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Empirical treatment of lower urinary tract infections in the face of spreading multidrug resistance: in vitro study on the effectiveness of nitroxoline

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ABSTRACT

The spread of antimicrobial resistance challenges the empirical treatment of urinary tract infections (UTIs). Among others, nitrofurantoin is recommended for first-line treatment, but acceptance among clinicians is limited due to chronic nitrofurantoin-induced lung toxicity and insufficient coverage of Enterobacteriaceae other than *Escherichia coli*. Nitroxoline appears to be an alternative to nitrofurantoin owing to its favourable safety profile, however data on its current in vitro susceptibility are sparse. In this study, susceptibility to nitroxoline was tested against 3012 urinary clinical isolates (including multidrug-resistant bacteria and *Candida* spp.) by disk diffusion test and/or broth microdilution. At least 91% of all Gram-negatives ($n = 2000$), Gram-positives ($n = 403$) and yeasts ($n = 132$) had inhibition zone diameters for nitroxoline ≥18 mm. Except for *Pseudomonas aeruginosa*, nitroxoline MIC_{90} values were ≤16 mg/L and were 2- to >16-fold lower compared with nitrofurantoin. In extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae and methicillin-resistant *Staphylococcus aureus* (MRSA), MIC_{90} values of nitroxoline were two-fold higher compared with non-ESBL-producing enterobacteria and methicillin-susceptible *S. aureus* (MSSA). The in vitro efficacies of nitroxoline and nitrofurantoin against ATCC strains of *E. coli*, *Enterococcus faecalis* and *Proteus mirabilis* were compared by time–kill curves in Mueller–Hinton broth and artificial urine. Nitroxoline was non-inferior against *E. coli*, *P. mirabilis* and *E. faecalis* in artificial urine. In conclusion, nitroxoline showed a broad antimicrobial spectrum, with inhibition zone diameters and MICs of nitroxoline well below the EUCAST breakpoint for *E. coli* for most organisms, and thus may also be a target for therapy of uncomplicated UTIs.

1. Introduction

Urinary tract infection (UTI) is a very common entity with a global prevalence of 0.7% for community-associated UTI [1] and 5.1% for healthcare-associated UTI in urology departments [1–3]. Apart from having an enormous financial impact, inadequate treatment practices for UTI will select for and foster the spread of emerging multidrug-resistant (MDR) bacterial strains. For practical and cost-effectiveness reasons, routine urinary cultures are recommended only in the setting of complicated or recurrent infections [4–6]. Successful empirical treatment regimens require resistance rates not exceeding 10–20% [7–9]. Current guidelines recommend antimicrobial agents that combine low 'collateral damage' potential and low resistance rates against the predominant pathogen *Escherichia coli*, such as nitrofurantoin, fosfomycin or pivmecillinam, for empirical treatment of uncomplicated UTI [3,9]. Nitrofurantoin is a broad-spectrum antibiotic active against Gram-positive and Gram-negative pathogens and has become the drug of choice treating lower UTIs caused by MDR pathogens [10]. Whereas susceptibility rates to nitrofurantoin in *E. coli* are excellent, other uropathogenic Enterobacteriaceae are mainly resistant [10]. Furthermore, nitrofurantoin has been associated with severe side effects, including pulmonary damage, hepatic toxicity, haemolytic anaemia, peripheral

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neuropathy and central nervous system disturbances [11–14]. Although adverse reactions are rare and mild when nitrofurantoin is given as short-term therapy, there is concern about treating an often self-limiting infection with a potentially fatal drug [15].

Nitroxoline (5-nitro-8-hydroxyquinoline) shares the positive aspects of nitrofurantoin but without its undesired toxicity. It is an orally administered, renally excreted antibiotic that does not reach significant plasma levels which was introduced into clinical practice in the 1960s but, in contrast to nitrofurantoin, no severe adverse effects have been reported far [10]. The antimicrobial activity of nitroxoline is mediated by the chelation of essential cations and involves multiple targets [16,17]. Although nitroxoline may be a good alternative, it was not included in treatment guidelines until 2016 owing to lack of information on resistance rates, minimum inhibitory concentration (MIC) distributions and European Committee on Antimicrobial Susceptibility Testing (EUCAST)-confirmed breakpoints. The update of the German interdisciplinary S3 guideline proposed to include nitroxoline in non-severe cases of uncomplicated UTI in adults [18], and the breakpoint for nitroxoline was included in the latest EUCAST clinical breakpoints table for bacteria (version 7, 2017; http://www.eucast.org/clinical_breakpoints/) for *E. coli* in uncomplicated UTI.

The aim of this analysis was to compare the in vitro antimicrobial activity of nitroxoline compared with nitrofurantoin against a large number of clinical UTI isolates including *E. coli* and other Enterobacteriaceae, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, Gram-positive isolates and *Candida* spp. A total of 3012 isolates were tested for nitroxoline susceptibility in vitro by disk diffusion assay and MIC testing. In addition, time–kill curves and minimum bactericidal concentrations (MBCs) for these two antibiotics were compared in selected ATCC reference strains.

2. Methods

2.1. Bacterial isolates

Clinical UTI isolates ($n = 3012$) were derived from consecutive urinary samples received at the Institute of Medical Microbiology of Jena University Hospital (Jena, Germany) for routine bacteriological testing between December 2009 and January 2012, including extended-spectrum β-lactamase (ESBL)-producing *E. coli* ($n = 146$), ESBL-producing *Klebsiella pneumoniae* ($n = 37$), vancomycin-resistant *Enterococcus faecium* ($n = 53$) and *Candida* spp. ($n = 132$). Because of the low prevalence of methicillin-resistant *S. aureus* (MRSA) UTI isolates, MRSA UTI isolates were additionally derived from the institute's clinical strain collection back to February 2008 ($n = 35$). In addition, *E. coli* ATCC 25922, *Proteus mirabilis* ATCC 29906 and *Enterococcus faecalis* ATCC 19433 were tested.

2.2. Antibiotic susceptibility testing

2.2.1. Routine diagnostic antibiotic susceptibility testing

Routine antibiotic susceptibility testing and species determination were performed using a VITEK®2 system (bioMérieux, Marcy-l'Étoile, France). An ESBL phenotype was confirmed by the double-disk synergy method employing cefotaxime, cefepime, ceftazidime and cefpodoxime with and without clavulanic acid (Mast Diagnostics, Reinfeld, Germany), and an MRSA phenotype was confirmed by subsequent agglutination of penicillin-binding protein 2a (PBP2a) using a SLIDEX® MRSA Detection Kit (bioMérieux), both according to the manufacturer's protocols.

2.2.2. Antimicrobial susceptibility testing for nitroxoline and nitrofurantoin

Routine nitroxoline susceptibility testing was conducted at our institution by the disk diffusion method between December 2009 and August 2010. Inhibition diameters were assessed manually using a ruler. In March 2010, routine nitroxoline susceptibility testing by broth microdilution was additionally implemented, which completely replaced the disk diffusion assay in August 2010.

Disk diffusion tests were carried out on cation-adjusted Mueller–Hinton agar plates by the direct colony suspension method according to Deutsches Institut für Normung (DIN) 58958-1:2008-2006 standards. Since nitroxoline disks could not be acquired commercially at the time of the analysis, customised paper disks were prepared by Oxoid Ltd. (Basingstoke, UK) using 30 µg nitroxoline provided by Rosen Pharma (Blieskastel, Germany). Inhibition zone diameters were interpreted as recommended by the manufacturer assuming a breakpoint at a zone diameter of 18 mm.

MIC testing for nitroxoline and nitrofurantoin was done by broth microdilution in accordance with EUCAST standards [ISO 20776-1:2006] using an automated MICRONAUT system (Merlin Diagnostika GmbH, Bornheim-Hersel, Germany) and customised microtitre plates containing lyophilised nitroxoline (0.0625–128 mg/L) or nitrofurantoin (0.25–256 mg/L). Nitroxoline and nitrofurantoin for MIC testing were purchased from Sigma-Aldrich (St Louis, MO).

MBCs of nitrofurantoin and nitroxoline were determined for *E. coli* ATCC 25922, *P. mirabilis* ATCC 29906 and *E. faecalis* ATCC 19433 by subsequent plating of the well contents from MIC testing on Columbia agar with 5% sheep blood (Becton, Dickinson & Co., Franklin Lakes, NJ). The MBC was assumed at nitroxoline and nitrofurantoin concentrations where no colonies grew on the plates.

2.3. Time–kill experiments

Time–kill kinetics of nitrofurantoin and nitroxoline were determined for *E. coli* ATCC 25922, *P. mirabilis* ATCC 29906 and *E. faecalis* ATCC 19433 at concentrations of 10, 50 and 200 mg/L. Stock solutions of nitroxoline and nitrofurantoin were made at 100 g/L in dimethyl sulfoxide (DMSO) or dimethylformamide (DMF), respectively, and were stored as aliquots at –20 °C. Cultures were inoculated with ca. 10^5 CFU/mL and were grown in glass flasks in 20 mL of Mueller–Hinton broth (MHB) or in artificial urine supplemented with 2% v/v tryptic soy broth (Liofilchem, Roseto degli Abruzzi, Italy) [19] at 37 °C under rotation (150 rpm). Samples of 1 mL were taken before incubation ($t = 0$) and after 3, 6, 9 and 24 h of incubation at 37 °C. Samples were immediately serially diluted in sterile saline (0.9% NaCl) and were plated on Columbia agar with 5% sheep blood (*E. coli* and *E. faecalis*) or cystine lactose electrolyte-deficient (CLED) agar (*P. mirabilis*), both from Becton Dickinson & Co. The number of CFU/mL was determined after 16–20 h of incubation at 35 °C.

2.4. Statistics

All statistical analyses and diagrams were performed using GraphPad Prism v.6.00 for Windows (GraphPad Software Inc., La Jolla, CA; http://www.graphpad.com). Differences in the MIC between susceptible and resistant isolates were investigated by the non-parametric Mann–Whitney test (two-tailed, 95% confidence intervals). Significance was assumed at a P-value of ≤0.05.

3. Results

3.1. Nitroxoline disk diffusion testing

Among the 3012 uropathogenic isolates, 1806 (98.4%) of 1835 Enterobacteriaceae, 34 (20.6%) of 165 non-fermenting bacteria (16.4% of *P. aeruginosa* and 75.0% and *A. baumannii*), 284 (88.5%) of 321 enterococci, and all 82 (100%) staphylococci and 132 (100%) yeasts tested had a nitroxoline inhibition zone diameter ≥18 mm (as recommended by the manufacturer as the susceptibility breakpoint before the EUCAST breakpoint for *E. coli* and uncomplicated UTI was