JOURNAL OF MEDICAL MICROBIOLOGY

REVIEW

Carter *et al.*, *Journal of Medical Microbiology* 2026;75:002129

DOI 10.1099/jmm.0.002129

MICROBIOLOGY SOCIETY

OPEN ACCESS

Trimethoprim: bactericidal or bacteriostatic activity is dependent on bacterial growth conditions

Cailean Carter[1,2,*], John Wain[1,2] and Gemma C. Langridge[1,2]

Abstract

Trimethoprim is a clinically important antibiotic used for the routine treatment of urinary tract infections as a cost-effective first-line choice for treatment. A unique feature of the drug is that it can have bacteriostatic or bactericidal effects depending upon the metabolites available in the environment. Bacteriostatic activity requires the absence of nucleoside from the growth media. Conversely, bactericidal activity requires the presence of a nucleoside and the amino acids glycine and methionine. Mechanistically, bacteriostatic action does not appear to be dependent upon protein or RNA synthesis, whereas protein synthesis does not appear to be essential for bactericidal activity. Instead, this is likely due to the inhibition of DNA synthesis and the triggering of programmed cell death, involving the suicide module *mazEF*. In summary, trimethoprim has a complex mechanism of action that should be considered when researching the antibiotic and informing growth media design to test susceptibility.

INTRODUCTION

Trimethoprim is of clinical significance as the most cost-effective treatment option for urinary tract infections (UTIs) (£0.75 for full course; Table 1). Further, trimethoprim was the second most prescribed first-line antibiotic (second to amoxicillin) in primary care in England between 2014 and 2022 among antibiotics used to treat UTIs and respiratory infections [1]. However, this statistic is likely inaccurate as the study excluded nitrofurantoin – the current first-line treatment for UTIs. Nitrofurantoin was recommended by Public Health England as the new first-line treatment for UTIs in 2014, taking over from trimethoprim due to rising resistance rates [2]. However, not all practices across England immediately adopted the changes [3]. In the past 5 years, nitrofurantoin prescriptions have exceeded trimethoprim prescriptions, with there being four to five prescriptions of trimethoprim for every ten prescriptions of nitrofurantoin across NHS England [4]. Many clinicians may have been hesitant in prescribing nitrofurantoin due to rising costs of the drug (personal correspondence with Dr Ngozi Elumogo, Consultant Microbiologist at the Clinical Microbiology Laboratory of Norfolk and Norwich University Hospital). So, despite changes in treatment recommendations towards nitrofurantoin, trimethoprim remains a prominent antibiotic of choice for treating UTIs in the UK. Trimethoprim has also been historically combined with another anti-folate drug, sulfamethoxazole, in the UK for routine treatment as co-trimoxazole [5, 6]. However, the combination fell out of use for monotherapy in clinical settings in the UK, but is still routinely used as a combination therapy in veterinary practice in the UK and worldwide for human UTIs and prophylaxis and treatment for other types of infections [7–9].

ROLE OF FOLATES IN BACTERIAL METABOLISM

Trimethoprim targets an important group of compounds called folates. Folates play a significant role in cell metabolism; they are essential for the synthesis of three of the four components of DNA (adenosine, guanosine and thymidine), two of four components of RNA and a major component of proteins (methionine) [10]. Besides being involved in the synthesis of methionine, folates also provide the formyl group for *N*-formylmethionine, which is a crucial protein synthesis initiator ($tRNA^{fMet}$). Therefore, trimethoprim directly affects DNA, RNA and protein synthesis through inhibition of folates.

The term folates is used to describe a group of molecules with a shared core structure (Fig. 1):

Received 21 October 2025; Accepted 31 January 2026; Published 03 March 2026

Author affiliations: [1]Microbes and Food Safety, Quadram Institute Bioscience, Norwich, NR4 7UQ, UK; [2]Centre for Microbial Interactions, Norwich Research Park, Norwich, NR4 7UG, UK.

***Correspondence:** Cailean Carter, cailean.carter@quadram.ac.uk

Keywords: anti-folate; bactericidal; bacteriostatic; metabolism; trimethoprim.

Abbreviations: DHF, dihydrofolate; DHFR, dihydrofolate reductase; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; pABA, para-aminobenzoic acid; PCD, programmed cell death; THF, tetrahydrofolate; TLD, thymineless death; UTIs, urinary tract infections.

- a pteridine ring (namely pterin), which can be reduced or oxidized,
- a para-aminobenzoic acid (pABA) linker that binds single carbon units at the 10 N position with the 5 N position of the pteridine ring and
- a polyglutamate chain that varies in length, which helps localize folates in the cell [11].

The role of folates is to provide carbon units in various reduced forms for the synthesis of purines, methionine and deoxythymidine monophosphate (dTMP). Biosynthesis of folates starts from dihydrofolate (DHF), which can be interpreted as an inactive form of folates, as DHF cannot accept carbon units [11]. DHF must be reduced to tetrahydrofolate (THF) before accepting carbon units, and THF forms the core structure for all active forms of folate (Fig. 1). Therefore, any compound preventing this process effectively limits the availability of active forms of folate; this includes the activity of trimethoprim. THF can accept carbon units from the amino acids serine and glycine. Serine carries one more carbon atom than glycine, so it can donate a methylene group (CH_2) to THF and then another methylene group after being converted to glycine (Fig. 2). The donation of a methylene group by glycine splits the amino acid into its carbon and nitrogen parts through the glycine cleavage system [12]. The donation of a methylene group by serine or glycine produces 5,10-methylene THF, which can be reduced or oxidized to meet the demands for synthesizing various metabolites that require carbon atoms [11]. For example, a methyl group (CH_3) is used for the synthesis of methionine, and a formyl group (CHO) is used twice for the synthesis of the purine ring. The transfer of methyl and formyl groups from THF to their respective metabolites maintains folates in their active form of THF, ready to accept further carbon units from serine or glycine. The exception to this is thymidylate synthase, which performs the essential conversion of deoxyuridine monophosphate (dUMP) to dTMP, requiring a methylene group and $2H^+$, which reverts THF to DHF [13]. Therefore, the conversion of DHF to THF is required to maintain the active folate pool. Folates are required for their own synthesis, as the pteridine ring is derived from the purine ring, more precisely, from guanosine triphosphate [11].

TRIMETHOPRIM MODE OF ACTION

The continued activity of reducing DHF to THF is essential for preventing the pool of active folates from depleting via the activity of thymidylate synthase. Humans take folate from their diet as vitamin B9, whereas pathogenic bacteria cannot transport folates in any form and are therefore required to synthesize it themselves [10]. The reduction of DHF to THF is performed by dihydrofolate reductase (DHFR; encoded by *folA*) and is the target for trimethoprim [13]. Trimethoprim is a structural analogue of the pteridine component of folates (Fig. 1), thereby acting through competitive inhibition of DHFR against DHF [14]. It has been reported that a separate enzyme encoded by *folM* can perform the reduction of DHF to THF; however, this has a V_{max} that is ~25% of *folA* [15]. Further, *folM* is not affected by trimethoprim and would continue to synthesize THF at a relatively slow rate, though not sufficiently to counteract trimethoprim. Ultimately, trimethoprim leads to an accumulation of DHF and depletion of active folates, thereby limiting the availability of carbon atoms for the synthesis of DNA, RNA and protein components [16]. Modelling of trimethoprim's metabolic perturbations suggests the *de novo* synthesis of further metabolites is affected: CoA, nicotinamide/flavin adenine dinucleotide, histidine and spermidine [17].

Table 1. Cost of antibiotics at recommended dosage and treatment course for uncomplicated UTIs in the UK

BD denotes twice daily. Gentamicin comes as a liquid injection; thus, dosage is mg of gentamicin per kg body weight. Source: NHS Business Services Authority and NHS Prescribing Services [63].

Drug (course for uncomplicated UTI)	Price for full course (£)
Ampicillin *500 mg every 4–6 h, 3-day course*	53.71
Cefalexin *500 mg BD for 7 days*	2.7
Ciprofloxacin *250–750 mg BD, 3-day course*	1.25
Co-amoxiclav *250/125 mg every 8 h*	1.67
Gentamicin *5–7 mg kg^{-1} once daily*	6.88
Nitrofurantoin *50 mg four times a day for 3 days*	3.43
Trimethoprim *200 mg BD, 3-day course*	0.75

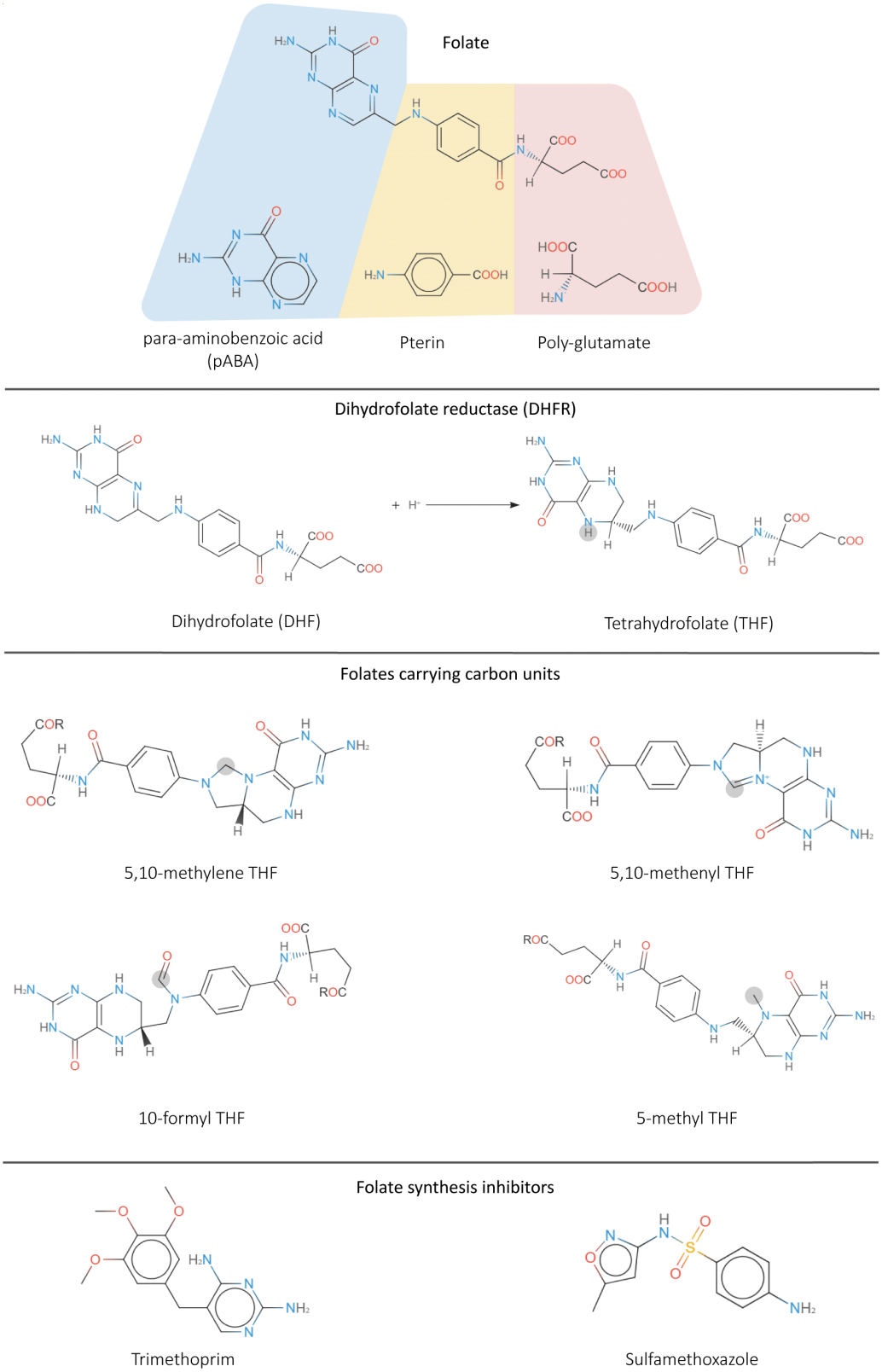

Fig. 1. Chemical structures of folates and folate synthesis inhibitors. Grey circles highlight the addition of hydrogen to THF or carbon units to active forms of folate. Chemical structures were drawn using the SmilesDrawer module from Faerun notebook (v0.1.6b0) using Python programming language on Google Colab. SMILES of metabolites were from EcoCyc.

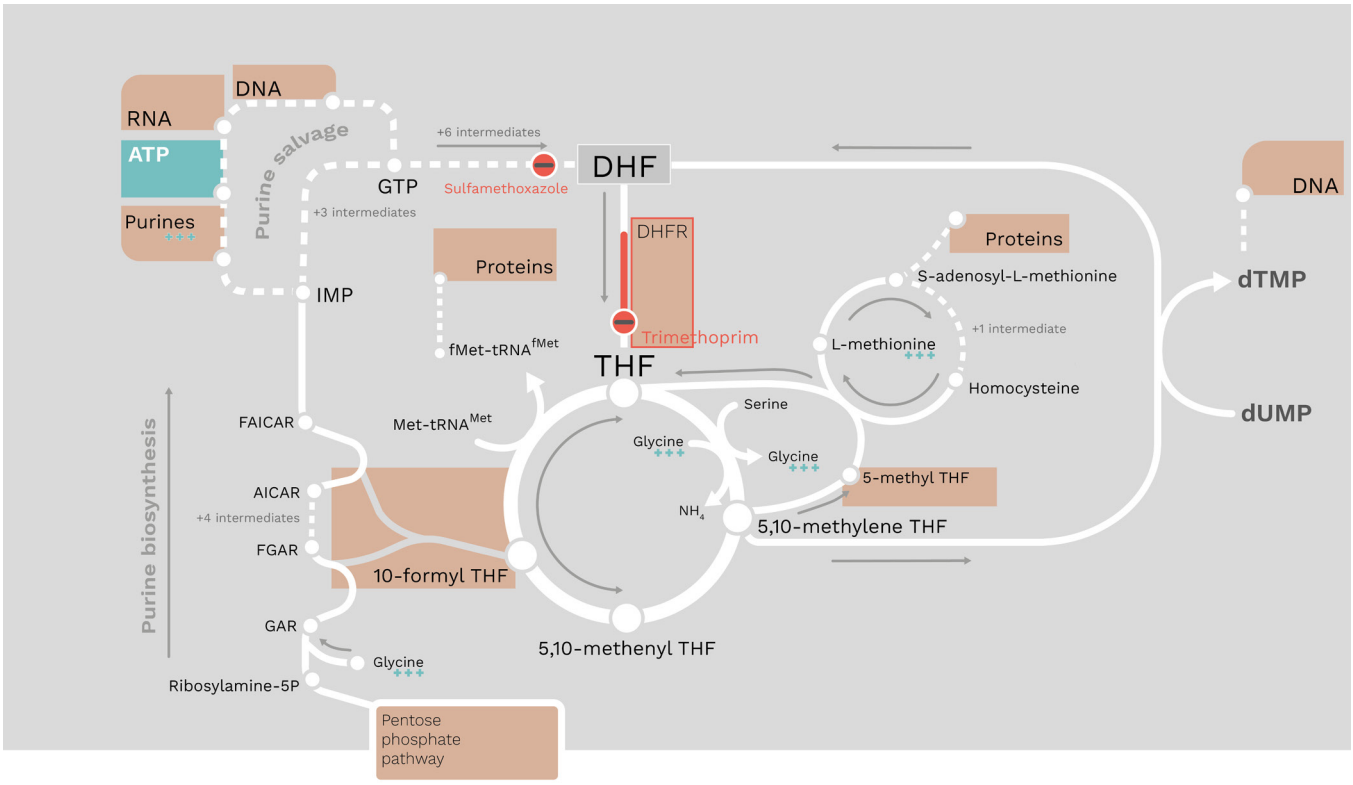

Fig. 2. A simplified network map of the folate pool and relevant pathways, including purine biosynthesis and salvage and folate biosynthesis. White lines denote reactions, and arrows for direction; sets of reactions have adjacent dark-grey arrows for direction. Dashed white lines denote several reactions and are condensed for clarity with labels stating number of intermediary reactions. Red circles with a black bar denote reactions where trimethoprim and sulfamethoxazole exert their anti-folate effects, and metabolites/macromolecules/reactions with a light brown box denote components of metabolism affected by anti-folate drugs. Metabolites that are present in growth media which elicit a bactericidal activity are annotated with three teal plus signs. ATP is highlighted with the same teal colour to emphasize its position in the network. DHFR is the only reaction labelled in the network map due to its significance as the target for trimethoprim. Reactions and pathways are derived from EcoCyc v21 [64].

Thymineless death

Cell death by trimethoprim action is thought to be caused by the inhibition of DNA synthesis primarily through the constraints applied to thymidylate synthase, thereby limiting availability of dTMP [18]. This aspect of trimethoprim activity draws parallels to a phenomenon described in the 1970s called thymineless death (TLD), whereby cells are artificially starved of thymine (a precursor to thymidine and dTMP) and become unviable [13, 19]. Originally described by Cohen and Barner [20], they reported how *Escherichia coli* 15T$^-$ with non-functional thymidylate synthase was dependent on an exogenous supply of thymine. In both TLD and trimethoprim conditions, the bacteria were unable to convert dUMP to dTMP, depriving DNA of an essential component, and were dependent on thymidine or thymine for viability. This means that thymidine and thymine can act as inhibitors of trimethoprim action, which is discussed in more detail later in this review [8] (Thymidine as a trimethoprim inhibitor). Thymidine counteracts TLD in a concentration-dependent manner [21]. By starving the bacteria of thymidine, newly synthesized DNA and DNA repair begin incorporating uracil in place of thymidine – giving DNA the weaker structural integrity of RNA [22]. Ultimately, the incorporation of uracil leads to DNA strand breakages, which the bacteria attempt to restore through recombination repair (encoded by *recBCD*) and provide some initial respite [23]. However, attempts at repair become futile, and the resulting cell death occurs through an unknown mechanism [21, 24]. *E. coli* with non-functional thymidylate synthase treated with hydroxyurea (which increases the number of simultaneous DNA replication events in a single cell) did not exacerbate TLD [21, 25]. This suggests the severity of TLD is not proportional to DNA replication activity. Nonetheless, TLD is believed to be the cause of death by trimethoprim and a group of antibiotics called sulphonamides share this same mode of action [26].

Synergy with sulfamethoxazole

Trimethoprim was historically combined with sulfamethoxazole for treating UTIs; a combination termed co-trimoxazole. Sulfamethoxazole belongs to the sulphonamide class of antibiotics. Sulphonamides target dihydropteroate synthase, an enzyme that links the pABA and pteridine ring together prior to the addition of glutamate [5, 27]; sulfamethoxazole is a structural

analogue of pABA (Fig. 1). Sulphonamides are distinct from trimethoprim, as they only restrict *de novo* synthesis of folates, while trimethoprim will also restrict the regeneration of DHF to THF. Because the two antibiotics have different targets involved in folate biosynthesis, trimethoprim and sulfamethoxazole can be used together and have a synergistic effect on the killing of bacteria [5, 28–31]. However, co-trimoxazole fell out of use for treating human UTIs in the UK, which can be attributed to two key findings. Firstly, there was growing evidence that trimethoprim alone had sufficient efficacy for treating UTIs in humans. Secondly, sulfamethoxazole led to frequent adverse side effects: a single-dose or 3-day course had a ~7% incidence rate, which jumped to ~25% for courses ≥5 days [6, 32]. As such, co-trimoxazole is limited to veterinary practice for treating UTIs.

BACTERIOSTATIC VS. BACTERICIDAL TRIMETHOPRIM CONDITIONS

After their initial observation of TLD (Cohen and Barner [20]), Cohen and Barner [33] explored how an *E. coli* strain with a functional thymidylate synthase responded to sulfanilamide (a sulphonamide) when certain metabolites were available or withheld. They used a custom media containing (metabolites directly affected by trimethoprim activity are denoted by †): glucose, methionine†, serine, valine, histidine, purines† (hypoxanthine and xanthine), vitamins (pantothenate and pyridoxine), thiamine and thymidine†. When *E. coli* was challenged with sulfanilamide on this media, they observed that cell viability would increase (as thymidine counteracted the drug), although an untreated control was not provided for comparison. Nonetheless, when purines were excluded from the media, cell viability arrested in the presence of sulfanilamide – a bacteriostatic effect. In contrast, when thymidine was excluded, cell viability rapidly declined in 3 h of exposure – a bactericidal effect (Fig. 3c, d shows a dose response of this effect). A summary of trimethoprim activity on viability in the presence of different metabolite combinations is shown in Table 2. The presence/absence of metabolites relating to the downstream effects of folate synthesis inhibitors meant it was possible to select whether DNA, RNA and/or protein synthesis would be affected. This outcome has been observed with sulfamethoxazole, trimethoprim and with the combination co-trimoxazole [13, 26, 29]. However, textbooks sometimes incorrectly claim that trimethoprim alone is bacteriostatic but only becomes bactericidal in the presence of sulfamethoxazole [34]. This misconception likely stems from turbidity (OD) measurements; bacteriostatic conditions will produce the expected plateau of turbidity after treatment; however, bactericidal conditions have been reported to have treated groups appear identical to untreated groups for up to 5 h post-exposure before declining in turbidity [33, 35]. Without further context, bactericidal conditions can appear bacteriostatic in the 8 h after trimethoprim exposure. However, within 2 h of exposure, ≥99.9% of the bacteria will no longer be viable [29]. The discrepancy between viability and turbidity could be explained by the elongation of bacteria observed under bactericidal conditions [36].

Bacteriostatic

Bacteriostatic trimethoprim activity requires the absence of purines from growth media for trimethoprim to visibly slow or arrest cellular growth [17, 30, 33]. This effect can be reproduced on a minimal growth medium containing only glucose, ammonium and salts (such as M9 minimal media). Under bacteriostatic conditions, the growth of bacteria is restricted in a dose-dependent manner and will saturate to a point where no growth is observed over several hours, but cells remain viable (Fig. 3a) [30].

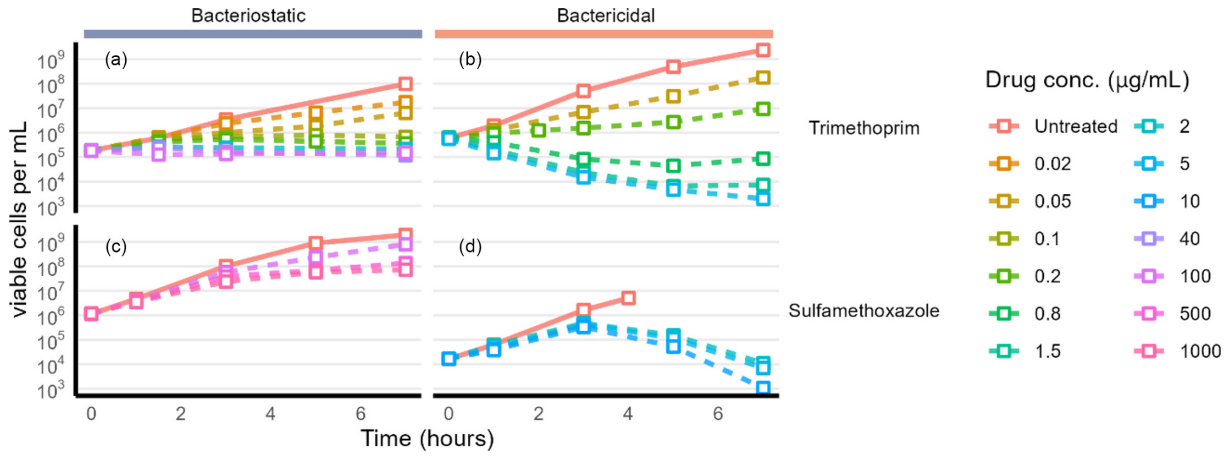

Fig. 3. Susceptible *E. coli* 1346 cell viability over time in response to varying concentrations of trimethoprim and sulfamethoxazole in bacteriostatic and bactericidal conditions. (a) Trimethoprim in bacteriostatic conditions (0.02–100 µg ml^{-1}), (b) trimethoprim in bactericidal conditions (0.02–5 µg ml^{-1}), (c) sulfamethoxazole in bacteriostatic conditions (40–1000 µg ml^{-1}) and (d) sulfamethoxazole in bactericidal conditions (2–10 µg ml^{-1}). Assays begin at 0 min after addition of antibiotic. Red solid line shows the untreated control; however, control data were not available for the full assay in sulfamethoxazole's bactericidal conditions. Data taken from Then and Angehrn [30] for trimethoprim and Then and Angehrn [26] for sulfamethoxazole.

Table 2. Outcome of trimethoprim activity on cell viability in the presence of glycine, methionine, a purine and thymidine in various combinations

Metabolites available	Trimethoprim activity	Loss of viability after 24 h (%)
None[1,2,3,4]	Bacteriostatic	≈0
Purine[1,2,4]	Bacteriostatic	~98
Glycine[1]	Bacteriostatic	≈0
Methionine[1]	Bacteriostatic	~40
Thymidine[4]	Bacteriostatic	≈0
Glycine+methionine[1,2,3,4]	Bacteriostatic	~80
Purine+methionine[1]	Bacteriostatic	~99
Purine+glycine[1]	Bactericidal*	≥99.9
Purine+glycine+methionine[1,2,4]	Bactericidal	≥99.9
Purine+glycine+methionine+thymidine[1,2]	None	None

*Conflicting evidence suggests delayed bactericidal activity as bacteriostatic in the first few hours of exposure. Sources: 1, Kwon *et al.* [35]; 2, Sangurdekar *et al.* [62]; 3, Then and Angehrn [30]; 4, Amyes and Smith [13].

As such, it does not appear that bacteriostatic conditions induce TLD on a population level and allow *E. coli* to survive in the presence of trimethoprim at relatively high concentrations. Indeed, *E. coli* treated with 100 μg ml^{-1} trimethoprim exhibited complete cessation of growth (Fig. 3a). For perspective, the clinical breakpoint for trimethoprim resistance is 4 μg ml^{-1} under 2021 EUCAST guidelines (v9.0) [37].

The mechanism behind bacteriostatic activity is unknown, and little evidence is available on this activity to draw a confident conclusion. Bacteriostatic action does not appear dependent on protein synthesis, as a bacteriostatic outcome occurs irrespective of whether methionine is available [13, 35]. This suggests that bacteriostatic activity is caused by the cell correctly recognizing that it can no longer synthesize new DNA and/or RNA due to purine starvation and results in halted growth [17]. Despite this, it leads to an energy-expensive outcome for *E. coli* that results in a rapid decline of intracellular ATP [17]. Some evidence suggests RNA synthesis is inhibited by trimethoprim activity under bacteriostatic conditions, but this does not support whether RNA is an essential component of the bacteriostatic activity [38].

Bactericidal

Bactericidal trimethoprim action is analogous to TLD, as the synthesis of dTMP appears to be the only metabolite directly affected [13, 35]. The minimum requirement to elicit a bactericidal trimethoprim action is the availability of a purine or nucleoside and amino acids glycine and methionine [13]. Bactericidal action is also dose-dependent and will result in a decline in viability within 2 h of exposure; however, lower concentrations can exhibit a bacteriostatic activity (Fig. 3b) [30]. The choice of purine or nucleoside is irrelevant, as the outcome is a decline of 1 to 10×10^3 from ~1.8×10^6 c.f.u. ml^{-1} after 7 h of exposure to 50 μg ml^{-1} trimethoprim regardless (Fig. 3b) [13]. The role of a purine/nucleoside is to replenish the purine pool, thereby restoring RNA and DNA synthesis [17]. This is supported by evidence of RNA synthesis occurring in bactericidal conditions for several hours post-exposure, as determined by accumulation of radiolabelled uracil [38, 39]. However, RNA synthesis has an inhibitory effect on cell killing, as *E. coli* L766 treated with trimethoprim in bactericidal conditions alongside the RNA synthesis inhibitor rifampicin exhibits a synergistic effect on killing compared to either drug alone [40]. The suicide module *mazEF* has been implicated as the killing mechanism behind TLD and bactericidal trimethoprim activity – a toxin (MazF)–antitoxin (MazE) system that results in programmed cell death (PCD) [39, 41]. Further, trimethoprim killing in bactericidal conditions is reversed in *E. coli mazEF* mutants [39]. The mechanism by which *mazEF* is triggered during TLD is unknown but is likely due to faulty DNA repair [24]. The inhibition of RNA synthesis by rifampicin likely prevents the unstable MazE antitoxin from being replenished, thereby activating *mazF* leading to PCD. This provides a plausible explanation as to why purine availability in minimal media (bacteriostatic conditions) results in significant cell death (Table 2).

Glycine and methionine, in addition to a purine, are a requirement for bactericidal activity (Table 2). Despite serine and glycine providing carbon units for the folate pool, the combination of adenine, serine and methionine does not result in a bactericidal action [13]. Nonetheless, the role of glycine is elusive, with some suggestions that it plays a regulatory role in the bactericidal action, and evidence is lacking to rule out other amino acids able to elicit bactericidal activity [13, 35]. Further, metabolic modelling of glycine's utilization during trimethoprim challenge suggested glycine did not play a specific role when the availability of active folates was constrained – reinforcing the notion of a regulatory role [17]. Methionine replenishes trimethoprim-induced

methionine depletion, thereby enabling protein synthesis, which is essential for bactericidal activity [13, 17]. Therefore, a bactericidal outcome requires DNA and protein synthesis via the availability of a purine, glycine and methionine [13, 33].

Trimethoprim activity in urine

Trimethoprim is an ideal antibiotic for treating UTIs, as it can persist at high concentrations in urine (10–90 mg l^{-1}), exceeding the minimum concentration needed to inhibit growth, over 24 h [42]. In the context of bacteriostatic and bactericidal trimethoprim activity, human urine in healthy adults contains metabolites that can influence this behaviour. Creatinine is used as a reference for the quantity of metabolites in urine, given the volume of water fluctuates greatly, and not all metabolites are always present in urine [43]. For each mM of creatinine in urine, there is 106 (range: 44–300) µM glycine with 100% occurrence, 1.4 (range: 0.5–25) µM methionine with 41% occurrence, 2.9 (range: 1.4–5.1) µM adenine with 45% occurrence (other purines and nucleosides at similar ranges and occurrence) and 2.2 (range: 0.7–39) µM thymidine with 36% occurrence [43]. The presence of key metabolites that elicit a bactericidal trimethoprim action suggests that bactericidal activity can occur in urine. Thymidine has been reported to be too low in concentration to impact the effect of trimethoprim in urine [44]. Several independent observations have been published that trimethoprim is bactericidal when urine is pooled from multiple participants, as determined by cell viability [40, 45, 46]. However, the bactericidal activity in these studies might be a result of the pooling, compensating for the lack of a purine or methionine in urine in some participants. Therefore, trimethoprim might act in a bacteriostatic fashion in many patients, which could explain the conflicting reports for bacteriostatic activity [47].

TRIMETHOPRIM RESISTANCE

Known resistance mechanisms to trimethoprim occur through point mutations in the DHFR enzyme encoding gene and are denoted as *dfr* to distinguish them from the wild-type *folA* [48]. There are two evolutionarily distinct groups: *dfrA* and *dfrB*, with the former being more common and often chromosomally encoded, whereas the latter is typically located on mobile genetic elements (especially next to integrons) [49, 50]. *dfrA* and *dfrB* are typed by their combination of mutations and numbered accordingly [48]. Amongst uropathogens, *dfrA1*, *dfrA5*, *dfrA12*, *dfrA14* and *dfrA17* were the most identified from genomic surveys [51–54]. Around 3–6% of *E. coli* isolated from urine carry two *dfrA* variants, with some evidence to suggest this provides a fitness benefit [51, 52, 54]. Thus far, the reported dual carriage cases are combinations of the predominant variants, typically containing *dfrA1*. Although *dfrA* variants are frequently found on the chromosome, they can also be highly mobile. Such variants are adjacent to integrons or carried on plasmids and frequently co-occur with aminoglycoside and extended-spectrum β-lactamase resistance genes, which minimizes treatment options for cases that are trimethoprim resistant (~20% incidence rate) [51, 55–57].

THYMIDINE AS A TRIMETHOPRIM INHIBITOR

The restrictions imposed on thymidylate synthase by trimethoprim limit the availability of dTMP – an essential precursor of DNA [13]. However, most organisms have a thymidine kinase which can synthesize dTMP from thymidine [20]. Therefore, trimethoprim killing has an inverse relationship with the quantity of thymidine available but only in bactericidal conditions (presumably as all biosynthesis components affected by trimethoprim are restored) [13, 19, 35, 58, 59]. In this context, trimethoprim killing can be restored by adding lysed horse blood, which contains thymidine phosphorylase that converts thymidine into its less effective counterpart thymine [60, 61]. Complex media often contains thymidine and should be used with caution when investigating trimethoprim activity [59]. Also, thymidine does not counteract bacteriostatic trimethoprim activity, which suggests it cannot act as a carbon source for folates [13, 35].

The counteractive properties of thymidine on bactericidal trimethoprim action led many to use thymidine as a control to assert that the observed behaviour was a result of trimethoprim [13, 29, 35, 39, 59]. However, there is insufficient evidence to suggest that thymidine is a valid control for short-term assays (under 3 h). For example, the time-course viability assay by Amyes and Smith [19] provided evidence to suggest thymidine is a valid control over several hours and matched the untreated sample. However, both metabolomics and protein expression data suggest that thymidine presence during bactericidal trimethoprim challenge does not match an untreated control in ≤120 min [35, 62]. Further, thymidine only counteracts the limited active folate available for thymidylate synthase and does not restore trimethoprim's constraint on purine and methionine synthesis [17]. This indicates thymidine is not an appropriate control for assays investigating trimethoprim action, particularly bacteriostatic activity.

DISCUSSION

Trimethoprim remains an important antibiotic for cost-effective, routine treatment of UTIs. The drug will have different effects on the bacterial cell depending on the metabolites available in the environment. The conventional bacteriostatic view of trimethoprim requires the absence of nucleosides from the environment and allows the bacteria to persist in the presence of relatively high concentrations of the drug. Conversely, trimethoprim becomes bactericidal in the presence of a nucleoside, glycine and methionine. Each behaviour has unique implications for the viability and omics data that can be collected from bacteria.

Trimethoprim has been suggested to be bactericidal in pooled human urine, but this might not reflect activity in individual cases due to changes in metabolite availability in urine. Therefore, it is important to understand the impact of media design on the activity of the drug to ensure comparability between published results and experimental design and interpretation. As such, it is prudent to have standardization of media design when investigating trimethoprim and limit usage of undefined growth media.

There are multiple paths that could be taken for future trimethoprim research. For example, it would be beneficial to revisit the early viability experiments of the 1970s and repeat these with a wider range of metabolites to understand if glycine and methionine are the only amino acids that can elicit bactericidal activity [13, 19, 26, 29, 30, 38, 61]. Further, glycine's role in bactericidal activity remains unknown. The means by which nucleoside limitation results in bacteriostatic activity is also unknown, alongside the killing mechanism of TLD in bactericidal conditions. A better understanding of how this antibiotic affects bacteria will enable greater exploitation of its activity for improved patient outcomes.

Funding information
All authors are supported by the Biotechnology and Biological Sciences Research Council Institute Strategic Programme Microbes and Food Safety BB/X011011/1 and its constituent project BBS/E/F/000PR13634 (Theme 1, Microbial threats from foods in established and evolving food systems).

Author contributions
C.C. – Conceptualization, Investigation, Visualization, Writing (original draft). J.W. – Writing (review and editing), Supervision. G.C.L. – Writing (review and editing), Supervision.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. McCloskey AP, Malabar L, McCabe PG, Gitsham A, Jarman I. Antibiotic prescribing trends in primary care 2014-2022. *Res Social Adm Pharm* 2023;19:1193–1201.
2. Public Health England. English surveillance programme for antimicrobial utilisation and resistance (ESPAUR) report. 2014.
3. Croker R, Walker AJ, Goldacre B. Why did some practices not implement new antibiotic prescribing guidelines on urinary tract infection? A cohort study and survey in NHS England primary care. *Journal of Antimicrobial Chemotherapy* 2019;74:1125–1132.
4. OpenPrescribing.net. Bennett Institute for Applied Data Science, University of Oxford; 2022. https://openprescribing.net
5. Bushby SRM. Trimethoprim-sulfamethoxazole: *in vitro* microbiological aspects. *J Infect Dis* 1973;128:S442–S462.
6. Gossius G, Vorland L. A randomised comparison of single-dose vs. three-day and ten-day therapy with trimethoprim-sulfamethoxazole for acute cystitis in women. *Scand J Infect Dis* 1984;16:373–379.
7. Church JA, Fitzgerald F, Walker AS, Gibb DM, Prendergast AJ. The expanding role of co-trimoxazole in developing countries. *Lancet Infect Dis* 2015;15:327–339.
8. Masters PA, O'Bryan TA, Zurlo J, Miller DQ, Joshi N. Trimethoprim-sulfamethoxazole revisited. *Arch Intern Med* 2003;163:402–410.
9. European Medicines Agency. Advice on the designation of antimicrobials or groups of antimicrobials reserved for treatment of certain infections in humans - in relation to implementing measures under Article 37(5) of Regulation (EU) 2019/6 on veterinary medicinal products. 2022.
10. Ducker GS, Rabinowitz JD. One-carbon metabolism in health and disease. *Cell Metab* 2017;25:27–42.
11. Green JM, Matthews RG. Folate biosynthesis, reduction, and polyglutamylation and the interconversion of folate derivatives. *EcoSal Plus* 2007;2.
12. Kikuchi G, Motokawa Y, Yoshida T, Hiraga K. Glycine cleavage system: reaction mechanism, physiological significance, and hyperglycinemia. *Proc Jpn Acad Ser B Phys Biol Sci* 2008;84:246–263.
13. Amyes SGB, Smith JT. Trimethoprim action and its analogy with thymine starvation. *Antimicrob Agents Chemother* 1974;5:169–178.
14. Dauber-Osguthorpe P, Roberts VA, Osguthorpe DJ, Wolff J, Genest M, *et al*. Structure and energetics of ligand binding to proteins: *Escherichia coli* dihydrofolate reductase-trimethoprim, a drug-receptor system. *Proteins* 1988;4:31–47.
15. Giladi M, Altman-Price N, Levin I, Levy L, Mevarech M. FolM, a new chromosomally encoded dihydrofolate reductase in *Escherichia coli*. *J Bacteriol* 2003;185:7015–7018.
16. Quinlivan EP, McPartlin J, Weir DG, Scott J. Mechanism of the antimicrobial drug trimethoprim revisited. *FASEB J* 2000;14:2519–2524.
17. Carter C, Singh D, Wain J, Langridge GC. Genome-scale metabolic modelling of trimethoprim's mode of action reveals free ATP salvaged to restore purine pool. *bioRxiv* 2025. 10.1101/2025.07.25.666800 [Preprint]
18. Giroux X, Su W-L, Bredeche M-F, Matic I. Maladaptive DNA repair is the ultimate contributor to the death of trimethoprim-treated cells under aerobic and anaerobic conditions. *Proc Natl Acad Sci USA* 2017;114:11512–11517.
19. Amyes SGB, Smith JT. Trimethoprim-sensitivity testing and thymineless mutants. *J Med Microbiol* 1974;7:143–153.
20. Cohen SS, Barner HD. Studies on unbalanced growth in *Escherichia coli*. *Proc Natl Acad Sci USA* 1954;40:885–893.
21. Khan SR, Kuzminov A. Thymineless death in *Escherichia coli* is unaffected by chromosomal replication Complexity. *J Bacteriol* 2019;201:00797–18.
22. Makino F, Munakata N. Deoxyuridine residues in DNA of thymine-requiring *Bacillus* subtilis strains with defective N-glycosidase activity for uracil-containing DNA. *J Bacteriol* 1978;134:24–29.
23. Kuong KJ, Kuzminov A. Stalled replication fork repair and misrepair during thymineless death in *Escherichia coli*. *Genes Cells* 2010;15:619–634.
24. Khodursky A, Guzmán EC, Hanawalt PC. Thymineless death lives on: new insights into a classic phenomenon. *Annu Rev Microbiol* 2015;69:247–263.
25. Khan SR, Mahaseth T, Kouzminova EA, Cronan GE, Kuzminov A. Static and dynamic factors limit chromosomal replication complexity in *Escherichia coli*, avoiding dangers of runaway overreplication. *Genetics* 2016;202:945–960.
26. Then R, Angehrn P. Sulphonamide-induced "thymineless death" in *Escherichia coli*. *J Gen Microbiol* 1973;76:255–263.
27. Fernández-Villa D, Aguilar MR, Rojo L. Folic acid antagonists: antimicrobial and immunomodulating mechanisms and applications. *Int J Mol Sci* 2019;20:4996.
28. Gallien R. Antibacterial action of trimethoprim-sulfamethoxazole. *J Infect Dis* 1973;128:Suppl.

29. Amyes SG. Bactericidal activity of trimethoprim alone and in combination with sulfamethoxazole on susceptible and resistant *Escherichia coli* K-12. *Antimicrob Agents Chemother* 1982;21:288–293.
30. Then R, Angehrn P. Nature of the bacterial action of sulfonamides and trimethoprim, alone and in combination. *J Infect Dis* 1973;128:Suppl.
31. Minato Y, Dawadi S, Kordus SL, Sivanandam A, Aldrich CC, *et al*. Mutual potentiation drives synergy between trimethoprim and sulfamethoxazole. *Nat Commun* 2018;9:1003.
32. Norrby SR. Short-term treatment of uncomplicated lower urinary tract infections in women. *Rev Infect Dis* 1990;12:458–467.
33. Cohen SS, Barner HD. Studies on the induction of thymine deficiency and on the effects of thymine and thymidine analogues in Escherichia coli. *J Bacteriol* 1956;71:588–597.
34. Kester M, Karpa KD, Vrana KE. 4 - treatment of infectious diseases. In: Kester M, Karpa KD and Vrana KE (eds). *Elsevier's Integrated Review Pharmacology*. Philadelphia: W.B. Saunders; 2012. pp. 41–78.
35. Kwon YK, Higgins MB, Rabinowitz JD. Antifolate-induced depletion of intracellular glycine and purines inhibits thymineless death in *E. coli*. *ACS Chem Biol* 2010;5:787–795.
36. Cassaro C. Imaging of Bacterial Responses to DNA Damage Caused by Antibacterial Agents (PhD Thesis). University of Oxford 2024.
37. European Committee on Antimicrobial Susceptibility Testing (EUCAST). EUCAST disk diffusion method (version 9). 2021.
38. Then R, Angehrn P. Effects of trimethoprim and its antagonists on RNA synthesis in *Escherichia coli*. *Biochimica et Biophysica Acta (BBA) - Nucleic Acids Protein Synthesis* 1972;287:98–105.
39. Sat B, Reches M, Engelberg-Kulka H. The *Escherichia coli* mazEF suicide module mediates thymineless death. *J Bacteriol* 2003;185:1803–1807.
40. Goldstein BP, Ripamonti IF, Bolzoni G, Carniti G, Arioli V. Rifampin plus trimethoprim: bactericidal activity and suppression of resistance in human urine in vitro. *Antimicrob Agents Chemother* 1979;16:736–742.
41. Engelberg-Kulka H, Hazan R, Amitai S. mazEF: a chromosomal toxin-antitoxin module that triggers programmed cell death in bacteria. *J Cell Sci* 2005;118:4327–4332.
42. Neu HC. Optimal characteristics of agents to treat uncomplicated urinary tract infections. *Infection* 1992;20 Suppl 4:S266–71.
43. Bouatra S, Aziat F, Mandal R, Guo AC, Wilson MR, *et al*. The human urine metabolome. *PLoS One* 2013;8:e73076.
44. Then R, Angehrn P. The biochemical basis of the antimicrobial action of sulfonamides and trimethoprim in vivo--I. Action of sulfonamides and trimethoprim in blood and urine. *Biochem Pharmacol* 1974;23:2977–2982.
45. Lacey RW, Rogerson JG, Stokes A. Antimicrobial effects of trimethoprim and sulphadiazine in infected urine and blood. *J Med Microbiol* 1980;13:121–132.
46. Drobot GR, Karlowsky JA, Hoban DJ, Zhanel GG. Antibiotic activity in microbiological media versus that in human urine: comparison of ampicillin, ciprofloxacin, and trimethoprim-sulfamethoxazole. *Antimicrob Agents Chemother* 1996;40:237–240.
47. Anderson JD, Lacey RW, Lewis EL, Sellin MA. Failure to demonstrate an advantage in combining sulphamethoxazole with trimethoprim in an experimental model of urinary infection. *J Clin Pathol* 1974;27:619–622.
48. Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, *et al*. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. *Nucleic Acids Res* 2020;48:D517–D525.
49. Toprak E, Veres A, Michel J-B, Chait R, Hartl DL, *et al*. Evolutionary paths to antibiotic resistance under dynamically sustained drug selection. *Nat Genet* 2012;44:101–105.
50. Toulouse JL, Edens TJ, Alejaldre L, Manges AR, Pelletier JN. Integron-associated DfrB4, a previously uncharacterized member of the trimethoprim-resistant dihydrofolate reductase B family, is a clinically identified emergent source of antibiotic resistance. *Antimicrob Agents Chemother* 2017;61:02665–16.
51. Carter C, Hutchison A, Rudder S, Trotter E, Waters EV, *et al*. Uropathogenic *Escherichia coli* population structure and antimicrobial susceptibility in Norfolk, UK. *J Antimicrob Chemother* 2023;78:2028–2036.
52. Spencer A, Wong Q, Lawson ST, Fry H, Ramchandani Ramchandani NM, *et al*. Trimethoprim resistance in *Escherichia coli* exhibits an allele-specific growth advantage. *J Med Microbiol* 2025;74:002021.
53. Somorin YM, Weir N-JM, Pattison SH, Crockard MA, Hughes CM, *et al*. Antimicrobial resistance in urinary pathogens and culture-independent detection of trimethoprim resistance in urine from patients with urinary tract infection. *BMC Microbiol* 2022;22:144.
54. Sundqvist M, Granholm S, Naseer U, Rydén P, Brolund A, *et al*. Within-Population distribution of trimethoprim resistance in *Escherichia coli* before and after a community-wide intervention on trimethoprim use. *Antimicrob Agents Chemother* 2014;58:7492–7500.
55. Nasrollahian S, Graham JP, Halaji M. A review of the mechanisms that confer antibiotic resistance in pathotypes of *E. coli Front Cell Infect Microbiol* 2024;14:1387497.
56. Poey ME, de Los Santos E, Aznarez D, García-Laviña CX, Laviña M. Genetics of resistance to trimethoprim in cotrimoxazole resistant uropathogenic *Escherichia coli*: integrons, transposons, and single gene cassettes. *Front Microbiol* 2024;15:1395953.
57. Goswami C, Fox S, Holden MTG, Connor M, Leanord A, *et al*. Origin, maintenance and spread of antibiotic resistance genes within plasmids and chromosomes of bloodstream isolates of *Escherichia coli Microb Genom* 2020;6:e000353.
58. Dale BA, Greenberg GR. Effect of the folic acid analogue, trimethoprim, on growth, macromolecular synthesis, and incorporation of exogenous thymine in *Escherichia coli*. *J Bacteriol* 1972;110:905–916.
59. Koch AE, Burchall JJ. Reversal of the antimicrobial activity of trimethoprim by thymidine in commercially prepared media. *Appl Microbiol* 1971;22:812–817.
60. Harper GJ, Cawston WC. The in-vitro determination of the sulphonamide sensitivity of bacteria. *J Pathol* 1945;57:59–66.
61. Amyes SGB, Smith JT. Trimethoprim antagonists: effect of uridine on thymine and thymidine uptake in mineral salts medium. *J Antimicrob Chemother* 1978;4:415–419.
62. Sangurdekar DP, Zhang Z, Khodursky AB. The association of DNA damage response and nucleotide level modulation with the antibacterial mechanism of the anti-folate drug trimethoprim. *BMC Genomics* 2011;12:583.
63. NHS Prescribing Services. Drug tariff. NHS Business Services Authority; 2022. https://www.nhsbsa.nhs.uk/pharmacies-gp-practices-and-appliance-contractors/drug-tariff
64. Karp PD, Billington R, Caspi R, Fulcher CA, Latendresse M, *et al*. The BioCyc collection of microbial genomes and metabolic pathways. *Brief Bioinformatics* 2019;20:1085–1093.