Trimethoprim and enterococci in urinary tract infections: new perspectives on an old issue

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The lack of oral treatment alternatives for enterococcal urinary tract infections (UTIs) has led to a renewed interest in trimethoprim. Enterococci can incorporate exogenously produced folates and thereby reverse the effect of trimethoprim. Although a large proportion of enterococci appear susceptible to trimethoprim in vitro using standard media devoid of folates, a 360-fold increase in the MIC can be seen when susceptibility testing is performed in media containing fresh urine. Even if trimethoprim has a favourable pharmacokinetic profile, with high serum and very high urine concentrations, pharmacodynamic (PD) estimates show that a large proportion of the apparent wild-type isolates (as categorized by standard susceptibility testing) have unfavourable PD indices. The clinical efficacy of trimethoprim in enterococcal UTI in the literature. The eradication rate was 82%. Case reports where patients on co-trimoxazole for UTI have developed bacteraemia with enterococci susceptible to trimethoprim seem to support experimental findings that standard antimicrobial susceptibility testing poorly predicts the clinical outcome of trimethoprim therapy. The European Committee on Antimicrobial Susceptibility Testing and the national breakpoint committees in Europe have recently debated the role of trimethoprim in the treatment of enterococcal UTI and agreed to categorize wild-type enterococci as intermediate to trimethoprim and trimethoprim/sulfamethoxazole. This allows the distinction between enterococci with and without acquired resistance mechanisms to trimethoprim. This review discusses the microbiological, experimental, clinical and PD aspects of the usage of trimethoprim for enterococcal UTI.

Keywords: MIC, PK/PD, drug susceptibility testing, antimicrobial activity

Introduction

Enterococci cause a wide variety of diseases with wound and urinary tract infections (UTIs) being the most common and bloodstream infections and endocarditis the most severe and therapeutically challenging. Resistance to most commonly used antimicrobial agents is a typical characteristic of enterococci and an important explanation for their success as nosocomial pathogens.1,2 Enterococci usually feature intrinsic low-level resistance to β-lactams and aminoglycosides and acquired resistance to most other classes of antibiotics.3,4

Systemic treatment options for serious enterococcal infections are ampicillin (although most Enterococcus faecium are resistant), glycopeptides, daptomycin, linezolid, dalfopristin/quinupristin (only E. faecium) and tigecycline.5–7 The newer compounds have limited approved clinical indications, some of them may require concentration monitoring and they are, in general, expensive and or difficult to administer and not suitable for oral therapy.

Oral treatment options are even fewer. Although basically all Enterococcus faecalis isolates are susceptible to amoxicillin and most to nitrofurantoin, resistance to other oral alternatives is frequently observed. Most E. faecium are resistant to all oral therapeutic alternatives (with the exception of linezolid), leaving few or no treatment options, depending on whether nitrofurantoin is perceived to be an efficacious agent in these cases, for relatively simple infections such as UTIs.

The lack of oral treatment alternatives for enterococcal infections has lead to a renewed interest in trimethoprim. In vitro, in test systems devoid of folates, a large proportion of enterococci typically exhibit low MICs for trimethoprim.8,9 However, the use of trimethoprim for the treatment of enterococcal urinary infections is controversial due the ability of enterococci to incorporate exogenous folates.10–12 As part of the process for harmonizing European breakpoints (through the European Committee on Antimicrobial Susceptibility Testing), breakpoint committees in Europe have been debating the role of...
trimethoprim in the treatment of UTI caused by enterococcal infections.

As trimethoprim is widely used for the empirical treatment of UTIs, and due to its favourable pharmacokinetic (PK) properties, the possible use of trimethoprim for enterococcal UTIs will be reviewed.

**Pharmacokinetics of trimethoprim**

Trimethoprim is a bacteriostatic compound that inhibits microbial dihydrofolate reductase and thereby renders the bacteria deficient in di- and tetrahydrofolic acid, essential derivatives in nucleotide and amino acid biosynthesis (Figure 1).

The PK properties of trimethoprim are summarized in Table 1. Peak plasma concentrations at steady state have been examined in a few studies and showed that an intravenous dose of 160 mg three-times daily gave peak plasma concentrations of ~8 mg/L, and if given orally, maximal levels of 5 mg/L were observed.\(^{13,14}\) Bruun et al.\(^{15}\) measured the human plasma concentration of trimethoprim in five healthy individuals throughout a 12 h period after an oral dose of 160 mg (twice daily). From this study, an approximate free AUC in serum of 31 mg·h/L could be calculated (Figure 2 and Table 1).

The tissue concentration of trimethoprim is usually higher than the corresponding concentration in serum (shown for bile, saliva and sputum). In particular, high concentrations can be found in lung and kidney tissues.\(^{16}\) In prostatic fluid, the levels are found to be two to three times the serum concentration.\(^{17}\) However, lower levels may be present in patients with chronic prostatitis.\(^{18}\)

Urine concentrations of trimethoprim are considerably higher than the corresponding plasma concentrations. Continuous dosing of 100 mg once daily gives urine concentrations ranging from 30 to 160 mg/L and a dose of 200 mg once daily produced levels twice as high. The urine concentration seems to be approximately 10 times as high as the corresponding serum concentration.\(^{19–21}\)

**Enterococci incorporate exogenously produced folates**

The activity of trimethoprim can be reversed if the bacteria can circumvent the blocked production of tetrahydrofolic acid by incorporating exogenously produced tetrahydrofolic acid or downstream components in the nucleic and amino acid synthesis pathway (Figure 1). In one study, it was shown that enterococci were more susceptible to the reversal of trimethoprim’s antibacterial activity by folates and related compounds than other species.\(^{22}\) *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *E. faecium*, *E. faecalis* and *Escherichia coli* could all incorporate the exogenous pyrimidine derivative thymidine at high levels. *E. coli* and the two tested enterococcal species could incorporate the pyrimidine derivative thymine. The ability to incorporate thymidine and thymine probably does not contribute to the resistance of enterococci and other species to trimethoprim, as

![Figure 1. Conversion of folate into tetrahydrofolate catalysed by dihydrofolate reductase.](image)

**Table 1. Trimethoprim PK characteristics**

<table>
<thead>
<tr>
<th>Pharmacokinetic variable</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption after oral administration</td>
<td>rapid, 90–100%</td>
</tr>
<tr>
<td>adults: 8–10 h</td>
<td>Drugdex(^{44})</td>
</tr>
<tr>
<td>children: 3–5.5 h</td>
<td>Kasanen et al.(^{27})</td>
</tr>
<tr>
<td>newborn: 19 h</td>
<td></td>
</tr>
<tr>
<td>Plasma protein binding</td>
<td>44%</td>
</tr>
<tr>
<td>Excretion</td>
<td>mainly renal (40–60% is excreted in its original form within 24 h) faecal (4%)</td>
</tr>
<tr>
<td>Metabolism</td>
<td>10–20% metabolized, primarily hepatic</td>
</tr>
<tr>
<td>adults: 1.3–1.8 L/kg</td>
<td>Drugdex(^{44},) Schwartz and Ziegler(^{45})</td>
</tr>
<tr>
<td>children (1–10 years): 1 L/kg</td>
<td>Drugdex(^{44})</td>
</tr>
<tr>
<td>newborn: 2.7 L/kg</td>
<td>Drugdex(^{44})</td>
</tr>
<tr>
<td>(f\text{AUC}) (^{a}) plasma</td>
<td>31 mg·h/L</td>
</tr>
<tr>
<td>(\text{AUC}) (^{b}) urine</td>
<td>552 mg·h/L</td>
</tr>
<tr>
<td>(C_{\text{AUC}}) plasma</td>
<td>2.3 mg·L (mean trough concentration) × 24 h × 0.56 (protein binding 44%). Trimethoprim dose 320 mg twice daily.</td>
</tr>
<tr>
<td>(C_{\text{AUC}}) urine</td>
<td>23 mg·L (approximated mean trough urine concentration) × 24 h. Trimethoprim dose 320 mg twice daily.</td>
</tr>
</tbody>
</table>

\(^{a}\)Calculated from Bruun et al.\(^{15}\) \(\text{Freet}f\text{AUC}\) \(_{\text{plasma}} = 2.3 \text{ mg·L (mean trough concentration)} \times 24 \text{ h} \times 0.56 \text{ (protein binding 44%). Trimethoprim dose 320 mg twice daily.}\)

\(^{b}\)Calculated from Bruun et al.\(^{15}\) and the approximation that the urine concentration, in general, is at least 10 times as high as the plasma concentration.\(^{19}\) \(\text{AUC}_{\text{urine}} = 23 \text{ mg·L (approximated mean trough urine concentration)} \times 24 \text{ h. Trimethoprim dose 320 mg twice daily.}\)
an animal model showed that these substances were rapidly degraded and consequently did not affect the antimicrobial activity of trimethoprim *in vivo*. E. faecalis and *E. faecium* were the only species tested that could incorporate dihydrofolate, tetrahydrofolate and formyl-tetrahydrofolate (folinic acid). By adding these folates to the media, the MIC of trimethoprim was increased in the order of 10-fold.

These results are consistent with those of Zervos and Schaberg who compared the MICs of trimethoprim in urine and in Mueller–Hinton broth (MHB) for 21 *E. faecalis* isolates. The study showed that when the MIC of trimethoprim was determined in urine, a more than 60-fold and 360-fold increase in MIC was seen in the two urine samples tested, respectively (Table 2). The addition of formyl-tetrahydrofolate to MHB increased the MIC of trimethoprim 25-fold. The study also demonstrated a sigmoid relationship between the concentration of formyl-tetrahydrofolate in the medium and the trimethoprim MIC of the enterococci. A linear increase in MIC in relation to formyl-tetrahydrofolate concentration was seen between 0.001 and 0.1 mg/L formyl-tetrahydrofolate, but not at higher concentrations (1–10 mg/L). These concentrations can be compared with the normal concentration of formyl-tetrahydrofolate in serum which is 6–20 mg/L and in urine 2–7 mg/L, i.e. in the range where a significant effect on the MIC of trimethoprim can be expected. Osmolality and pH were also shown to affect the MIC of trimethoprim. An increased pH partially reversed the resistance of *E. faecalis* to trimethoprim.

### Table 2. Susceptibility of clinical isolates of *E. faecalis* (n = 21) to trimethoprim when tested in MHB, MHB supplemented with tetrahydrofolate and in two different, arbitrarily selected urines

<table>
<thead>
<tr>
<th>Medium</th>
<th>Trimethoprim/ sulfamethoxazole MIC (mg/L) expressed as the TMP MIC (mean, range)</th>
<th>Increase in MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHB</td>
<td>0.13 (0.002–0.625)</td>
<td>1</td>
</tr>
<tr>
<td>MHB + tetrahydrofolate</td>
<td>3.3 (0.1–12.5)</td>
<td>25</td>
</tr>
<tr>
<td>(1 mg/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine 1</td>
<td>8.1 (1.6–50)</td>
<td>62</td>
</tr>
<tr>
<td>Urine 2</td>
<td>46.4 (25–100)</td>
<td>357</td>
</tr>
</tbody>
</table>

TMP, trimethoprim.

Adapted from Zervos and Schaberg with kind permission from the authors and the American Society for Microbiology.

**Clinical studies**

No large randomized prospective clinical studies have been performed in which the effect of trimethoprim or trimethoprim/sulfamethoxazole on UTIs caused by *Enterococcus* spp. has been studied. However, a few clinical studies have evaluated the effect of trimethoprim alone or in combination with various sulphonamides on UTI. Sulphonamides have no *in vitro* activity against enterococci, and only sulfamethoxazole has been demonstrated to have an additive effect if combined with trimethoprim (ratio 1:19) and only on *E. faecalis*. Thus, only the contributions of the trimethoprim component will be reviewed below. As there are no control groups in these studies, the results are difficult to interpret. Only in three of the studies could the therapeutic efficacy of trimethoprim be evaluated in relation to the infecting organism, leaving a total of 38 evaluable enterococcal UTIs.

Sitzhen and Rugendorf looked at the effect of trimethoprim/sulfadiazine and trimethoprim/sulfamethoxazole in the treatment of UTIs. The trimethoprim/sulfadiazine group received 180 mg of trimethoprim (and 820 mg of sulfadiazine) (once daily) (enterococci; n = 13), and the trimethoprim/sulfamethoxazole group received 320 mg of trimethoprim (and 800 mg of sulfamethoxazole) (twice daily) (enterococci; n = 12). At follow-up, 3–4 weeks after therapy was initiated, and 10 of 12 (83%) and 11 of 13 (85%) patients on the respective dosage were cured microbiologically. This can be compared with UTIs due to *E. coli* and *S. aureus*, in which 90% and 100%, respectively, were eradicated.

Nielsen *et al.* studied the effect of trimethoprim in combination with sulfamethoxazole. Six patients received 320 mg of trimethoprim daily (and 800 mg of sulfamethoxazole twice daily). The patients were with (n = 5) or without (n = 1) factors predisposing to UTI. Three of five patients (and the only patient without predisposing factors) were cured microbiologically at follow-up on day 14.

Stratford and Dixon studied trimethoprim and sulfamethoxazole in combination, and patients were given ‘two tablets Bactrim every six or every eight hours’. Most likely, this corresponds to a dose of 80 mg of trimethoprim (and 400 mg of sulfamethoxazole) per tablet and daily doses of 240–320 mg of trimethoprim. Eight patients were identified as having enterococcal UTIs. Of these, seven (87.5%) were cleared at follow-up. The authors also looked at re-infection after Gram-negative UTI and pointed out that a large proportion of re-infections were due to enterococci.

Of the total of 38 enterococcal UTIs in the three studies together, 31 were eradicated at follow-up, which gives an overall eradication rate of 82%.

A study by Reid and Howard looked at the use of trimethoprim as prophylaxis (40 mg twice daily) for UTIs in patients with spinal cord injuries (n = 30). Although the prophylactic therapy significantly reduced the incidence of symptomatic UTIs, the regimen resulted in a 2-fold increase in enterococcal UTIs.

### Case reports of clinical failures

Two separate reports have been published in which infections with enterococcal isolates with apparent trimethoprim *in vitro*...
susceptibility failed on trimethoprim/sulfamethoxazole treatment. Goodhart described two patients with uncomplicated UTIs caused by apparently susceptible enterococci, both treated with trimethoprim/sulfamethoxazole. Both patients developed bacteraemia during trimethoprim/sulfamethoxazole treatment, and the in vitro susceptible enterococci could be isolated from the respective blood cultures. The patients recovered after trimethoprim/sulfamethoxazole was discontinued and exchanged for vancomycin plus streptomycin in the first case and penicillin trimethoprim in the placebo group) when compared with 40% for ampicillin.

Animal models

Two animal models investigating trimethoprim/sulfamethoxazole in the treatment of enterococci have been published. In a rat enterococcal endocarditis model, the efficacy of trimethoprim sulfamethoxazole and ampicillin was compared \((n = 31)\). Trimethoprim/sulfamethoxazole had no effect on enterococcal endocarditis in this study. In the trimethoprim/sulfamethoxazole group, the cure rate was 0% (same as placebo) when compared with the ampicillin group for which the cure rate was 38%. Chenoweth et al. compared trimethoprim sulfamethoxazole and ampicillin in a mouse peritonitis lethal model \((n = 20)\). With sulfamethoxazole treatment, the mortality was 95% (similar to 100% in the placebo group) when compared with 40% for ampicillin.

Acquired resistance

Although a large proportion of enterococci are susceptible to trimethoprim in vitro, high-level resistance has been reported from many parts of the world. Resistance rates of 25–50% are common. The resistance mechanisms have not been fully elucidated. In other species, mutational changes in the dihydrofolate reductase gene leading to changes in the affinity for trimethoprim and/or overproduction of dihydrofolate reductase have been reported.

PK and pharmacodynamic modelling

Whether the effect of trimethoprim best correlates with time above MIC \((%T/MIC)\) or is concentration-dependent \((AUC/MIC)\) has not been studied. In order to obtain an estimate as to whether reasonable target levels can be reached, approximate \(AUC/MIC\) and \(%T/MIC\) were calculated for both serum and urine (Table 3). Hypothetical MIC values range from 0.5 mg/L (the highest MIC value of wild-type isolates on standard media) to 100 mg/L (the highest MIC value of wild-type isolates in fresh urine). For serum, the free trimethoprim concentration was used, but since the protein bound fraction in urine is unknown, the total trimethoprim concentration was used in these calculations. Though never determined for trimethoprim, we assumed that trimethoprim, similar to other nucleic acid synthesis inhibitors, would require a target AUC/MIC of 40–60. For Gram-positive pathogens, the required \(%T/MIC\) is, in general, 30% to 40% for \(\beta\)-lactams, which we tentatively used for trimethoprim. Assuming a UTI and a trimethoprim dose of 160 mg every 12h, these criteria can be met with strains having MIC values up to 10 and 45 mg/L, respectively, depending on whether AUC/MIC or \(%T/MIC\) is used as the pharmacodynamic (PD) target. Using these theoretical PD MIC breakpoints and the MIC values obtained when testing clinical isolates in fresh urine, a large proportion of the apparent wild-type isolates [as categorized by standard antimicrobial susceptibility testing (AST)] would be categorized as resistant.

Conclusions

MIC values of trimethoprim in MHB range from 0.002 to 0.63 mg/L. When performing MIC determinations in urine, the range increased to 2 to 100 mg/L. As the activity of trimethoprim is heavily influenced by the presence of folates and the degree of osmolality and acidity, all of which are factors that vary widely in urine, it is difficult to ascertain the predictive value of susceptibility testing when the isolate is categorized as susceptible to trimethoprim using standard AST. In vitro testing can, however, predict when trimethoprim should not be used, as the test can accurately distinguish between isolates with and without resistance mechanisms.

Animal models and reports of clinical failures with in vitro susceptible _E. faecalis_ strains indicate a lack of clinical success with trimethoprim in systemic infections. Due to the high trimethoprim concentrations in the urine, trimethoprim could be a therapeutic option for UTIs. The few clinical studies that exist have no control groups, and the number of trimethoprim-treated enterococcal UTIs is limited. However, the clinical studies indicate that there is clinical success with trimethoprim in some patients with enterococcal UTI. Although desirable, it seems difficult to envisage a susceptibility test procedure that takes into account individual differences in folate content, pH and/or osmolality. Even if the predictive value of a susceptible categorization is doubtful, an epidemiological cut-off value which on standard media can distinguish wild-type (WT \(\leq 0.5\) mg/L) from non-wild-type enterococci would be useful. In the ongoing harmonization process for European breakpoints, the currently agreed approach among breakpoint committees in Europe is to categorize wild-type enterococci as intermediate to trimethoprim.

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**Table 3. Trimethoprim (TMP)—calculated AUC/MIC and %T/MIC for urine and plasma of six hypothetical MIC values**

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>(f\text{AUC}^{\text{a}}/\text{MIC}) (plasma)</th>
<th>(\text{AUC}^{\text{b}}/\text{MIC}) (urine)</th>
<th>%(T^{\text{c}}) (free concentration)/MIC (plasma)</th>
<th>%(T^{\text{d}}) (urine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>62</td>
<td>1104</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>3.1</td>
<td>55</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>25</td>
<td>1.2</td>
<td>22</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>45</td>
<td>0.7</td>
<td>12</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>50</td>
<td>0.6</td>
<td>11</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>100</td>
<td>0.31</td>
<td>5.5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^{a}\)Calculations are based on a trimethoprim dose of 160 mg every 12 h.\(^{15}\)

\(^{b}\)\(AUC_{\text{plasma}} = 31\) mg h/L (Table 1).

\(^{c}\)\(AUC_{\text{urine}} = 552\) mg h/L (Table 1).

\(^{d}\)Approximated from Bruun et al.\(^{15}\)

\(^{e}\)Approximated from Bruun et al.\(^{15}\) and Jodal and Fellner.\(^{19}\)
and trimethoprim/sulfamethoxazole and that the R-breakpoint \( R > 1 \text{ mg/L} \) will be helpful in identifying strains with acquired high-grade resistance to trimethoprim.\textsuperscript{23}

### Acknowledgements

We are grateful to Annelie Brauner for critical reading of the manuscript.

### Transparency declarations

None to declare.

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