli. These few mutations may have wide-ranging implications for broad cross-resistance. Zidovudine-resistant E. coli, for example, had the broadest phenotypic resistance profile to all antiviral and antibiotic drugs tested, but had only two unique genomic mutations in coding regions, namely in the thymidine kinase gene and in bifunctional aspartate kinase/homoserine dehydrogenase I.

Although several of the observed genetic mutations in antiviral-resistant isolates occurred in proteins or pathways known or suspected to play a role in antimicrobial resistance, most genetic alterations occurred in genes not known to be involved in antibiotic resistance. Many of the genes in which the mutations occurred are known to be involved in metabolism and nutrient transport, such as the galactofuranose-binding protein, sodium/glutamate transporter and bifunctional aspartate kinase/homoserine dehydrogenase I. The researchers emphasized that the existing literature implicates a broad range of metabolic pathways in the development of antibiotic resistance. The capacity of bacteria to adapt to environmental

stressors, including drugs may result in many different alterations and adaptations for survival. Antiviral drugs may have similar effects as stressors that lead to resistance phenotypes.

In conclusion, this study has shown that non-antibiotic drugs such as antivirals can contribute to antimicrobial resistance. The phenotypic changes in conjunction with the genotypic results suggest that bacteria exposed to antiviral drugs with antibacterial properties *in vitro* may develop multiple resistance mutations that confer cross-resistance to antibiotics. The genes identified in this study may point to pathways involved in resistance development and serve as targets for novel therapeutics.

These results suggest that the widespread use of antiviral drugs may contribute to the development and spread of antimicrobial resistance in humans and in the environment. The risk of developing antimicrobial resistance due to the presence of antivirals is greatest where the burden of viral disease— and concomitant antiviral drug use– is high.

Journal Reference

Wallace VJ.et al. Bacteria exposed to antiviral drugs develop antibiotic cross-resistance and unique resistance profiles. COMMUNICATIONS BIOLOGY | (2023) 6:837 (Open Access) https://doi.org/10.1038/s42003-023-05177-3