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Effect of chemotherapeutic agents on natural transformation frequency in *Acinetobacter baylyi*

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Abstract

Natural transformation is the ability of a bacterial cell to take up extracellular DNA which is subsequently available for recombination into the chromosome (or maintenance as an extrachromosomal element). Like other mechanisms of horizontal gene transfer, natural transformation is a significant driver for the dissemination of antimicrobial resistance. Recent studies have shown that many pharmaceutical compounds such as antidepressants and anti-inflammatory drugs can upregulate transformation frequency in the model species *Acinetobacter baylyi*. Chemotherapeutic compounds have been shown to increase the abundance of antimicrobial resistance genes and increase colonization rates of potentially pathogenic bacteria in patient gastrointestinal tracts, indicating an increased risk of infection and providing a pool of pathogenicity or resistance genes for transformable commensal bacteria. We here test for the effect of six cancer chemotherapeutic compounds on *A. baylyi* natural transformation frequency, finding two compounds, docetaxel and daunorubicin, to significantly decrease transformation frequency, and daunorubicin to also decrease growth rate significantly. Enhancing our understanding of the effect of chemotherapeutic compounds on the frequency of natural transformation could aid in preventing the horizontal spread of antimicrobial resistance genes.

DATA SUMMARY

Supporting data and method of analysis with Supplementary Material for Effect of Chemotherapeutic Agents on Natural Transformation Frequency in *Acinetobacter baylyi* are deposited at 10.6084/m9.figshare.24468091 [1].

INTRODUCTION

Antimicrobial resistance (AMR) is a global threat to modern medicine and is accelerated greatly by rapid dissemination of antimicrobial resistance genes via horizontal gene transfer (HGT) [2–6]. The increased prevalence of AMR has severe consequences for modern medicine; for instance, AMR infections were linked to an estimated 1.27 million deaths worldwide in 2019 [7]. Natural transformation, the process whereby prokaryotes take up extracellular DNA from the environment [8–10], is an underrecognized driver of AMR dissemination worldwide despite conferring the ability to acquire cell-free chromosomal DNA, plasmids and transposons [11]. While only approximately 80–90 species are known to be naturally transformable [10], the WHO's list of priority multidrug-resistant pathogens is composed mostly of transformable species, indicating that this mechanism could be important in the acquisition of resistance [12].

Transformation frequency in bacteria can be up- or down-regulated in response to a range of stimuli [10, 13]. A wide variety of anthropogenic pollutants including pharmaceutical products have been demonstrated to increase transformation frequencies, particularly in *Acinetobacter baylyi* [10, 14–18]. For example, pharmaceutical compounds such as anti-inflammatory drugs [14]

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Abbreviations: AMR, antimicrobial resistance; DMSO, dimethylsulfoxide; HGT, horizontal gene transfer; ssDNA, single stranded DNA; WHO, World Health Organisation.
Three supplementary tables are available with the online version of this article.

Table 1. List of the drugs used in this study, their mechanisms of action and concentrations reported for clinical samples

Drug	Mechanism of action	Clinically relevant concn
Cytarabine	Cytosine analogue	Blood plasma – 17.8 μg ml^{-1} [58]
Daunorubicin	Topoisomerase II inhibitor	Concentration inside leukaemic cells – 10.6 μg ml^{-1} [53]
Docetaxel	Disrupts microtubule function	Blood plasma – 2.42 ng ml^{-1} [51]
Exemestane	Aromatase inhibitor (oestrogen synthesis inhibitor)	Blood plasma – 4.1 ng ml^{-1} [59]
Imatinib	Tyrosine kinase inhibitor	Blood plasma – 1 μg ml^{-1} [60]
Methotrexate	Folate synthesis inhibitor	Blood plasma – 13.63 μg ml^{-1} [61]

and antidepressants [19] can increase natural transformation frequency two- to threefold. However, no data are available on the possible effect of chemotherapy compounds on natural transformation. Chemotherapy compounds are cytotoxic agents which target a range of human cell functions which are often upregulated in – or unique to – malignant cells to induce cell death [20]. The use of chemotherapy compounds to treat malignancies in humans may lead to increased levels of AMR in gut microbiota through increasing rates of *de novo* mutation, HGT or by acting as a selective pressure [21–23]. Consequently, this may increase the risk of contracting resistant bloodstream infections, a cause of death in approximately 1 in 10 cancer patients [24, 25]. A proposed mechanism for AMR acquisition in response to exposure to chemotherapy compounds is the induction of the SOS response pathway via genotoxic or cytotoxic damage which consequently leads to increased bacterial mutation rates [22, 26, 27]. Although the SOS stress response pathway is atypical in *A. baylyi* [28, 29], it is considered to be an inducer of competence for natural transformation [30], as it is in other species [31–33]. Therefore, exposure to chemotherapeutic compounds could be hypothesized to increase transformation frequencies.

Here, we use the model organism *A. baylyi* to test the effects of chemotherapeutic drugs on natural transformation. *A. baylyi* is a ubiquitous environmental bacterium capable of opportunistic infection [34, 35], and is constitutively naturally transformable [13, 36]. *Acinetobacter* species, particularly *A. baumannii*, can colonize human gastrointestinal systems [37] and can cause severe and even fatal infections in patients undergoing cancer chemotherapy [38, 39]. We exposed *A. baylyi* to six chemotherapy compounds currently used to treat malignancies in humans to test for dose-dependent changes in transformation frequency and growth rate in *A. baylyi*. Each drug belongs to a different class with different mechanisms of action: cytarabine, a cytosine analogue [40]; daunorubicin, a DNA topoisomerase II inhibitor [41]; docetaxel, a disruptor of microtubule function [42]; exemestane, an aromatase inhibitor [43]; imatinib, a tyrosine kinase inhibitor [44]; and methotrexate, a folate synthesis inhibitor [45] (Table 1). As the varied pharmacokinetic properties of these six drugs can indicate differences in diffusion between blood plasma and tissues [46], a range of concentrations spanning previously measured blood plasma concentrations were used.

METHODS

Chemotherapeutic drugs

Cytarabine (Abcam), daunorubicin (Cayman Chemical Company), docetaxel (Cambridge Bioscience), exemestane (Merck), imatinib (Cambridge Bioscience) and methotrexate (Cayman Chemical Company) were stored at −20°C in single-use aliquots dissolved in DMSO (Fisher) at 100× the concentration used in each treatment. Aliquots of drug stocks were added as 1% of the final volume of culture to ensure an equal final concentration of DMSO across all treatments. DMSO at 1% (v/v) had no effect on transformation frequency or growth rate in *A. baylyi*.

Transformation assay

Genomic DNA as a substrate for natural transformation was isolated from an *A. baylyi* construct labelled with red fluorescence and spectinomycin resistance [47]. An isogenic transformable green fluorescent *apraR* wild-type *A. baylyi* ADP1 was grown overnight in LB broth (Formedium) and diluted fivefold into 2 ml of LB broth in a universal 30 ml container (see Winter *et al.* [47] for strain construction details). Cultures were amended with chemotherapeutic drugs on a $\log_{10}$ dilution range. In the no-drug control, DMSO was added to be consistent with the 1% DMSO concentration in drug treatment groups. Spectinomycin resistance-conferring DNA was obtained by lysis following the Qiagen Genomic DNA Handbook (April 2012) protocol. DNA from the eluate was precipitated by adding two volumes of ice-cold isopropanol and centrifuged at 26 000 ***g*** for 15 min to pellet the DNA. DNA was dissolved in TE buffer to a final concentration of 342.4 ng μl^{-1} (Nanodrop 2000, Thermo Scientific) and frozen at −20°C in single-use aliquots for addition to each experiment at a final concentration of 100 ng ml^{-1} for each sample. Samples were incubated at 30°C and 180 r.p.m. for 5 h. Recipients and transformants were enumerated before and after incubation by plating on LB agar amended with 240 μg ml^{-1} apramycin (Duchefa), and LB agar amended with both 240 μg ml^{-1} apramycin and 360 μg ml^{-1} spectinomycin (Melford), respectively. Concentrations of antibiotics far exceeding the MICs required to

inhibit *A. baylyi* growth were used to reduce the chance of false positives caused by contamination. All treatments were sampled at a minimum of sixfold biological replication. To determine the highest concentration of DMSO which had no observable effect on cell viability and growth, a transformation assay was conducted as above, but without chemotherapy compounds (five-fold dilution of *A. baylyi* into LB broth for 3 h at 30°C, 180 r.p.m., with 100 ng ml^{-1} DNA and 10, 1, 0.01, 0.001 or 0% DMSO; data not shown).

Statistical analyses

The effects of chemotherapy compound presence and concentration on *A. baylyi* transformation frequencies and growth rate were determined using Kruskal–Wallis and paired Wilcoxon testing, respectively. For analyses measuring growth rate, the Malthusian parameter was calculated and used as a response variable [48]. Fold changes in transformation frequency were calculated using the means of the respective compared treatment groups. In all analyses, P values of <0.05 were considered significant. False discovery rate adjustment for multiple testing was used in all instances where multiple tests were conducted in the same analysis.

RESULTS

Contrasting effects of different chemotherapy drugs on natural transformation frequency in *A. baylyi*

We tested the effect of six chemotherapeutic compounds on natural transformation and growth rate at varied concentrations pertaining to those found in patient blood plasma. Cytarabine was not observed to have a significant effect on transformation frequency (Kruskal–Wallis test, H=2.22, df=3, P=0.528; Fig. 1a) or growth rate (Kruskal–Wallis test, H=0.878, df=3, P=0.831; Fig. 1b). Daunorubicin resulted in significantly decreased transformation frequency (Kruskal–Wallis test, H=28.4, df=3, P<0.0001; Fig. 1c) and growth rate (Kruskal–Wallis test, H=17.3, df=3, P<0.001; Fig. 1d). Compared to the control group, transformation frequency of *A. baylyi* in 5.63 µg ml^{-1} daunorubicin significantly reduced by over 16-fold [Wilcoxon pairwise comparison, H=194, P<0.001; Fig. 1c and Table S1 (available in the online version of this article)]. Growth rate of *A. baylyi* in the presence of 56.3 µg ml^{-1} daunorubicin was significantly reduced compared to the control group (Wilcoxon pairwise comparison, W=200, P<0.001; Fig. 1d and Table S2), and is the only treatment group in this study where a net loss of cells was observed. Growth rates of *A. baylyi* in 0.563 and 5.63 µg ml^{-1} daunorubicin were not significantly different to the control group (Wilcoxon pairwise comparisons, P>0.05; Fig. 1d and Table S2). Transformation frequency of *A. baylyi* in 56.3 µg ml^{-1} daunorubicin was below the detectable limit (10^{-7}) and significantly lower than all other concentrations and the no-drug control (Wilcoxon pairwise comparisons, P<0.05; Fig. 1c and Table S1). Docetaxel significantly decreased the transformation frequency over sevenfold at 80.7 µg ml^{-1} (Wilcoxon pairwise comparison, W=181, P<0.01; Fig. 1e and Table S3), but did not affect the growth rate (Kruskal–Wallis test, H=2.47, P=0.481, df=3; Fig. 1f). Exemestane did not have a significant effect on transformation frequency (Kruskal–Wallis test, H=2.35, P=0.504, df=3; Fig. 1g) or growth rate (Kruskal–Wallis test, H=2.56, P=0.465, df=3; Fig. 1h). Imatinib had no significant effect on transformation frequency (H=3.51, P=0.319, df=3; Fig. 1i) or growth rate (Kruskal–Wallis test, H=1.29, P=0.732, df=3; Fig. 1j). Methotrexate had no significant effect on transformation frequency (Kruskal–Wallis test, H=3.71, P=0.294, df=3; Fig. 1k) or growth rate (Kruskal–Wallis test, H=0.101, P=0.992, df=3; Fig. 1l).

DISCUSSION

In this study, six chemotherapeutic drugs with diverse mechanisms of action were tested for their effect on natural transformation and growth rate in *A. baylyi*. Four compounds, cytarabine, exemestane, imatinib and methotrexate, demonstrated no observable effect on natural transformation or growth at clinically relevant concentrations, while two compounds, docetaxel and daunorubicin, caused a dose-dependent decrease in transformation frequency, with daunorubicin significantly decreasing growth rate.

Docetaxel acts on eukaryotic cells by causing disruption of microtubule function leading to reduced cell proliferation [42]. If cell proliferation in *A. baylyi* was arrested by docetaxel, we expected to see a reduction in competence as competence is linked to growth phase in this species [36, 49, 50]. Docetaxel negatively affected transformation frequency, but not growth rate, suggesting that its mechanism of action primarily affects transformation machinery and is not an indirect effect of reduced growth rate. Clinically relevant blood concentrations of docetaxel are around 2.42 ng ml^{-1} [51] and are therefore lower than those used in this study. Exposure to daunorubicin could in theory lead to altered rates of *gyrA* and *gyrB* transcription which are also upregulated in the SOS response in *A. baylyi* [19]. Daunorubicin showed strong effects on both growth rate and transformation frequency where transformation frequency reduced to below detectable levels at 56.3 µg ml^{-1} daunorubicin. As growth rate is unaffected at lower concentrations of daunorubicin, its effects on transformation may be independent of effects on growth rate, indicating that the mechanism of action of daunorubicin may act directly on DNA uptake machinery. Intracellular concentrations of daunorubicin may also be sufficient for the promotion of secondary structures of ssDNA internalized by cells caused by binding of the drug to ssDNA [52], thereby limiting the accessibility of ssDNA for recombination. The mean concentration of daunorubicin found in leukaemic cells during treatment is around 18.8 µmol l^{-1} [53] or 10.6 µg ml^{-1} which is within the range tested here and so the observed effects may be clinically relevant. However, it is unclear how concentrations of cancer chemotherapeutics in blood are related to those in blood serum. To our knowledge, only one study to date has attempted to estimate non-antibiotic drug concentrations in the gut [54], but exclusively considered oral drug administration. As both daunorubicin and docetaxel are

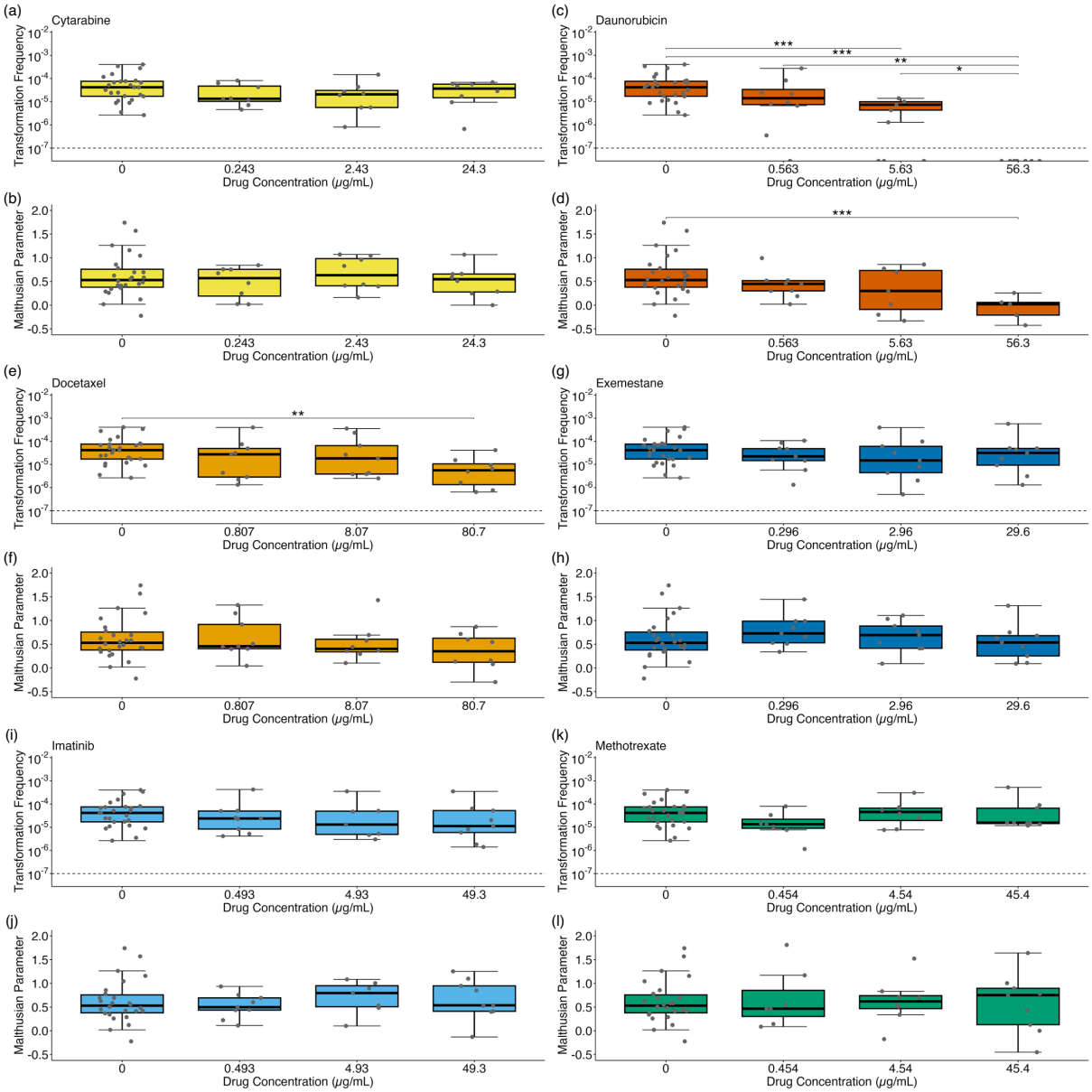

Fig. 1. Effect of six chemotherapeutic agents on the transformation frequency and growth rate of *A. baylyi*. Effect of cytarabine on transformation frequency (a) and growth rate (b); daunorubicin on transformation frequency (c) and growth rate (d); docetaxel on transformation frequency (e) and growth rate (f); exemestane on transformation frequency (g) and growth rate (h); imatinib on transformation frequency (i) and growth rate (j); methotrexate on transformation frequency (k) and growth rate (l). Plotted points represent individual replicates and are horizontally scattered for improved visibility only. Values plotted below 10^{-7} (dashed line) indicate frequencies below the detectable limit (*P<0.05; **P<0.01; ***P<0.001; ****P<0.0001).

administered intravenously, we cannot estimate the concentrations at which these drugs are found in the gut and thus the clinical relevance of the concentrations tested in this study. *A. baylyi* is a ubiquitous environmental bacterium which may come into contact with cancer chemotherapeutic pollutants in wastewater systems [28, 55].

The routine use of last-resort antibiotics as a prophylactic treatment for cancer patients increases the resistance of gut microbiota to those antibiotics [56] and has been known to increase colonization of potentially pathogenic bacteria in the gut [57]. This can lead to infection but could also potentially act as a donor pool of resistance or pathogenicity genes to resident species that are able to engage in natural transformation. The limited effect the drugs tested in this study have on transformation frequency at putatively clinically relevant concentrations is favourable, as it may reduce or not affect the rate of transformation events which can lead to the acquisition of traits which are beneficial to pathogens. Future work is needed to close the knowledge gap on

in vitro concentrations of non-antibiotic drugs in different organ systems and different human-associated bacteria. Additionally, our data identify a need to further investigate the effects and mechanisms of both these and currently untested chemotherapeutic drugs to help monitor and establish preventative measures which can limit the spread of AMR genes.

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Author contributions
M.W.: methodology, validation, formal analysis, investigation, data curation, writing – original draft, writing – review and editing, visualization, project administration, funding acquisition. M.V.: validation, supervision, writing – review and editing, visualization, funding acquisition. A.B.: validation, writing – review and editing. P.J: methodology, validation, resources, writing – review and editing, conceptualization. K.H.: methodology, validation, resources, conceptualization, writing – review and editing, supervision, funding acquisition.

Conflicts of interest
The authors have no conflicts of interest to declare.

References

1. Winter M. R script with.Csv file for the analysis of data for the paper titled 'effect of chemotherapeutic agents on natural transformation frequency in Acinetobacter Baylyi". *Figshare*. 2023. DOI: 10.6084/m9.figshare.24468091.v1.
2. von Wintersdorff CJH, Penders J, van Niekerk JM, Mills ND, Majumder S, *et al*. Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Front Microbiol* 2016;7:173.
3. Ventola CL. The antibiotic resistance crisis: causes and threats. *P & T J* 2015;40:277–283.
4. Jiang X, Ellabaan MMH, Charusanti P, Munck C, Blin K, *et al*. Dissemination of antibiotic resistance genes from antibiotic producers to pathogens. *Nat Commun* 2017;8:15784.
5. Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, *et al*. Call of the wild: antibiotic resistance genes in natural environments. *Nat Rev Microbiol* 2010;8:251–259.
6. Vinayamohan PG, Pellissery AJ, Venkitanarayanan K. Role of horizontal gene transfer in the dissemination of antimicrobial resistance in food animal production. *Curr Opin Food Sci* 2022;47:100882.
7. Murray CJL, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, *et al*. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* 2022;399:629–655.
8. Johnsborg O, Eldholm V, Håvarstein LS. Natural genetic transformation: prevalence, mechanisms and function. *Res Microbiol* 2007;158:767–778.
9. Seitz P, Blokesch M. Cues and regulatory pathways involved in natural competence and transformation in pathogenic and environmental Gram-negative bacteria. *FEMS Microbiol Rev* 2013;37:336–363.
10. Johnston C, Martin B, Fichant G, Polard P, Claverys JP. Bacterial transformation: distribution, shared mechanisms and divergent control. *Nat Rev Microbiol* 2014;12:181–196.
11. Winter M, Buckling A, Harms K, Johnsen PJ, Vos M. Antimicrobial resistance acquisition via natural transformation: context is everything. *Curr Opin Microbiol* 2021;64:133–138.
12. Blokesch M. In and out-contribution of natural transformation to the shuffling of large genomic regions. *Curr Opin Microbiol* 2017;38:22–29.
13. Blokesch M. Natural competence for transformation. *Curr Biol* 2016;26:R1126–R1130.
14. Wang Y, Lu J, Engelstädter J, Zhang S, Ding P, *et al*. Non-antibiotic pharmaceuticals enhance the transmission of exogenous antibiotic resistance genes through bacterial transformation. *ISME J* 2020;14:2179–2196.
15. Zhang S, Wang Y, Lu J, Yu Z, Song H, *et al*. Chlorine disinfection facilitates natural transformation through ROS-mediated oxidative stress. *ISME J* 2021;15:2969–2985.
16. Jin M, Liu L, Wang D-N, Yang D, Liu W-L, *et al*. Chlorine disinfection promotes the exchange of antibiotic resistance genes across bacterial genera by natural transformation. *ISME J* 2020;14:1847–1856.
17. Li J, Zhang K, Zhang H. Adsorption of antibiotics on microplastics. *Environ Pollut* 2018;237:460–467.
18. Yu Z, Wang Y, Henderson IR, Guo J. Artificial sweeteners stimulate horizontal transfer of extracellular antibiotic resistance genes through natural transformation. *ISME J* 2022;16:543–554.
19. Lu J, Ding P, Wang Y, Guo J. Antidepressants promote the spread of extracellular antibiotic resistance genes via transformation. *ISME Commun* 2022;2:63.
20. Nygren P, SBU-group. Swedish Council on Technology Assessment in Health Care. What is cancer chemotherapy? *Acta Oncol* 2001;40:166–174.
21. Papanicolas LE, Gordon DL, Wesselingh SL, Rogers GB. Not just antibiotics: is cancer chemotherapy driving antimicrobial resistance? *Trends Microbiol* 2018;26:393–400.
22. Meunier A, Nerich V, Fagnoni-Legat C, Richard M, Mazel D, *et al*. Enhanced emergence of antibiotic-resistant pathogenic bacteria after in vitro induction with cancer chemotherapy drugs. *J Antimicrob Chemother* 2019;74:1572–1577.
23. Guðmundsdóttir JS, Fredheim EGA, Koumans CIM, Hegstad J, Tang P-C, *et al*. The chemotherapeutic drug methotrexate selects for antibiotic resistance. *EBioMedicine* 2021;74:103742.
24. Danai PA, Moss M, Mannino DM, Martin GS. The epidemiology of sepsis in patients with malignancy. *Chest* 2006;129:1432–1440.
25. Williams MD, Braun LA, Cooper LM, Johnston J, Weiss RV, *et al*. Hospitalized cancer patients with severe sepsis: analysis of incidence, mortality, and associated costs of care. *Crit Care* 2004;8:R291–R298.
26. Thi TD, López E, Rodríguez-Rojas A, Rodríguez-Beltrán J, Couce A, *et al*. Effect of recA inactivation on mutagenesis of *Escherichia coli* exposed to sublethal concentrations of antimicrobials. *J Antimicrob Chemother* 2011;66:531–538.
27. Bjedov I, Tenaillon O, Gérard B, Souza V, Denamur E, *et al*. Stress-induced mutagenesis in bacteria. *Science* 2003;300:1404–1409.
28. Mantilla-Calderon D, Plewa MJ, Michoud G, Fodelianakis S, Daffonchio D, *et al*. Water disinfection byproducts increase natural transformation rates of environmental DNA in acinetobacter baylyi ADP1. *Environ Sci Technol* 2019;53:6520–6528.
29. Robinson A, Brzoska AJ, Turner KM, Withers R, Harry EJ, *et al*. Essential biological processes of an emerging pathogen: DNA

replication, transcription, and cell division in *Acinetobacter* spp. *Microbiol Mol Biol Rev* 2010;74:273–297.

30. Lin L, Ringel PD, Vettiger A, Dürr L, Basler M. DNA uptake upon T6SS-dependent prey cell lysis induces SOS response and reduces fitness of *Acinetobacter baylyi*. *Cell Rep* 2019;29:1633–1644.
31. Charpentier X, Polard P, Claverys J-P. Induction of competence for genetic transformation by antibiotics: convergent evolution of stress responses in distant bacterial species lacking SOS? *Curr Opin Microbiol* 2012;15:570–576.
32. Charpentier X, Kay E, Schneider D, Shuman HA. Antibiotics and UV radiation induce competence for natural transformation in *Legionella pneumophila*. *J Bacteriol* 2011;193:1114–1121.
33. Prudhomme M, Attaiech L, Sanchez G, Martin B, Claverys JP. Antibiotic stress induces genetic transformability in the human pathogen *Streptococcus pneumoniae*. *Science* 2006;313:89–92.
34. Chen T-L, Siu L-K, Lee Y-T, Chen C-P, Huang L-Y, *et al*. *Acinetobacter baylyi* as a pathogen for opportunistic infection. *J Clin Microbiol* 2008;46:2938–2944.
35. Zhou Z, Du X, Wang L, Yang Q, Fu Y, *et al*. Clinical carbapenem-resistant *Acinetobacter baylyi* strain coharboring blaSIM-1 and blaOXA-23 from China. *Antimicrob Agents Chemother* 2011;55:5347–5349.
36. Palmen R, Vosman B, Buijsman P, Breek CKD, Hellingwerf KJ. Physiological characterization of natural transformation in *Acinetobacter calcoaceticus*. *J Gen Microbiol* 1993;139:295–305.
37. Ketter PM, Yu J-J, Guentzel MN, May HC, Gupta R, *et al*. *Acinetobacter baumannii* gastrointestinal colonization is facilitated by secretory IgA which is reductively dissociated by bacterial thioredoxin A. *mBio* 2018;9:e01298-18.
38. Fukuta Y, Muder RR, Agha ME, Clarke LG, Wagener MM, *et al*. Risk factors for acquisition of multidrug-resistant Acinetobacter baumannii among cancer patients. *Am J Infect Control* 2013;41:1249–1252.
39. Freire MP, de Oliveira Garcia D, Garcia CP, Campagnari Bueno MF, Camargo CH, *et al*. Bloodstream infection caused by extensively drug-resistant *Acinetobacter baumannii* in cancer patients: high mortality associated with delayed treatment rather than with the degree of neutropenia. *Clin Microbiol Infect* 2016;22:352–358.
40. Crisp LB, Smith SM, Mathers MA, Young GA, Lyons SD, *et al*. Effects of cytosine arabinoside on human leukemia cells. *Int J Biochem Cell Biol* 1996;28:1061–1069.
41. Agrawal K. Daunorubicin. In: *xPharm: The Comprehensive Pharmacology Reference*. 2020. pp. 1–4.
42. Herbst RS, Khuri FR. Mode of action of docetaxel - a basis for combination with novel anticancer agents. *Cancer Treat Rev* 2003;29:407–415.
43. Lombardi P. Exemestane, a new steroidal aromatase inhibitor of clinical relevance. *Biochim Biophys Acta* 2002;1587:326–337.
44. de Kogel CE, Schellens JHM. Imatinib. *Oncologist* 2007;12:1390–1394.
45. Cronstein BN. The mechanism of action of methotrexate. *Rheum Dis Clin North Am* 1997;23:739–755.
46. Chillistone S, Hardman JG. Factors affecting drug absorption and distribution. *Anaesth Intens Care Med* 2017;18:335–339.
47. Winter M, Harms K, Johnsen PJ, Buckling A, Vos M. Testing for the fitness benefits of natural transformation during community-embedded evolution. *Microbiology* 2023;169:1375.
48. Lenski RE, Rose MR, Simpson SC, Tadler SC. Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2,000 generations. *Am Nat* 1991;138:1315–1341.
49. Utnes ALG, Sørum V, Hülter N, Primicerio R, Hegstad J, *et al*. Growth phase-specific evolutionary benefits of natural transformation in *Acinetobacter baylyi*. *ISME J* 2015;9:2221–2231.
50. Ray JL, Nielsen KM. Experimental methods for assaying natural transformation and inferring horizontal gene transfer. *Methods Enzymol* 2005;395:491–520.
51. Morgan C, Lewis PD, Jones RM, Bertelli G, Thomas GA, *et al*. The *in vitro* anti-tumour activity of zoledronic acid and docetaxel at clinically achievable concentrations in prostate cancer. *Acta Oncologica* 2007;46:669–677.
52. Adamcik J, Valle F, Witz G, Rechendorff K, Dietler G. The promotion of secondary structures in single-stranded DNA by drugs that bind to duplex DNA: an atomic force microscopy study. *Nanotechnology* 2008;19:384016.
53. Tidefelt U, Liliemark J, Gruber A, Liliemark E, Sundman-Engberg B, *et al*. P-Glycoprotein inhibitor valspodar (PSC 833) increases the intracellular concentrations of daunorubicin in vivo in patients with P-glycoprotein-positive acute myeloid leukemia. *J Clin Oncol* 2000;18:1837–1844.
54. Maier L, Pruteanu M, Kuhn M, Zeller G, Telzerow A, *et al*. Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature* 2018;555:623–628.
55. Schuster D, Axtmann K, Holstein N, Felder C, Voigt A, *et al*. Antibiotic concentrations in raw hospital wastewater surpass minimal selective and minimum inhibitory concentrations of resistant *Acinetobacter baylyi* strains. *Environ Microbiol* 2022;24:5721–5733.
56. Hobson CA, Bonacorsi S, Hocquet D, Baruchel A, Fahd M, *et al*. Impact of anticancer chemotherapy on the extension of beta-lactamase spectrum: an example with KPC-type carbapenemase activity towards ceftazidime-avibactam. *Sci Rep* 2020;10:589.
57. van Vliet MJ, Tissing WJE, Dun CAJ, Meessen NEL, Kamps WA, *et al*. Chemotherapy treatment in pediatric patients with acute myeloid leukemia receiving antimicrobial prophylaxis leads to a relative increase of colonization with potentially pathogenic bacteria in the gut. *Clin Infect Dis* 2009;49:262–270.
58. Slevin ML, Piall EM, Aherne GW, Harvey VJ, Johnston A, *et al*. Effect of dose and schedule on pharmacokinetics of high-dose cytosine arabinoside in plasma and cerebrospinal fluid. *J Clin Oncol* 1983;1:546–551.
59. Luo S, Chen G, Truica CI, Baird CC, Xia Z, *et al*. Identification and quantification of novel major metabolites of the steroidal aromatase inhibitor, exemestane. *Drug Metab Dispos* 2018;46:1867–1878.
60. Gotta V, Widmer N, Decosterd LA, Chalandon Y, Heim D, *et al*. Clinical usefulness of therapeutic concentration monitoring for imatinib dosage individualization: results from a randomized controlled trial. *Cancer Chemother Pharmacol* 2014;74:1307–1319.
61. Hornung N, Ellingsen T, Attermann J, Stengaard-Pedersen K, Poulsen JH. Patients with rheumatoid arthritis treated with methotrexate (MTX): concentrations of steady-state erythrocyte MTX correlate to plasma concentrations and clinical efficacy. *J Rheumatol* 2008;35:1709–1715.