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Pharmacodynamic evaluation of fosfomycin against *Enterobacteriaceae* from urinary tract infections and the influence of pH on fosfomycin activity

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Dissertação apresentada ao Programa de Pós-Graduação em Biociências e Fisiopatologia do Departamento de Análises Clínicas e Biomedicina, Centro de Ciências da Saúde da Universidade Estadual de Maringá, como requisito parcial para obtenção do título de Mestre em Biociências e Fisiopatologia.
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"Talvez não tenha conseguido fazer o melhor, mas lutei para que o melhor fosse feito. Não sou o que deveria ser, mas graças a Deus, não sou o que era antes". (Marthin Luther King)

Avaliação farmacodinâmica da fosfomicina contra *Enterobacteriaceae* isoladas a partir de infecções do trato urinário e a influência do pH sobre a atividade da fosfomicina

RESUMO

A fosfomicina é amplamente utilizada no tratamento de infecção do trato urinário (ITU) e recentemente tem sido recomendada até mesmo para o tratamento bacilos Gram-negativos multirresistentes (MDR). Os regimes posológicos de fosfomicina em organismos MDR e a influência do pH urinário não estão estabelecidos. Assim, o estudo avaliou a farmacodinâmica da fosfomicina contra enterobactérias urinárias e a otimização do pH do ambiente para proporcionar um melhor alcance do resultado terapêutico. Um total de 314 isolados consecutivos não-duplicados (158 *Escherichia coli*, 87 *Klebsiella* spp., 30 *Enterobacter cloacae*, 23 *Proteus mirabilis*, 11 *Citrobacter* spp., 3 *Morganella morganii* e 2 *Serratia marcescens*) identificados através do sistema automatizado BD Phoenix™ entre janeiro 2011 e junho de 2015 foram incluídos no estudo. A concentração mínima inibitória (CIM) foi determinada através do método de ágar diluição em pH 7.0 e 6.0 conforme recomendado pelo *Clinical and Laboratory Standards Institute* (CLSI) 2016. O CIM_{50} e CIM_{90} foram determinadas para os regimes de dosagem de fosfomicina (4 g cada 8h [q8h], 6 g q6h, 8 g q8h) em bolus (0,5-h) e infusão prolongada (4-h) utilizando a simulação de Monte Carlo para avaliar a porcentagem tempo que a concentração de fármaco livre permance acima da CIM durante o intervalo de dosagem (*f*T>CIM). A fosfomicina foi eficaz contra *E. coli* ($CIM_{90} \leq$ 16μg/mL) e também *Citrobacter* spp. e *P. mirabilis* (> 82% dos isolados), mas não contra *Klebsiella* spp. e *E. cloacae* (<27% dos isolados). A acidificação do pH do ambiente aumentou a sensibilidade bacteriana para 65% dos isolados e favoreceu uma diminuição estatisticamente significativa na sobrevivência dos isolados de *E. coli* e *Klebsiella* spp.. Os regimes de fosfomicina de 6g q6h e 8g q8h em ambas as infusões de 0,5 h e 4 h contra CIM 32μg/mL foram capazes de alcançar ≥90% de uma probabilidadede de atingir o alvo (PTA) de 70% *f*T>CIM. Nenhuma dosagem conseguiu PTA adequada contra a CIM ≥ 64μg/mL. As análises PK/PD de fosfomicina mostraram que a diminuição do pH melhora a PTA e o alcance do índice PD na maioria dos isolados de *Enterobacteriaceae*, exceto para espécies de *Klebsiella* e *E. cloacae*, deste modo o uso da fosfomicina associado a acidificação da urina pode tornar mais eficiente o tratamento de ITU.

Palavras - chave: Fosfomicina. *Enterobacteriaceae*. Simulação de Monte Carlo. Infecção do trato urinário. PH ácido.

Pharmacodynamic evaluation of fosfomycin against *Enterobacteriaceae* from urinary tract infections and the influence of pH on fosfomycin activity

ABSTRACT

Fosfomycin is widely used to treat urinary tract infection (UTI) and recently have been recommended even for treating multidrug-resistant (MDR) Gram-negative bacilli. Fosfomycin dosing regimens in challenging MDR organisms and the influence of urinary pH have not been established. Thus the study assessed the pharmacodynamics of fosfomycin against urinary enterobacteria and pH environment optimization to provide better achievement of therapeutic outcome. A total of 314 consecutive nonduplicate isolates (158 *Escherichia coli*, 87 *Klebsiella* spp., 30 *Enterobacter cloacae*, 23 *Proteus mirabilis*, 11 *Citrobacter* spp., 3 *Morganella morganii* e 2 *Serratia marcescens*) identified by means of the BD Phoenix^{TM} automated microbiology system between January 2011 and June 2015 were included in the study. Minimal inhibitory concentration (MIC) was determined using agar dilution in pH 7.0 and 6.0 as recommended by the Clinical and Laboratory Standards Institute (CLSI) 2016. The MIC_{50} and MIC_{90} were challenged against short (0.5-h) and prolonged (4-h) infusion regimens of fosfomycin (4 g every 8h [q8h], 6 g q6h, 8 g q8h) using Monte Carlo simulation to evaluate the time above the MIC of the free drug concentration as a percentage of the dosing interval (*f*T>MIC). Fosfomycin was effective against *E. coli* ($MIC_{90} \leq$ 16µg/mL) and also *Citrobacter* spp. and *P. mirabilis* isolates (>82% of isolates) but not against *Klebsiella* spp. and *E. cloacae* (<27% of isolates). Acidification of pH environment increased bacterial susceptibility to 65% of isolates and favored a statistically significant decrease in the survival of *E. coli* and *Klebsiella* spp. isolates. Fosfomycin regimens of 6g q6h and 8g q8h as both 0.5-h and 4-h infusion against MIC 32µg/mL were able to achieving ≥90% probability of target attainment (PTA) of 70% *f*T>MIC. No dosage achieved adequate PTA against the MIC ≥ 64µg/mL. PK/PD analyses of fosfomycin showed that decreased pH improves PTA of the target PD index in majority of *Enterobacteriaceae* isolates, except *Klebsiella* species and *E. cloacae*, thus the use of fosfomycin associated with urine acidification can make the UTI treatment more efficient.

Keywords: Fosfomycin. *Enterobacteriaceae.* Monte Carlo simulation. Urinary tract infections. Acid pH.

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SUMÁRIO

CAPÍTULO I

INTRODUÇÃO:

Infecções do trato urinário (ITUs) estão entre as infecções bacterianas mais comuns, afetando 150 milhões de pessoas a cada ano em todo mundo [(1)]. Em 2003, um estudo multicêntrico realizado na América Latina a partir de dados reportados pelo MYSTIC *Program Brazil* revelou que as ITUs estão entre as infecções bacterianas mais frequentes representando mais de 30% do total de infecções hospitalares [(2)]. A ITU também é considerada uma das importantes fontes de sepse, refletindo nas altas taxas de mortalidade e custos para os sistemas de saúde [(3,4)].

A ITU caracteriza-se pela invasão e multiplicação bacteriana em qualquer seguimento do aparelho urinário (uretra, bexiga, ureteres ou rins). De maneira geral, a ITU pode ser classificada quanto à localização em ITU baixa (cistite) e ITU alta (pielonefrite) e quanto à presença de fatores complicadores em ITU não complicada e ITU complicada [(5,6)]. Esta categorização baseia-se na interpretação clínica e nos fatores de risco associados ao hospedeiro como, por exemplo, sexo, idade, reincidência da infecção e uso de cateter vesical [(7)].

A grande maioria das infecções urinárias é causada por espécies da família *Enterobacteriaceae*, sendo a *Escherichia coli* a mais comum, isolada em cerca de 70 a 90% dos casos e esta frequentemente associada a ITU não complicada. Além de *E. coli*, as ITUs têm sido relacionadas com espécies de *Klebsiella*, *Enterobacter* e *Proteus*, bem como *Staphylococcus saprophyticus*. Nas infecções urinárias complicadas o espectro de bactérias envolvidas é mais amplo incluindo bactérias Gram-positivas e Gram-negativas e com uma elevada frequência de microrganismos multirresistentes [(8-10)].

Recentemente, o nível de resistência dos patógenos causadores de infecção urinária tem aumentado significativamente, devido principalmente ao tratamento empírico, pois na maioria das vezes somente em casos agravados os testes de sensibilidade de rotina são realizados. Assim, verificou-se nos últimos anos, um grande aumento na utilização de fluoroquinolonas, isto por agirem de forma potente em amplo espectro para o tratamento

destas infecções. Esta prática, por sua vez, conduziu a um aumento de bactérias resistentes, particularmente de *E. coli* resistente as fluoroquinolonas [10-12].

Infelizmente, na atualidade o investimento da indústria farmacêutica na descoberta de novos antibacterianos é muito pequeno enquanto a resistência bacteriana tem aumentado a cada dia [13,14]. Esta dificuldade terapêutica, principalmente de infecções causadas por bacilos Gram-negativos multidroga resistentes (MDR) na última década, favoreceu um renovado interesse em antimicrobianos antigos, como a classe das polimixinas e mais recentemente a fosfomicina [15-17].

A fosfomicina é um antimicrobiano fosfônico descoberto na Espanha no final da década de 1960 em culturas de *Streptomyces* sp., apresenta estrutura química bastante distinta das outras classes de antimicrobianos (Figura 1) [18,19]. Possui ação bactericida de amplo espectro, não apresenta resistência cruzada, possui baixo peso molecular apresentando baixa ligação a proteínas [20] e frequentemente demonstra sinergismo quando usado em combinação com outros antimicrobianos, incluindo β-lactâmicos, aminoglicosídeos e fluoroquinolas [21-23].

Atualmente a fosfomicina é administrada na forma oral, através de um sal hidrossolúvel denominada fosfomicina-trometamol [24]. Essa forma é usada exclusivamente contra ITU não complicada e é mais facilmente absorvida pelo trato gastrointestinal diferente de sua forma em sal de cálcio que é menos solúvel e, portanto menos absorvida [25].

Em outros países, principalmente na Europa, é relatado o uso de fosfomicina intravenosa para o tratamento de ITU complicada sendo conhecida como fosfomicina dissódica [26]. Esta formulação também possui uma boa distribuição em tecidos, sendo capaz de atingir valores clinicamente relevantes em rins, parede da bexiga, próstata, pulmões, tecidos inflamados, ossos, fluidos de abcessos e válvulas cardíacas [20,27].

A fosfomicina exerce sua atividade bloqueando a fase inicial de síntese da parede celular de bactérias, tanto em Gram-positivas como Gram-negativas. Especificamente, fosfomicina inibe a enzima citoplasmática difosfato de uridina N-acetilglicosamina (UDP-GlcNAc) enolpiruvil transferase (MurA) que esta envolvida na síntese do peptídeoglicano [28]. No entanto, para alcançar seu sítio de ação, a fosfomicina precisa penetrar na membrana celular bacteriana que acontece por meio de dois sistemas transportadores distintos: *L-α-glycerophosphate* (GlpT) e o *hexose-phosphate uptake system* (UhpT), que envolve a presença de glicose-6-fosfato [29].

Sua farmacodinâmica é definida como tempo dependente, significando que a ótima atividade bactericida é proporcional ao período de tempo em que as concentrações permanecem acima da concentração inibitória mínima (CIM) do microrganismo, expressada pelo índice farmacodinâmico %*f*T>CIM e uma cobertura ideal de 70% [30].

Infecções urinárias têm sido tratadas com fosfomicina, pois ela apresenta amplo espectro de atividade antimicrobiana atuando tanto em bacilos Gram-negativos (BGN) como em cocos Gram-positivos (CGP). Entretanto, nos últimos anos a fosfomicina tem sido resgatada para o tratamento de enterobactérias MDR (do inglês, "*multidrug resistante"*) bem como para produtoras de enzimas β-lactamases do tipo ESBL (do inglês, "*extended spectrum beta-lactamases"*) [31], KPC (do inglês, "*Klebsiella pneumoniae carbapenemase*") [32] e até mesmo NDM (do inglês, "*New Delhi Metallobetalactamase*") [33].

Outro aspecto importante da fosfomicina é a sua utilização para o tratamento de bactérias MDR de maneira associada com outros fármacos como, por exemplo, em estudo publicado por Albeiro et al. que demonstraram o efeito sinérgico da fosfomicina em combinação com o meropenem contra isolados de *Klebsiella pneumoniae* KPC-2 [23].

O uso de agentes não antimicrobianos tais como suco de Cranberry e extrato de Echinacea também têm sido utilizados como estratégias para o tratamento e prevenção de ITU recorrente com o objetivo de se preservar os agentes antibacterianos e minimizar a emergência de resistência. Acredita-se que estes métodos de terapia alternativa provoquem a estimulação da resposta imune, alterações do pH urinário, a prevenção do crescimento e modificação na adesão de patógenos nas células uroteliais [34].

O pH do fluído corporal deve ser considerado um fator importante para o alcance da eficácia terapêutica [35]. A Tabela 2 apresenta os valores de pH que já foram descritos para os diferentes fluídos e órgãos. Entretanto, em alguns casos o pH do fluído onde as bactérias estão presentes durante o processo infeccioso pode variar e assumir característica ácida ou básica [36].

A acidificação urinária tem sido muito utilizada como um suporte para o tratamento e prevenção de ITU, embora a evidência para apoiar a sua eficácia não seja totalmente comprovada [35,37]. Como tentativa de otimizar o pH urinário durante o processo infeccioso vários agentes têm sido usado, por exemplo, bicarbonato de sódio, citrato de potássio (alcalinizantes) [38] ácido ascórbico, cloreto de amônio (acidificantes) [37,38].

Pouco tem sido investigado sobre a real influência do pH no tratamento antimicrobiano. Sabemos que a fosfomicina tem sido indicada como primeira escolha terapêutica em ITU não complicada e recentemente tem sido utilizada em combinação com outro antimicrobiano quando em ITU complicadas. Entretanto, não é do nosso conhecimento nenhum estudo que avalie influência do pH sobre a atividade *in vitro* da fosfomicina associado a uma análise farmacodinâmica. Estudos desta natureza são de extrema importância, pois podem direcionar os clínicos na definição de esquemas mais adequados para o tratamento de ITU.

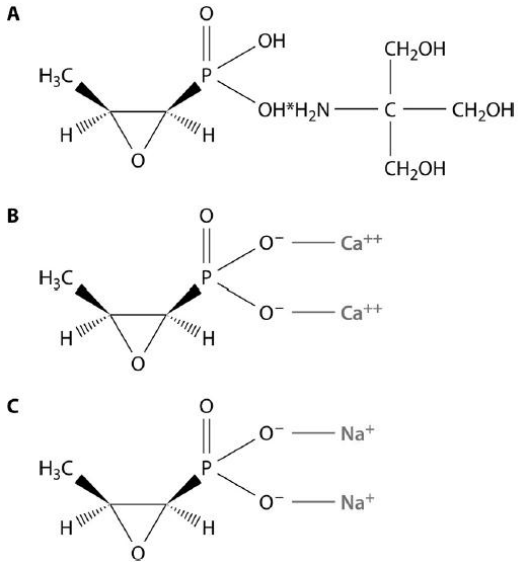

Figura 1**.** Estrutura molecular da fosfomicina trometamol (A), cálcica (B) e dissódica (C) [19].

Tabela 1. Valores de pH de fluídos e órgãos.

Órgão ou fluído	**pH**	**Referência**
Urina	4,5 - 8	
	Ácida 4,5 - 6	35
	Alcalina 6,5 - 8	
Estômago	1,35 – 3,5	39
Bile	7,6 – 8,8	39
Fluído cérebro espinhal	7,3	39
Fluído intracelular	6 – 7,2	40
Sanguíneo	7,35 – 7,4	39

JUSTIFICATIVA

Infecções do trato urinário representam um sério problema de saúde para os pacientes e um alto custo para a sociedade. A crescente disseminação de enterobactérias multirresistentes, causando graves infecções, tem provocado a redução das opções terapêuticas e altas taxas de mortalidade;

Como resultado clínicos têm voltado seu interesse para antigos antimicrobianos, no entanto, existe a falta de dados na literatura que os direcionem a estabelecer posologias adequadas para esses antimicrobianos, principalmente para a fosfomicina, que no momento vêm sendo resgatada para o tratamento destas infecções;

Considerando que, embora já tenha sido mostrado que o pH do fluído corporal durante o processo infeccioso pode influenciar na atividade do antimicrobiano, ainda são poucos os trabalhos que tenham investigado o pH e o melhor alcance do resultado terapêutico rem relação a fosfomicina;

Considerando a necessidade de estudos que avaliem tal situação, pretendemos investigar o índice farmacodinâmico alcançado pelos esquemas posológicos de fosfomicina e a influência do pH sobre a atividade *in vitro* da fosfomicina contra enterobactérias urinárias.

OBJETIVOS

GERAL

O presente estudo teve como objetivo avaliar índice farmacodinâmico alcançado pelos esquemas posológicos por meio de simulação de Monte Carlo e a influência do pH sobre a atividade *in vitro* da fosfomicina contra enterobactérias urinárias.

ESPECÍFICOS

Determinar a concentração inibitória mínima (CIM) para fosfomicina pela metodologia de ágar diluição para estes isolados;

Avaliar a influência do pH sobre a atividade da fosfomicina contra enterobactérias urinárias;

Gerar por meio de simulação de Monte Carlo uma população de 10.000 pacientes doentes apresentando características farmacocinéticas populacionais para fosfomicina;

Aplicar modelos matemáticos farmacodinâmicos que descrevem a %*f*T>CIM para os diferentes esquemas posológicos identificando os respectivos índices farmacodinâmicos obtidos;

Demonstrar qual o ponto de corte da fosfomicina mais adequado para que ocorra uma cobertura ideal, ou seja, %*f*T>CIM maior ou igual a 70%, com probabilidade de atingir o alvo (PTA) ≥ 0,9.

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CAPÍTULO II

Manuscrito: "PHARMACODYNAMIC EVALUATION OF FOSFOMYCIN AGAINST *ENTEROBACTERIACEAE* FROM URINARY TRACT INFECTIONS AND THE INFLUENCE OF PH ON FOSFOMYCIN ACTIVITIES"

Pharmacodynamic evaluation of fosfomycin against *Enterobacteriaceae* from urinary tract infections and the influence of pH on fosfomycin activities

Running title: PK/PD of fosfomycin against urinary enterobacteria

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Abstract

Fosfomycin is widely used to treat urinary tract infection (UTI) and recently have been recommended even for treating multidrug-resistant Gram-negative bacilli (MDR). Fosfomycin dosing regimens in challenging MDR organisms and the influence of urinary pH have not been established. Minimal inhibitory concentration (MIC) was determined using agar dilution in pH 6.0 and 7.0 for 314 *Enterobacteriaceae* isolated from UTI. The MIC_{50} and MIC_{90} were challenged against short (0.5-h) and prolonged (4-h) infusion regimens of fosfomycin (4 g every 8h [q8h], 6 g q6h, 8 g q8h) using Monte Carlo simulation to evaluate the time above the MIC of the free drug concentration as a percentage of the dosing interval (*f*T>MIC). Fosfomycin was effective against *E. coli* ($MIC_{90} \leq 16\mu g/mL$) and also *Citrobacter* spp. and *P. mirabilis* isolates (>82% of isolates) but not against *Klebsiella* spp. and *E. cloacae* (<27% of isolates). Acidification of pH environment increased bacterial susceptibility to 65% of isolates and favored a statistically significant decrease in the survival of *E. coli* and *Klebsiella* spp. isolates. Fosfomycin regimens of 6g q6h and 8g q8h as both 0.5-h and 4-h infusion against MIC 32µg/mL were able to achieving ≥90% probability of target attainment (PTA) of 70% *f*T>MIC. No dosage achieved adequate PTA against the MIC ≥ 64µg/mL. PK/PD analyses of fosfomycin showed that decreased pH improves PTA of the target PD index in majority of *Enterobacteriaceae* isolates, except *Klebsiella* and *E. cloacae* species. Urine acidification is recommended in the treatment of UTI using fosfomycin.

Keywords: Fosfomycin. *Enterobacteriaceae.* Monte Carlo simulation. Urinary tract infections. Acid pH.

Introduction

Urinary tract infections (UTI) are the most common infections worldwide in which *Enterobacteriaceae* are the main pathogens (1). The rise in antibiotic resistance over the last several years limited treatment options currently available against multi-drug resistant (MDR) bacteria. Fosfomycin is an "old" antibiotic agent frequently used in UTI therapy and has been re-evaluated as a potential treatment option against MDR Gram-negative bacteria (2).

Fosfomycin is a phosphonic acid derivate (cis-1,2-epoxypropyl phosphonic acid) isolated from the *Streptomyces* species (3). The action of this molecule is via inhibition of an enzyme-catalyzed reaction in the first step of the synthesis of bacterial cell wall. Fosfomycin shows potent bactericidal action against both Gram-positive and Gram-negative pathogens (4). This drug shows favorable pharmacokinetic properties against UTI due to high drug concentrations in the urine (5, 6).

Therapeutic response of antibacterial agents may be affected by the pH of body fluids (7) including the pH environment in the urine. Previous studies have shown that fosfomycin presented an optimal antimicrobial activity in an acidic urine (pH: 5.0–6.0) (8, 9). However, there is a lack of information related to the pharmacodynamics (PD) of fosfomycin dosing regimens at various pH in the treatment of UTI.

The objectives of the current study are (1) assessed the pharmacodynamics of fosfomycin against urinary enterobacteria and (2) pH environment optimization to provide better achievement of therapeutic outcome.

Materials and Methods

Bacterial isolates

A total of 314 consecutive nonduplicate isolates of *Enterobacteriaceae* recovered from UTIs were selected from the medical microbiology laboratory organism bank of the university

hospital. All isolates were identified by means of the BD Phoenix^{TM} automated microbiology system and were stored at - 20°C in Trypticase Soy Broth (Difco Laboratories, Detroit, MI, USA) with 30% glycerol until they were tested. The isolates were recovered on MacConkey Agar (Difco Laboratories, Detroit, MI, USA) plates to verify purity of the culture. These plates were incubated at 35 ± 2°C in ambient air for 24h. The isolates, which were collected between January 2011 and June 2015, included 158 *Escherichia coli*, 87 *Klebsiella* spp., 30 *Enterobacter cloacae*, 23 *Proteus mirabilis*, 11 *Citrobacter* spp., 2 *Serratia marcescens* and 3 *Morganella morganii*. Only one isolate per patient was included in the study.

Antimicrobial agents

Fosfomycin (Sigma-Aldrich, St. Louis, MO, USA) was purchased from LabCompany (Londrina, Paraná, Brasil). Fosfomycin was dissolved in water to form 10 µg/mL stock solution, which was stored at -20°C (stock solution).

Antimicrobial susceptibility testing

The susceptibilities of isolates from UTIs to fosfomycin were determined by the agar dilution method described in the CLSI guidelines (10, 11) utilizing pH 7.0 and Mueller-Hinton agar (Difco Laboratories, Detroit, MI, USA) supplemented with additional 25 µg/mL of glicose-6-phosphate. The technique was done using the Steers replicator. The inoculated plates were incubated in ambient air at 35 ± 2°C for 16 to 20 h. Fosfomycin susceptibility testing was carried out for each isolates at pH 6.0 and some isolates at pH 8.0. The pH of the media was adjusted by adding either 1N HCl or NaOH. Fosfomycin concentrations tested ranged from 0.25 to 1024 µg/mL. The MIC of antimicrobial agent was defined as the lowest concentration that completely inhibits growth of the organism in duplicate tests. Control strains, including *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 29212, were included in each set of tests (10).

Simulation of fosfomycin pharmacokinetics in critically ill patients

Critically ill population consisting of 10,000 virtual patients had a 50/50 ratio of males and females. Male heights were $176.3 \pm 0.17\sqrt{4{,}482}$ cm (mean $\pm$ SD) and female heights were $162.2 \pm 0.16\sqrt{4{,}857}$ cm, assuming normal distribution for both genders (12). The weight-height relationships for both genders were: $WT_{male} = \exp(3.28 + 1.92 \log HT_{male})$ and $WT_{female} = \exp(3.4 + 1.45 \log HT_{female})$, for males and females, respectively,(13) where WT refers to weight and HT refers to height. Interindividual variability of weight was log-normally distributed such that $WT_i = WT \exp(\eta_i)$, wherein η is normally distributed with a mean of 0 and SD of 0.14 and 0.17, for males and females, respectively, and i represents an individual (14). Patient's ages was uniformly distributed between 50 and 90 years of age. Serum creatinine (sCr) levels in critically ill patients were assumed to be bi-modally distributed in order to generate a bi-modal nature of creatinine clearance (15); the two normal distributions were: (1) normal renal function were 0.8 ± 0.07 and 0.7 ± 0.07 mg/dl for males and females, respectively, (2) renal impairment were 1.2 ± 0.13 and 1.1 ± 0.13 mg/dl for males and females, respectively. Creatinine clearance (CL_{CR}) was estimated using the modification of renal disease (MDRD) equation:(16) $CL_{CR} = 186 \times S_{CR}^{-1.154} \times age^{-0.203}$ ($\times$ 0.742 if the patient is female).

Fosfomycin population pharmacokinetic model in critically ill patients (17) was a two compartment model parameterized on clearance (CL), intercompartmental clearance (Q), volumes of central (V_C) and peripheral compartments (V_P), and was used to simulate 10,000 virtual patient's concentration-time profiles at steady-state. The population CL and V_C equations incorporated CL_{CR} and WT as covariates: CL (liters/h) $= 5.57 \times (CL_{CR}/90)$, and V_C (liters) $= 26.5 \times (WT/70)^{0.75}$. V_P and Q were 22.3 liters and 19.8 liters/h, respectively. Interindividual variability in CL and V_C, was log-normally distributed with CVs of 91.9% and 39%, respectively. Fosfomycin protein binding was negligible (17, 18).

Pharmacodynamics

Pharmacodynamic index that was shown to be correlated with therapeutic efficacy of fosfomycin was time above MIC of the free drug concentration as a percentage of the dosing interval with a target magnitude of 70% *f*T> MIC (19, 20). Pharmacodynamic analyses of antimicrobial regimens in 0.5-h and 4-h infusions at the MIC_{50} or MIC_{90} against this isolate population were conducted to evaluate *f*T> MIC for each dosage regimen. The fosfomycin dosage regimens evaluated were 4 g and 8 g q8h and 6g q6h. These regimens were chosen based on the most common regimens reported in the literature (21).

Monte Carlo simulation

The simulation of various distributions to characterize the demographics of the 10,000 virtual patients was performed in R v.3.1.1 (R Foundation for Statistical Computing, Vienna, Austria). Plasma fosfomycin concentration-time profiles of these patients were generated using NONMEM v.7.2 (ICON, Ellicot City, MD) with Advan 3 subroutines. Linear interpolation was used to determine the time in the ascending and descending phases of the concentration-time profiles in which the concentration is at the MIC. The difference between the two time points was the time above MIC; and its percentage over the dosing interval was determined for each individual's profile. Probability of target attainment (PTA) for each regimen was evaluated to determine the percentage of the simulated profiles that achieved or exceeded the pharmacodynamic surrogate indices for fosfomycin of ≥70% *f*T>MIC at increasing MICs. A 90% PTA was set for therapeutic success (22, 23). Cumulative fraction of response (CFR) for each dosing regimen at 70% *f*T>MIC of fosfomycin was computed as the summation of the density or percentage of bacteria at each MIC across the distribution multiplied by the PTA value at the MIC for the regimen (24-26).

Statistical analysis

Survival analysis for interval-censored data was used to compare the effect of pH on the survival curve for all bacteria used in the study. For the comparison of the survival curves, log-rank test was used to determine whether the curves were significantly different (27). A $p < 0.05$ was considered significant.

Results

In vitro susceptibility

Table 1 presents the antimicrobial susceptibility profile of 314 *Enterobacteriaceae* urinary isolates to fosfomycin at different pH values. At pH 7.0, the MIC_{50} and MIC_{90} against *Enterobacteriaceae* ranged from 2 to 256 µg/mL and from 8 to > 512 µg/mL, respectively. Fosfomycin was highly active against *E. coli* with a $MIC_{90} \leq 16$ µg/mL. *Citrobacter* spp. and *P. mirabilis* were also susceptible to fosfomycin (> 82% of isolates). In contrast, fosfomycin was not active against *Klebsiella* spp. and *E. cloacae* with only 27% or less of the isolates considered susceptible. High fosfomycin MIC_{90} of ≥ 512 µg/mL were observed against these isolates.

Effect of pH on MIC

Using agar dilution, the *in vitro* activity of fosfomycin was affected by acidification of the growth media for six of the seven bacterial species tested (Table 1). Fosfomycin MIC against *Enterobaceriaceae* was reduced in 65% (206/314) of the isolates. The MIC_{90} was 2-fold lower against *E. coli*, *Klebsiella* spp., *Citrobacter* spp. and *E. cloacae* in the lower pH environment. Several strains that were previously resistant to fosfomycin at pH 7.0 became susceptible at pH 6.0, with the greatest effects observed for the *Klebsiella* spp. and *E. cloacae*, given the CLSI breakpoint value of ≥ 64 µg/mL.

To evaluate whether the decrease in MIC values were statistically significant, we utilized survival analysis approach, replacing the time component with MIC values. Figure 1

shows the survival curves for *E. coli* and *Klebsiella* spp. isolated from UTIs at pH 6.0 and 7.0. Applying log-rank test to compare the two curves, we rejected the hypothesis that survival curves were equal (both P = .0001; log-rank test). In contrast, acidic pH conditions did not improve fosfomycin activity against *Citrobacter* spp., *Proteus mirabilis* and *Enterobacter cloacae* isolates (P = 0.3403, 0.9079 and 0.0877; log-rank tests, respectively).

In this study, we also evaluated the effect of pH 8.0 on fosfomycin activity against twelve clinical isolates of *E. coli* and *Klebsiella pneumoniae*; the results are shown in Figure 2, indicating that the activity of fosfomycin decreased sharply against both microorganisms going from pH 6.0 to 8.0.

Pharmacodynamics analyses

Table 2 and Figure 3 show the PTA of 70% *f*T>MIC for fosfomycin in various dosing regimens as 0.5-h and 4-h infusions and the MIC frequency of fosfomycin by microorganism type at pH 6.0 and 7.0. All fosfomycin regimens achieved ≥90% PTA for ≥70% *f*T>MIC at MIC ≤16 μg/mL, indicating a sufficient antimicrobial coverage for the MIC_{50} against *Citrobacter* spp. and *P. mirabilis* as well as MIC_{90} against *E. coli* isolates at both pH 6.0 and 7.0. None of the fosfomycin dosing regimens achieved ≥ 90% PTA at $MIC_{50/90}$ against *Klebsiella* spp. and *E. cloacae* isolates at pH 6.0 or 7.0. Only the higher doses, 6g qh6h and 8g qh8h as either 0.5-h or 4-h infusions, demonstrated ≥90% PTA at MIC ≤ 32 μg/mL which is the susceptible breakpoint based on EUCAST and none of the fosfomycin regimens achieved ≥ 90% PTA at MIC of 64 μg/mL, the susceptible breakpoint based on CLSI. In general, an acidic pH and prolonged infusion of 4 h demonstrated improvement in achieving higher PTA at MIC_{50} and MIC_{90}. However, these two conditions were not sufficient for fosfomycin regimens against *Klebsiella* spp. and *E. cloacae* isolates to achieve 90% PTA.

The summary of CFR by dosing regimens of fosfomycin at pH 6.0 and 7.0 are shown in Table 3. A greater than 80% CFR was estimated for *E. coli*, *P. mirabilis* and *Citrobacter*

spp. for all fosfomycin regimens. The 6g q6h regimen had marginal improvement in CFR over the 8g q8h regimen. Low fosfomycin activity against *Klebsiella* spp. and *E. cloacae* was observed, regardless of pH conditions and dosing regimens.

Discussion

MDR Gram-negative bacterial infections have prompted the revival of fosfomycin, which is used either as monotherapy or in combination (2, 28). Our study showed that fosfomycin dosing regimens that were commonly used in the clinical practice were more likely to achieve the PTA at MIC_{90} against *E. coli*. When administrated at the maximum daily dose of 24g, fosfomycin was shown to be effective against majority of *Citrobacter* spp. and *P. mirabilis* isolates. However fosfomycin has no utility against *Klebsiella* spp. and *E. cloacae* as shown by PTA falling below the 90% PTA for fosfomycin PD target index at 70% *f*T>MIC.

Our findings were consistent with those reported in recent studies (1, 29, 30). Fosfomycin presented considerable activity against *Citrobacter* spp. and *P. mirabilis* tested, wherein activity against these bacterial types has been previously demonstrated (31). Fosfomycin was less active against *Klebsiella* spp. and *E. cloacae*, than other *Enterobacteriaceae* isolated in our study. In general, *Klebsiella* spp. and *E. cloacae* displayed a slightly higher MIC distribution (32-34). According to Falagas et al., fosfomycin MIC distribution can be quite variable and can also be influenced by several factors, including bacterial species (35).

The success of antimicrobial therapy against UTIs in a population can be estimated by PK-PD profiles inferred from the plasma drug concentrations (36). All fosfomycin dosing regimens tested showed sufficient antimicrobial coverage up to MIC of 16 μg/mL; only higher dosages of 6g qh6h and 8g qh8h as either 0.5-h or 4-h infusions were able to achieve ≥ 90% PTA at MIC ≤ 32 μg/mL. A study by Matzi et al. found that a single i.v. dose of 4g was

suitable against pathogens with an MIC value of up 32 µg/mL in infected lung tissues (37). In our study, fosfomycin was effective against a majority of the *Citrobacter* spp. and *P. mirabilis* and *E. coli* isolates at both pH 6.0 and 7.0; however none of the fosfomycin dosing regimens evaluated would achieve satisfactory PTA at 64 µg/mL MIC. Consequently, PTA≥90% was unattainable for MIC_{50} against *Klebsiella* spp. and *E. cloacae* isolates.

Albiero and colleagues evaluated treatment regimens of the fosfomycin alone and in combination with meropenem and also showed that administration of fosfomycin in monotherapy against KPC-2-producing *K. pneumoniae* (MIC_{50} of 64 µg/mL) was not able to achieve ≥ 90% PTA not even at higher dosages and 3-h infusions in patients with normal renal function or renal impairment (21). Combination with a carbapenem is required to confer susceptibility to both fosfomycin and meropenem in KPC-producing *K. pneumonaie* (21). Several studies have shown that factors such as a prolonged infusion, increased dosage, more divided doses and inclusion of a second antimicrobial increased probability of target attainment (19, 21, 38). In addition to the list, the site of infection should also be considered, particularly when there are large variations in pH of body fluids.

The CLSI recommends a breakpoint of ≤ 64 µg/mL for fosfomycin against *E. coli* and *E. faecalis* isolates from UTIs to delineate susceptibility from resistant classification of bacteria isolates (10). The EUCAST MIC "susceptible" breakpoint of was lower at < 32 µg/mL for intravenous fosfomycin against *Enterobacteriaceae* and *Staphylococcus* spp., irrespective of the site of infection (39). Our analyses showed that only the higher doses (6g qh6h and 8g qh8h) in both short or prolonged infusion were able to achieve ≥90% PTA at MIC ≤ 32 µg/mL (susceptible breakpoint for EUCAST) and none of the fosfomycin dosage regimens tested can achieve a 90% probability of target attainment against CLSI susceptible breakpoint at MIC 64 µg/mL. Fosfomycin is excreted in the active form via the kidneys in the urine and might achieve *in vivo* concentrations above the usual MIC against common

uropathogens (5, 6). The same studies demonstrated that serum susceptibility data overestimated the resistance of urinary isolates in the presence of high urinary antibiotic level (6, 40-42). Even though only the higher doses may be required to achieve PTA against MIC at 64 µg/mL, fosfomycin becomes highly concentrated in the urine and the current dosing regimens may be sufficient against *Klebsiella* spp. It remains to be evaluated in a clinical setting to determine whether the current dosing regimens were sufficient to treat MDR-UTIs.

Acidification of the bacterial growth medium was an important factor affecting the efficacy of fosfomycin and consequently improved the antimicrobial coverage for majority of *Enterobacteriaceae* (65%). Lower pH environment increased the PTA against *Klebsiella* spp. (up to 27%) administrated in a prolonged infusion when compared to an alkaline pH. There was a significant difference in MIC between pH 6.0 and 7.0 for *E. coli* and *Klebsiella* spp. isolates, which corroborate with other studies that demonstrated pH effect on *in vitro* activity of antimicrobial agents and therapeutic response (8, 43-49). The enhanced activity of fosfomycin in acidic environment can be explained by its physicochemical properties. The molecular structure of fosfomycin contains an epóxide ring linked to a phosphate group that is ionized depending on the pH. It has two pKa values: pKa 1 = 1.25 and pKa 2 = 7.82 (Figure 4). Based on Chemicalize database (https://chemicalize.com/#/calculation), fosfomycin molecule is less protonated in pH 6.0 (predominant microspecies with electric charge of -1) than in pH 7.0 (predominant microspecies with electric charge -2) (Figure 5). In acidic pH wherein fosfomycin is in its least ionized and more lipophilic state, a major fraction of the available antibiotic molecules can enter the bacteria, resulting in a greater antimicrobial activity in acidic urine (8, 9).

It is known that some urinary pathogens such as *P. mirabilis* and *Klebsiella* species are capable of producing ammonia from urea, resulting in an increased urine pH (50). Urine alkalization caused by these microorganisms can hinder antimicrobial treatment using

fosfomycin. Alternative complimentary strategies have been used for treatment of UTIs, including the use of agents that acidify the urine (51). Ascorbic acidic (vitamin C) is regarded as safe and effective in altering urinary pH (49, 52). It is often used as an agent to prevent UTI, although there is no evidence to support this indication (53). Few studies have shown the benefits of using vitamin C together with antimicrobials. Carlsoon et al. investigated growth inhibition in different bacterial strains, including *E. coli*, by ascorbic acid at various pH levels in human urine and demonstrated that vitamin C may be used in the treatment and prophylaxis of UTI (52). However use should not be excessive because excess ascorbic acid can induce tissue damage and salt precipitation causing urinary stones and/or encrustation in humans (49).

The present study has some limitations. First, the low number of isolates evaluated for each bacteria species can potentially influence the MIC_{50} and MIC_{90} statistic. However, *E. coli* and *Klebsiella* spp. demonstrated good distribution and variability in MIC ranges for fosfomycin. The isolates came from a public hospital that provides services for the population of 754,570 residing in the Maringa metropolitan region, but may not be representative of the Brazilian population. Second, the narrow range of pH values investigated to all *Enterobacteriaceae* isolates (6.0 – 7.0) precludes a whole spectrum of pH range regarding the behavior of fosfomycin in relation to the pH environment. We verified that there was decreased antibacterial activity of fosfomycin when tested at pH 8.0 to some *E. coli* and *Klebsiella pneumoniae* isolates (twelve of each species), reaffirming our findings that fosfomycin is active in lower pH environment. The third limitation is that the pharmacodynamic evaluations were carried out assuming fosfomycin pharmacokinetic in plasma but not in urine. Our approach is valid and may even veer on the conservative side, given that fosfomycin tends to be concentrated in the urine.

In conclusion, PK/PD analyses of fosfomycin showed that decreased pH improved attainment of the target PD index in the majority of *Enterobacteriaceae* isolates, except to *Klebsiella* species and *E. cloacae*. In addition, our study clearly demonstrated the improvement in the activity of fosfomycin at an acidic pH.

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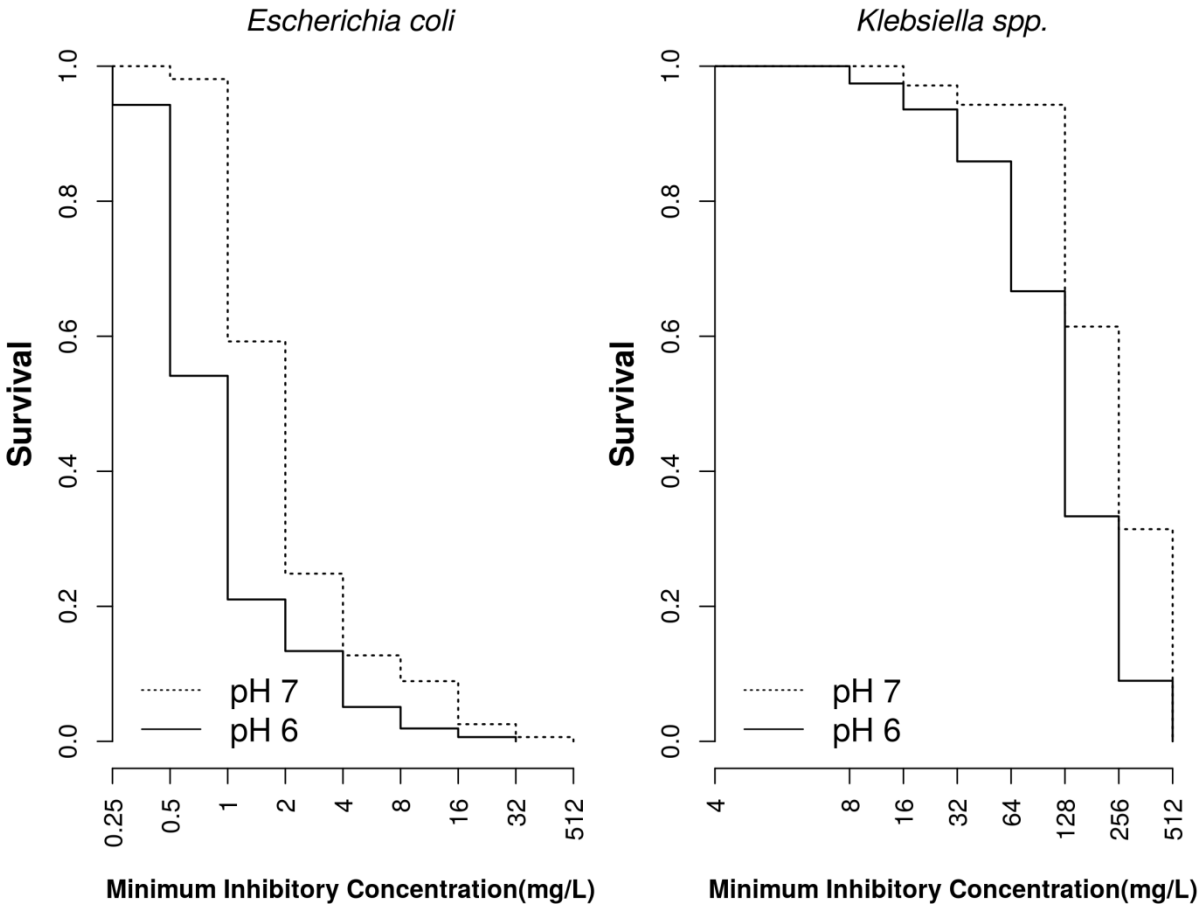

Figure 1: Survival-type antimicrobial susceptibility curves for *Escherichia coli* (n = 158) and *Klebsiella* spp. (n = 87) isolated from urinary tract infection and stratified on the basis of pH conditions at 6.0 (solid line) and 7.0 (dotted line).

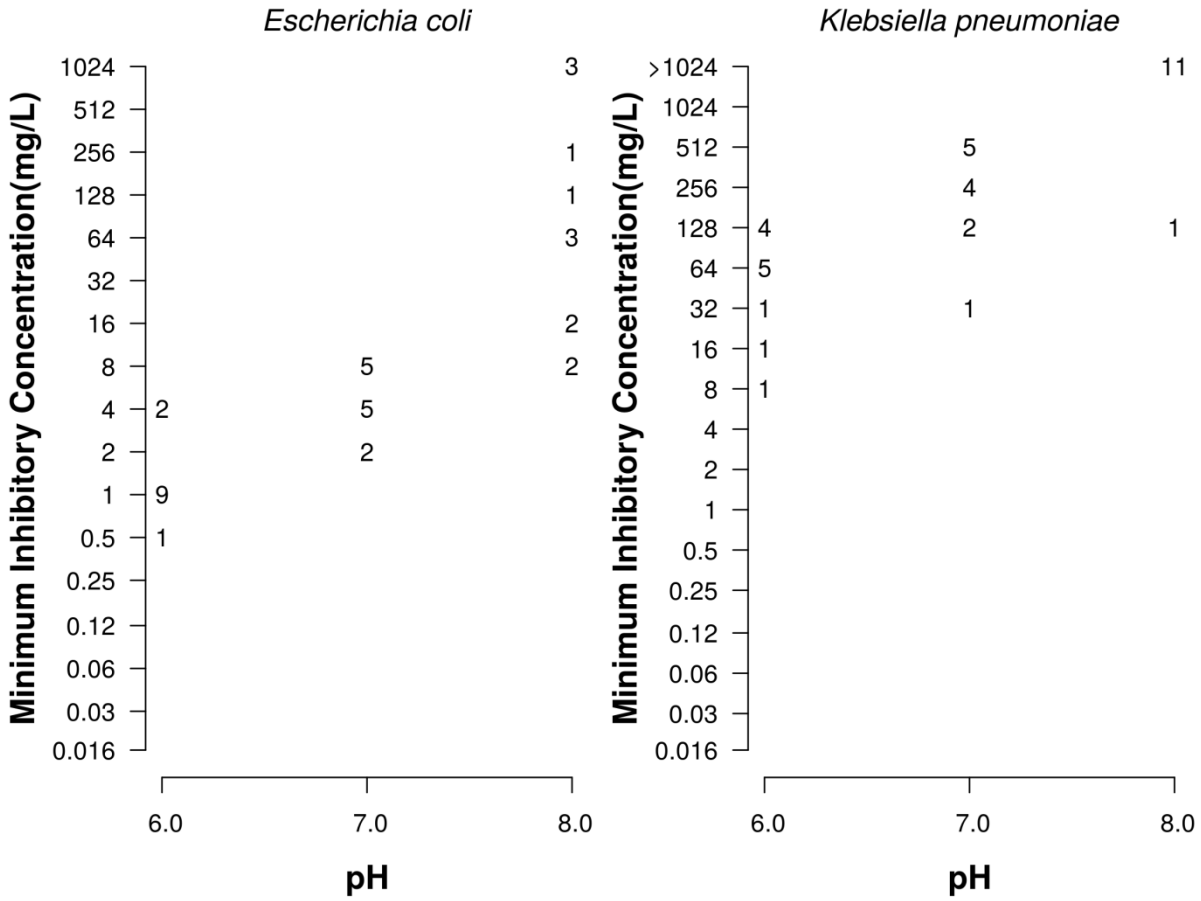

Figure 2: Effect of pH on the MIC of fosfomycin against twelve *E. coli* and *Klebsiella pneumoniae* clinical isolates

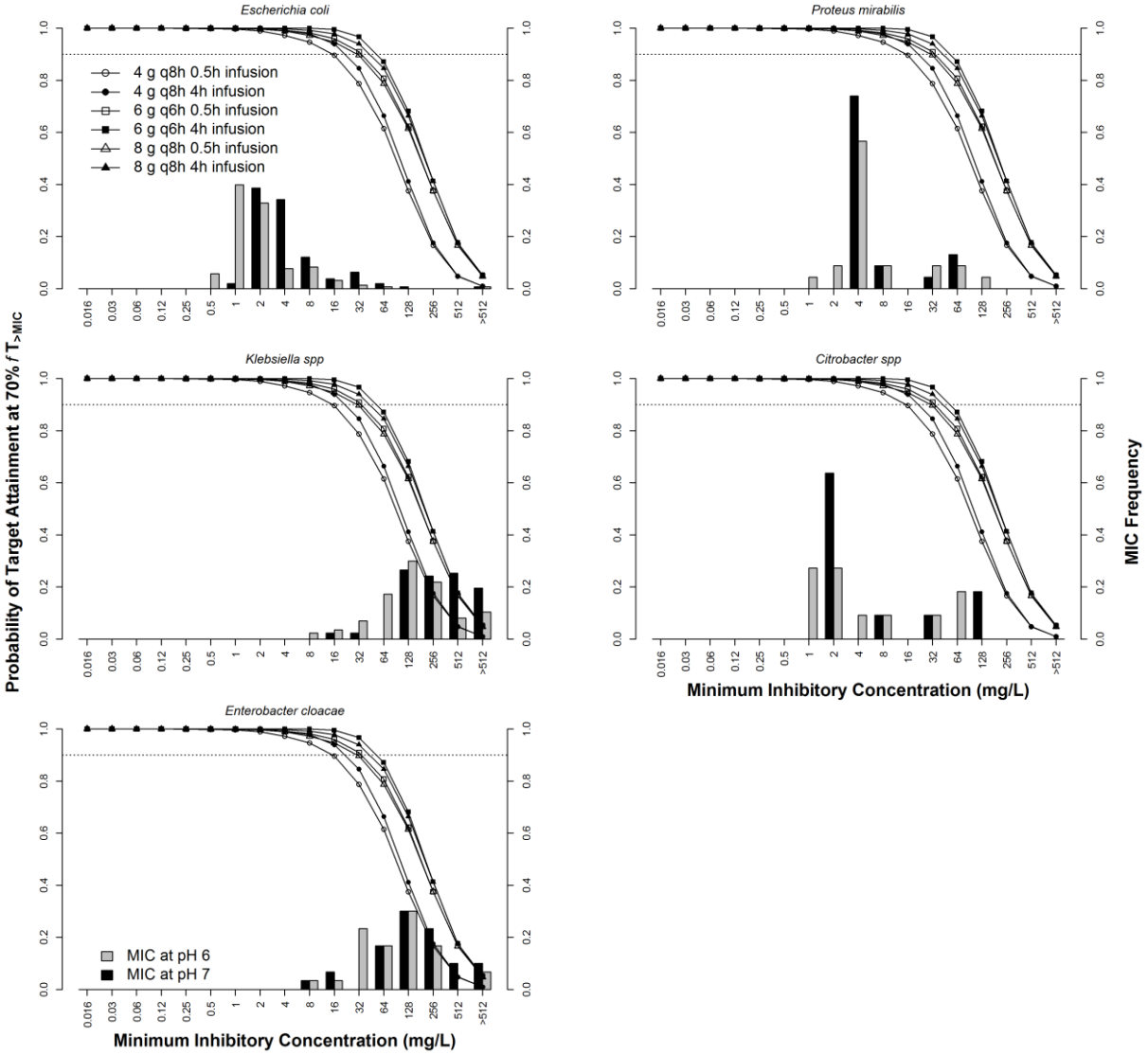

Figure 3: Fosfomycin MIC frequency in 314 *Enterobacteriaceae* clinical isolates in pH 6.0 and 7.0 and probability of target attainment of 70% *f*T>MIC for the fosfomycin dosing regimens of 4g q8h, 6g q6h, and 8g q8h in critically ill virtual patients. Open symbols represent a 0.5-h infusion, and filled symbols indicate a 4-h infusion. The dotted line indicates 90% probability of target attainment.

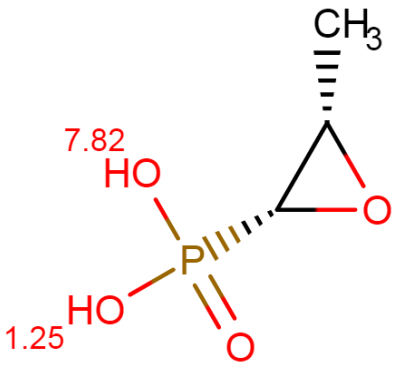

Figure 4: Molecular structure of fosfomycin and pKa values derived from Chemicalize database [https://chemicalize.com/#/calculation]

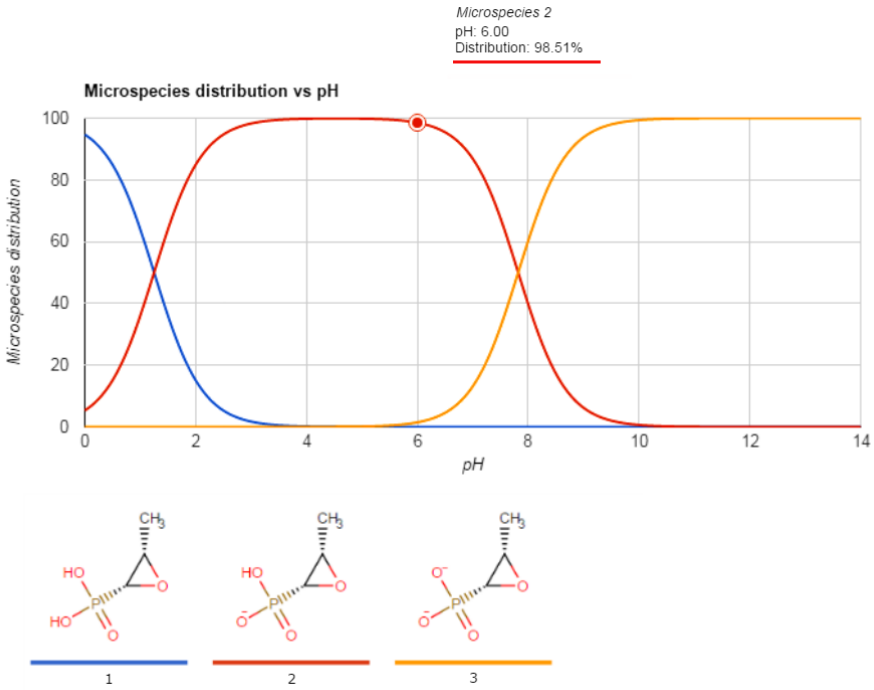

Figure 5: Relationship between microspecies distribution percentage of fosfomycin and pH derived from Chemicalize database [https://chemicalize.com/#/calculation]

Table 1: *In vitro* susceptibility at pH 7.0 and 6.0 for fosfomycin against 314 *Enterobacteriaceae* clinical isolates from urinary tract infections

Microrganisms	No. of strains	pH of test	No. of isolates with the following MIC (µg/ml):													MICs (µg/ml)			% of isolates CLSI			EUCAST	
			0.25	0.5	1	2	4	8	16	32	64	128	256	512	>512	MIC_{50}	MIC_{90}	Range	S	I	R	S	R
Escherichia coli	158	7.0	-		3	61	54	19	6	10	3	1	-	-	1	4	16	1 - >512	98	1	1	97	3
		6.0	-	9	63	52	12	13	5	2	1	-	-	-	1	2	8	0.5 - >512	99	-	1	98	2
Proteus mirabilis	23	7.0	-	-	-	-	17	2	-	1	3	-	-	-	-	4	64	4 - 64	100	-	-	87	13
		6.0	-	-	1	2	13	2	-	2	2	1	-	-	-	4	64	1 - 128	96	4	-	87	13
Citrobacter spp. [a]	11	7.0	-	-	-	7	-	1	-	1	-	2	-	-	-	2	128	2 - 128	82	18	-	82	18
		6.0	-	-	3	3	1	1	-	1	2	-	-	-	-	2	64	1 - 64	100	-	-	82	18
Klebsiella spp. [b]	87	7.0	-	-	-	-	-	-	2	2	-	23	21	22	17	256	>512	16 - >512	5	26	69	5	95
		6.0	-	-	-	-	-	2	3	6	15	26	19	7	9	128	512	8 - >512	30	30	40	13	87
Enterobacter cloacae	30	7.0	-	-	-	-	-	1	2	-	5	9	7	3	3	128	512	8 - >512	27	30	43	10	90
		6.0	-	-	-	-	-	1	1	7	5	9	5		2	128	256	8 - >512	47	30	23	30	70
Morganella morganii	3	7.0	-	-	-	-	-	-	-	-	-	-	3	-	-	ND	ND	ND	-	-	100	-	100
		6.0	-	-	-	-	-	-	-	-	-	2	1	-	-	ND	ND	128 - 256	-	67	33	-	100
Serratia marcescens	2	7.0	-	-	-	-	-	-	-	1	1	-	-	-	-	ND	ND	32 - 64	100	-	-	50	50
		6.0	-	-	-	-	-	-	-	1	1	-	-	-	-	ND	ND	32 - 64	100	-	-	50	50

ND: not determinated; (-): no isolate; S, susceptible; I, intermediate susceptibility; R, resistant; MIC_{50}, concentration that inhibits 50% of isolates; MIC_{90}, concentration that inhibits 90% of isolates; CLSI, Clinical and Laboratory Standards Institute interpretative criteria (susceptible, MIC of ≤ 64 mg/L; intermediate, MIC of 128 mg/L; resistant, MIC of ≥ 256 mg/L; EUCAST, European Committee on Antimicrobial Susceptibility interpretative criteria (susceptible, MIC of ≤ 32 mg/L; resistant, MIC of ≥ 32 mg/L).

[a] Species isolated*: Citrobacter koseri* (4), *C. freundii* (7)

[b] Species isolated: *Klebsiella pneumoniae* (81), *K. oxytoca* (6)

Table 2: Probability of target attainment at target pharmacodynamic indice of 70% *f*T > MIC for fosfomycin dosing regimens by infusion duration and pH values against urinary *Enterobacteriaceae* isolates.

		Fosfomycin regimens and achieved PTA (%)											
		4g q8h				6g q6h				8g q8h			
		pH 7.0		pH 6.0		pH 7.0		pH 6.0		pH 7.0		pH 6.0	
Microorganisms		0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
E. coli	MIC_{50}	97	99	99	100	99	100	100	100	99	100	99	100
	MIC_{90}	90	94	95	98	96	99	98	100	95	99	97	99
P. mirabilis	MIC_{50}	97	99	97	99	99	100	99	100	98	100	98	100
	MIC_{90}	61	66	61	66	**80**	**87**	**80**	**87**	78	**84**	78	**84**
Citrobacter spp.	MIC_{50}	99	100	98	100	100	100	100	100	99	100	99	100
	MIC_{90}	37	41	61	66	62	68	**81**	**87**	79	66	79	**84**
Klebsiella spp.	MIC_{50}	16	17	37	41	37	41	62	68	37	41	61	66
	MIC_{90}	0.0	0.0	4.7	4.8	4.9	5.2	16	17	4.7	4.8	16	17
E. cloacae	MIC_{50}	37	41	37	41	62	68	62	68	61	66	61	66
	MIC_{90}	4.7	4.8	16	17	16	17	37	41	16	17	37	41

*f*T ≥ MIC, percentage of the dosing interval that free antimicrobial concentrations remain above MIC of the bacteria; Dark grey shade indicates ≥90% probability and boldface indicates 80% to <90% probability.

Table 3: Cumulative fraction of bacterial response at 70% *f*T>MIC for fosfomycin regimens as 0.5-h and 4-h infusion against collection of clinical isolates by bacteria types

	pH 6		**pH 7**	
Antimicrobial Regimens	**0.5 h**	**4 h**	**0.5 h**	**4 h**
E. coli				
4 g q8h	97%	98%	94%	97%
6 g q6h	99%	99%	97%	99%
8 g q8h	98%	99%	97%	98%
P. mirabilis				
4 g q8h	90%	92%	92%	94%
6 g q6h	95%	97%	96%	98%
8 g q8h	95%	96%	96%	97%
***Citrobacter* spp.**				
4 g q8h	90%	92%	**85%**	**88%**
6 g q6h	95%	97%	92%	94%
8 g q8h	95%	97%	92%	93%
***Klebsiela* spp.**				
4 g q8h	37%	39%	19%	21%
6 g q6h	54%	59%	35%	38%
8 g q8h	54%	57%	35%	37%
Enterobacter cloacae				
4 g q8h	49%	52%	35%	38%
6 g q6h	66%	71%	53%	57%
8 g q8h	65%	70%	52%	56%

Dark grey shade indicates ≥90% CFR and boldface indicates 80% to <90% CFR.

CAPÍTULO III

CONCLUSÕES

O estudo da análise farmacodinâmica por meio de simulação de Monte Carlo avaliando os esquemas posológicos de fosfomicina contra enterobactérias urinárias e a influência do pH sobre a atividade da fosfomicina mostrou que:

1) A redução do pH melhorou a probabilidade de alcançar os índices farmacodinâmicos desejados e consequente resultado terapêutico, quando comparado ao pH alcalino do meio;

2) A fosfomicina pode ser uma importante escolha no tratamento de infeções do trato urinário causada por enterobactérias uma vez que a grande maioria dos isolados, particularmente de *E. coli*, apresentaram MIC onde todos os esquemas posológicos alcançaram PTA adequadas;

3) Em relação à *Klebsiella* spp. e *E. cloacae* as MIC_{50} e MIC_{90} foram elevadas apresentando baixas PTAs nos esquemas terapêuticos analisados;

4) A otimização do pH, uso de infusão prolongada, esquemas posológicos com dosagens elevadas e fracionadas demonstraram maiores probabilidades de sucesso terapêutico no tratamento de ITU causadas por enterobacterias.

PERSPECTIVAS FUTURAS

- Avaliar e propor modelo matemático farmacodinâmico que descreva a %fT>CIM para diferentes esquemas posológicos de fosfomicina oral (formulação disponível no Brasil) considerando as concentrações alcançadas na urina;

- A partir dos resultados obtidos nos modelo matemático, propor estudos *in vivo*, tanto em animais quanto em pacientes atendidos no Hospital Universitário a fim de verificar a possibilidade do uso clínico desses esquemas posológicos e a melhor utilização deste importante antibacteriano.

ANEXOS

Normas para publicação do periódico:

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

August 2016, Instructions to Authors, pages 1–24

INSTRUCTIONS TO AUTHORS

SCOPE

Antimicrobial Agents and Chemotherapy (AAC) is an interdisciplinary journal devoted to the dissemination of knowledge relating to all aspects of antimicrobial and antiparasitic agents and chemotherapy. Within the circumscriptions set forth below, any report involving studies of or with antimicrobial, antiviral (including antiretroviral), antifungal, or antiparasitic agents as these relate to human disease is within the purview of AAC. Studies involving animal models, pharmacological characterization, and clinical trials are appropriate for consideration.

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(i) Papers which describe the use of antimicrobial agents as tools for elucidating the basic biological processes of bacteria are considered more appropriate for the *Journal of Bacteriology*.

(ii) Manuscripts that (a) describe the use of antimicrobial or antiparasitic agents as tools in the isolation, identification, or epidemiology of microorganisms associated with disease; (b) are concerned with quality control procedures for diffusion, elution, or dilution tests for determining susceptibilities to antimicrobial agents in clinical laboratories; and (c) deal with applications of commercially prepared tests or kits to assays performed in clinical laboratories to measure the activities of established antimicrobial agents or their concentrations in body fluids are considered more appropriate for the *Journal of Clinical Microbiology*. Manuscripts concerned with the development or modification of assay methods (e.g., plasma antimicrobial concentrations and high-throughput screening techniques, etc.) and validation of their sensitivity and specificity with a sufficiently large number of determinations or compounds are considered appropriate for AAC.

(iii) Manuscripts describing new or novel methods or improvements in media and culture conditions will not be considered for publication in AAC unless these methods are applied to the study of problems related to the production or activity of antimicrobial agents. Such manuscripts are more appropriate for *Applied and Environmental Microbiology* or the *Journal of Clinical Microbiology*.

(iv) Manuscripts dealing with properties of unpurified natural products, with entities that are primarily antitumor agents, or with immunomodulatory agents that are not antimicrobial agents are not appropriate for AAC.

(v) Manuscripts dealing with novel small molecular antimicrobials must provide at least some data showing that the proposed new agents or scaffolds have the potential to become therapeutic agents. Appropriate demonstrations will vary but generally should be some combination of data on physical properties (solubility, protein binding, log *P* [logarithm of the ratio of the concentrations of un-ionized solutes in solvents]), pharmacological properties (Caco2 predictions of bioavailability, pharmacokinetics in an animal species), or tolerability (mammalian cell toxicity, likelihood of hepatic metabolism, potential for receptor interactions, potential for human ERG liability). Initial presentations of compounds are not expected to address all these areas but rather to show an appropriate initial subset. For example, the first publication of a novel com-pound or compound series might address selected physical properties plus mammalian cell toxicity. Subsequent publica-tions are expected to add progressively to the proof of the agent's therapeutic potential.

(vi) Biochemical analyses for -lactamases that determine kinetic parameters (e.g., K_m, k_{cat}) must be performed on purified enzyme preparations. The enzyme must be in its native form, without any leader sequences or fusions used for purifi-cation (e.g., His tag). The determination of relative rates of hydrolysis may be performed on crude extracts.

(vii) Authors of papers describing enzymological studies should review the standards of the STRENDA Commission for information required for adequate description of experimental conditions and for reporting enzyme activity data (http://www.beilstein-institut.de/en/projects/strenda/guidelines).

(viii) A manuscript limited to the nucleic acid sequence of a gene encoding an antibiotic target, receptor, or resistance mechanism may be submitted as a Short-Form paper (see "Short-Form Papers") or a New-Data Letter to the Editor (see "Letters to the Editor"), depending on its length. Formatting instructions for nucleic acid sequences are given below (see "Presentation of Nucleic Acid Sequences"). Repetition of sequences already in a database should be avoided.

Questions about these guidelines may be directed to the editor in chief of the journal being considered.

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Challenging Clinical Cases in Antimicrobial Resistance

Challenging Clinical Cases are brief articles (limit of three printed pages) designed to familiarize and provide guidance to the reader on the clinical approach to the treatment of real, challenging cases involving multidrug-resistant organisms (bacteria, viruses, fungi, and parasites). This section is focused on providing an up-to-date scientific rationale for choosing specific antimicrobials based on available clinical, microbio-logical, and pharmacological data and on discussing the im-pact of mechanisms of resistance on the outcomes for infected patients. These articles may discuss novel therapeutic strategies for treating patients infected with multidrug-resistant organ-isms. Only highly interesting cases that have important mech-anistic and epidemiological or novel microbiological insights will be selected for review.

The article should include (i) a brief abstract (limit of 75 words); (ii) a case section describing a single clinical case up to the point when the organism is isolated, characterization of the organism, and information about susceptibility testing, when appropriate; (iii) interesting photos, figures, and/or tables (limit of two combined) highlighting the clinical presentation (see "Illustrations and Tables" below for guidelines on accept-able file types, resolution, size, etc.); (iv) a single multiple-choice question addressing the most relevant therapeutic is-sues (How would you interpret the susceptibility report? Which antimicrobials would be best for the patient presented in the case and why? What are the underlying mechanisms of resistance? Are there any particular pharmacological strategies, in terms of drug administration, delivery, etc., that could help in treating this patient?) with several possible answers as choic-es; (v) a description of the treatment strategy and patient out-come; and (vi) a reference list containing no more than 10 references. Sections ii and v above (case presentation and strategy/outcome) must not exceed 1,200 words combined.

An expert in the field (a reviewer) will discuss the case in a brief commentary section and explore answers to the questions posed by the author. (The commentator's name and role will appear at the end of the published article byline.)

These articles will be made freely available to readers at the time of publication. No page charges will be associated with these articles, but the standard fee for accepted supplemental material, if any, applies. In an attempt to stimulate conversations and engagement, readers will be able to add comments via an online feature.

Letters to the Editor

Two types of Letters to the Editor may be submitted. The first type (Comment Letter) is intended for comments on final, typeset articles published in the journal (not on accepted manuscripts posted online) and must cite published references to support the writer's argument. The second type (New-Data Letter) may report new, concise findings that are not appropriate for publication as full-length or Short-Form papers.

Letters may be **no more than 500 words long and must be typed double-spaced**. Refer to a recently published Letter for correct formatting. Note that authors and affiliations are listed below the title.

All Letters to the Editor must be submitted electronically, and the type of Letter (New Data or Comment) must be selected from the drop-down list in the submission form. For Letters commenting on published articles, the cover letter should state the volume and issue in which the article was published, the title of the article, and the last name of the first author. In the Abstract section of the submission form, put "Not Applicable." Letters to the Editor do not have abstracts. Both types of Letter must have a title, which must appear on the manuscript and on the submission form. Figures and tables should be kept to a minimum.

If the Letter is related to a published article, it will be sent to the editor who handled the article in question. If the editor believes that publication is warranted, he/she will solicit a reply from the corresponding author of the article and give approval for publication.

New-Data Letters will be assigned to an editor according to subject matter and will be reviewed by that editor and/or a reviewer.

Please note that some indexing/abstracting services do not include Letters to the Editor in their databases.

Errata

Errata provide a means of correcting errors that occurred during the writing, typing, editing, or publication (e.g., a mis-spelling, a dropped word or line, or mislabeling in a figure) of a published article. Submit Errata via the eJP online manu-script submission and peer review system (see "Submission, Review, and Publication Processes"). In the Abstract section of the submission form (a required field), put "Not Applicable." Upload the text of your Erratum as a Microsoft Word file. Please see a recent issue for correct formatting.

Author Corrections

Author Corrections provide a means of correcting errors of omission (e.g., author names or citations) and errors of a scientific nature that do not alter the overall basic results or con-clusions of a published article (e.g., an incorrect unit of measurement or order of magnitude used throughout, contamination of one of numerous cultures, or misidentification of a mutant strain, causing erroneous data for only a [noncritical] portion of the study). Note that the addition of new data is not permitted.

For corrections of a scientific nature or issues involving authorship, including contributions and use or ownership of data and/or materials, all disputing parties must agree, in writing, to publication of the Correction. For omission of an author's name, letters must be signed by the authors of the article and the author whose name was omitted. The editor who handled the article will be consulted if necessary.

Submit an Author Correction via the eJP online manuscript submission and peer review system (see "Submission, Review,

and Publication Processes"). Select Author Correction as the manuscript type. In the Abstract section of the submission form (a required field), put "Not Applicable." Upload the text of your Author Correction as a Microsoft Word file. Please see a recent issue for correct formatting. Signed letters of agree-ment must be supplied as supplemental material not for pub-lication (scanned PDF files).

Retractions

Retractions are reserved for major errors or breaches of eth-ics that, for example, may call into question the source of the data or the validity of the results and conclusions of an article. Submit Retractions via the eJP online manuscript submission and peer review system (see "Submission, Review, and Publi-cation Processes"). In the Abstract section of the submission form (a required field), put "Not Applicable." Upload the text of your Retraction as a Microsoft Word file. Letters of agree-ment signed by all of the authors must be supplied as supple-mental material not for publication (scanned PDF files). The Retraction will be assigned to the editor in chief of the journal, and the editor who handled the paper and the chairperson of the ASM Journals Board will be consulted. If all parties agree to the publication and content of the Retraction, it will be sent to the Journals Department for publication.

CrossMark

ASM has implemented CrossMark. CrossMark is a multi-publisher initiative to provide a standard way for readers to locate the current version of an article. Clicking on the Cross-Mark logo will indicate whether an article is current or whether updates have been published. Additional information about CrossMark can be found on CrossMark's website and on ASM's CrossMark policy page.

ILLUSTRATIONS AND TABLES

Illustrations

Image manipulation. Digital images submitted for publi-cation may be inspected by ASM production specialists for any manipulations or electronic enhancements that may be con-sidered to be the result of scientific misconduct based on the guidelines provided below. Any images/data found to contain manipulations of concern will be referred to the editor in chief, and authors may then be requested to provide their primary data for comparison with the submitted image file. Investiga-tion of the concerns may delay publication and may result in revocation of acceptance and/or additional action by ASM.

Linear adjustments to contrast, brightness, and/or color are generally acceptable, as long as the measures taken are neces-sary to view elements that are already present in the data and the adjustments are applied to the entire image and not just specific areas. Unacceptable adjustments to images include, but are not limited to, the removal or deletion, concealment, duplication (copying and pasting), addition, selective en-hancement, or repositioning of elements within the image.

Nonlinear adjustments made to images, such as changes to gamma settings, should be fully disclosed in the figure legends at the time of submission. In addition, images created by com-piling multiple files, including noncontiguous portions of the same image, should clearly distinguish that these multiple files are not a single image. This can be done by "tooling," or inserting thin lines, between the individual images.

File types and formats. Illustrations may be continuous-tone images, line drawings, or composites. Color graphics may be submitted. Suggestions about how to ensure accurate color reproduction are given below.

On initial submission, figures may be uploaded as individual PDF files or combined and uploaded as a single PDF file. Place each legend in the text file, as well as on the same page with the corresponding figure to assist review. At the modification stage, production-quality digital files must be provided. Be-cause the legends will be copyedited and typeset for final pub-lication, they should appear within the main text, after the References section, and should not be included as part of the figure itself at this stage. All graphics submitted with modified manuscripts must be bitmap, grayscale, or in the RGB (pre-ferred) or CMYK color mode. See "Color illustrations." Half-tone images (those with various densities or shades) must be grayscale, not bitmap. AAC accepts TIFF or EPS files but dis-courages PowerPoint for either black-and-white or color im-ages.

For instructions on creating acceptable EPS and TIFF files, refer to the Cadmus digital art website, http://art.cadmus.com/da/index.jsp. PowerPoint requires users to pay close attention to the fonts used in their images (see the section on fonts below). If instructions for fonts are not followed exactly, images prepared for publication are subject to missing characters, improperly con-verted characters, or shifting/obscuring of elements or text in the figure. For proper font use in PowerPoint images, refer to the Cadmus digital art website, http://art.cadmus.com/da/instructions/ppt_disclaimer.jsp. Note that, due to page compo-sition system requirements, you must verify that your PowerPoint files can be converted to PDF without any errors.

We strongly recommend that before returning their mod-ified manuscripts, authors check the acceptability of their digital images for production by running their files through Rapid Inspector, a tool provided at the following URL: http://rapidinspector.cadmus.com/RapidInspector/zmw/index.jsp. Rapid Inspector is an easy-to-use, Web-based application that identifies file characteristics that may ren-der the image unusable for production. Please note when using Rapid Inspector to check PowerPoint files that there is a known bug in the application that can occasionally fail PowerPoint Presentation (.pptx) files, even though the files meet all required production criteria. If you experience this bug, the issue can be corrected by saving the PowerPoint files as an older version, PowerPoint 97-2004 Presentation (.ppt), during the Save As process (use the drop-down for-mat menu and select this format). Once you save your files as .ppt, they will pass Rapid Inspector if all required produc-tion criteria have been met.

If you have additional questions about using the Rapid In-spector preflighting tool, please send an e-mail inquiry to helpdesk.digitalartsupport@cenveo.com.

Minimum resolution. It is extremely important that a high enough file resolution is used. All separate images that you import into a figure file must be at the correct resolution before they are placed. (For instance, placing a 72-dpi image in a 300-dpi EPS file will not result in the placed image meeting the minimum requirements for file resolution.) Note, however, that the higher the resolution, the larger the file and the longer the upload time. Publication quality will not be improved by using a resolution higher than the minimum. Minimum reso-lutions are as follows:

- 300 dpi for grayscale and color
- 600 dpi for combination art (lettering and images)
- 1,200 dpi for line art

Size. All graphics **should be submitted at their intended publication size** so that no reduction or enlargement is neces-sary. Resolution must be at the required level at the submitted size. Include only the significant portion of an illustration. White space must be cropped from the image, and excess space between panel labels and the image must be eliminated.

- Maximum width for a 1-column figure: 20.6 picas (ca. 8.7 cm)
- Maximum width for a 2-column figure: 42 picas (ca. 17.8 cm)
- Minimum width for a 2-column figure: 26 picas (11.1 cm)
- Maximum height for a standard figure: 54.7 picas (ca. 23.2 cm)
- Maximum height for an oversized figure (no running title): 57.4 picas (ca. 24.3 cm)

Contrast. Illustrations must contain sufficient contrast to be viewed easily on a monitor or on the printed page.

Labeling and assembly. All final lettering and labeling must be incorporated into the figures. On initial submission, illus-trations should be provided as PDF files, with the legends in the text file and with a legend beneath each image to assist review. At the modification stage, production-quality digital figure files (without legends) must be provided. Put the figure num-ber well outside the boundaries of the image itself. (Number-ing may need to be changed at the copyediting stage.) Each figure must be uploaded as a separate file, and any multipanel figures must be assembled into one file; i.e., rather than up-loading a separate file for each panel in a figure, assemble all panels in one piece and supply them as one file.

Fonts. To avoid font problems, set all type in one of the following fonts: Arial, Helvetica, Times Roman, European PI, Mathematical PI, or Symbol. Courier may be used but should be limited to nucleotide or amino acid sequences in which a nonproportional (monospace) font is required. All fonts other than these must be converted to paths (or outlines) in the ap-plication with which they were created.

Color illustrations. All figures submitted in color will be processed as color. Adherence to the following guidelines will help to ensure color reproduction that is as accurate as possible. The final online version is considered the version of record for AAC and all other ASM journals. To maximize online re-production, color illustrations should be supplied in the RGB color mode as either (i) RGB TIFF images with a resolution of at least 300 pixels per inch (raster files, consisting of pixels) or (ii) Illustrator-compatible EPS files with RGB color elements (vector files, consisting of lines, fonts, fills, and images). CMYK files are also accepted. Other than in color space, CMYK files must meet the same production criteria as RGB files. The RGB color space is the native color space of computer monitors and of most of the equipment and software used to capture scien-tific data, and it can display a wider range of colors (especially bright fluorescent hues) than the CMYK (cyan, magenta, yel-low, black) color space used by print devices that put ink (or toner) on paper. For reprints, ASM's print provider will auto-matically create CMYK versions of color illustrations from the supplied RGB versions. Color in the reprints may not match that in the online journal of record because of the smaller range of colors capable of being reproduced by CMYK inks on a printing press. For additional information on RGB versus CMYK color, refer to the Cadmus digital art site, http: //art.cadmus.com/da/guidelines_rgb.jsp.

Drawings

Submit graphs, charts, complicated chemical or mathemat-ical formulas, diagrams, and other drawings as finished prod-ucts not requiring additional artwork or typesetting. All ele-ments, including letters, numbers, and symbols, must be easily readable, and both axes of a graph must be labeled. When creating line art, please use the following guidelines:

(i) All art must be submitted at its intended publication size. For acceptable dimensions, see "Size," above.

(ii) **Avoid using screens (i.e., shading) in line art.** It can be difficult and time-consuming to reproduce these images with-out moire´ patterns. Various pattern backgrounds are prefera-ble to screens as long as the patterns are not imported from another application. If you must use images containing screens,

(a) Generate the image at line screens of 85 lines per inch or less.

(b) When applying multiple shades of gray, differentiate the gray levels by at least 20%.

(c) Never use levels of gray below 5% or above 95% as they are likely to fade out or become totally black when output.

(iii) Use thick, solid lines that are no finer than 1 point in thickness.

(iv) No type should be smaller than 6 points at the final publication size.

(v) Avoid layering type directly over shaded or textured ar-eas.

(vi) Avoid the use of reversed type (white lettering on a black background).

(vii) Avoid heavy letters, which tend to close up, and unusual symbols, which the printer may not be able to reproduce in the legend.

(viii) If colors are used, avoid using similar shades of the same color and avoid very light colors.

In figure ordinate and abscissa scales (as well as table column headings), avoid the ambiguous use of numbers with exponents. Usually, it is preferable to use the Syste`me International d'Unite´s (SI) symbols (for 10^6, m for 10^3, k for 10^3, and M for 10^6, etc.). Thus, a representation of 20,000 cpm on a figure ordinate should be made by the number 20 accompanied by the label kcpm. A complete listing of SI symbols can be found in the International Union of Pure and Applied Chemistry (IUPAC) publication *Quantities, Units and Symbols in Physical Chemistry*, 3rd ed. (RSC Publishing, Cambridge, United Kingdom, 2007), and at http://www.nist.gov/pml/pubs/sp811/.

When powers of 10 must be used, the journal requires that the exponent power be associated with the number shown. In representing 20,000 cells per ml, the numeral on the ordinate should be "2" and the label should be "10^4 cells per ml" (not "cells per ml 10^4"). Likewise, an enzyme activity of 0.06 U/ml might be shown as 6 accompanied by the label "10^2 U/ml." The preferred designation is 60 mU/ml (milliunits per milliliter).

Presentation of Nucleic Acid Sequences

Long nucleic acid sequences must be presented as figures in the following format to conserve space. Print the se-quence in lines of approximately 100 to 120 nucleotides in a nonproportional (monospace) font that is easily legible when published with a line length of 6 inches (ca. 15.2 cm). If possible, lines of nucleic acid sequence should be further subdivided into blocks of 10 or 20 nucleotides by spaces within the sequence or by marks above it. Uppercase and lowercase letters may be used to designate the exon-intron structure or transcribed regions, etc., if the lowercase letters remain legible at a 6-inch (ca. 15.2-cm) line length. Number the sequence line by line; place numerals representing the first base of each line to the left of the lines. Minimize spacing between lines of sequence, leaving room only for annotation of the sequence. Annotation may include boldface, underlining, brackets, and boxes, etc. Encoded amino acid sequences may be presented, if necessary, immediately above or below the first nucleotide of each codon, by using the single-letter amino acid symbols. Comparisons of multiple nucleic acid sequences should conform as nearly as possible to the same format.

Figure Legends

On initial submission, each legend should be placed in the text file *and* be incorporated into the image file beneath the figure to assist review.

Legends should provide enough information so that the figure is understandable without frequent reference to the text. However, detailed experimental methods must be described in the Materials and Methods section, not in a figure legend. A method that is unique to one of several experiments may be set forth in a legend only if the description is very brief (one or two sentences). Define all symbols used in the figure and define all abbreviations that are not used in the text.

TABLE 1 Distribution of protein and ATPase in fractions of dialyzed membranes[a]

Membrane	Fraction	ATPase	
		U/mg of protein	Total U
Control	Depleted membrane	0.036	2.3
	Concentrated supernatant	0.134	4.82
E1 treated	Depleted membrane	0.034	1.98
	Concentrated supernatant	0.11	4.6

[a] Specific activities of ATPase of nondepleted membranes from control and treated bacteria were 0.21 and 0.20, respectively.

Tables

Tables that contain artwork, chemical structures, or shading must be submitted as illustrations in an acceptable format at the modification stage. The preferred format for regular tables is Microsoft Word; however, WordPerfect and Acrobat PDF are also acceptable. Note that a straight Excel file is not cur-rently an acceptable format. Excel files must be either embed-ded in a Word or WordPerfect document or converted to PDF before being uploaded.

Tables should be formatted as follows. Arrange the data so that **columns of like material read down, not across.** The headings should be sufficiently clear so that the meaning of the data is understandable without reference to the text. See the "Abbreviations" section of these Instructions for those that should be used in tables. Explanatory footnotes are acceptable, but more-extensive table "legends" are not. Footnotes should not include detailed descriptions of the experiment. Tables must include enough information to warrant table format; those with fewer than six pieces of data will be incorporated into the text by the copy editor. Table 1 is an example of a well-constructed table.

Avoid tables (or figures) of raw data on drug susceptibility, therapeutic activity, or toxicity. Such data should be analyzed by an approved procedure, and the results should be presented in tabular form.

NOMENCLATURE

Chemical and Biochemical Nomenclature

The recognized authority for the names of chemical com-pounds is *Chemical Abstracts* (CAS; http://www.cas.org/) and its indexes. *The Merck Index Online* (https://www.rsc.org/ merck-index) is also an excellent source. For guidelines to the use of biochemical terminology, consult *Biochemical Nomen-clature and Related Documents* (Portland Press, London, United Kingdom, 1992), available at http://www.chem.qmul

.ac.uk/iupac/bibliog/white.html, and the instructions to authors of the *Journal of Biological Chemistry*.

Molecular weight should not be expressed in daltons; molecular weight is a unitless ratio. Molecular mass is expressed in daltons.

For enzymes, use the recommended (trivial) name as as-signed by the Nomenclature Committee of the International Union of Biochemistry (IUB) as described in *Enzyme No-menclature* (Academic Press, Inc., New York, NY, 1992) and its supplements and at http://www.chem.qmul.ac.uk/iubmb /enzyme/. If a nonrecommended name is used, place the proper (trivial) name in parentheses at first use in the abstract and text. Use the EC number when one has been assigned. Authors of papers describing enzymological studies should re-view the standards of the STRENDA Commission for informa-tion required for adequate description of experimental condi-tions and for reporting enzyme activity data (http://www .beilstein-institut.de/en/projects/strenda/guidelines).

Nomenclature of Microorganisms

Binary names, consisting of a generic name and a specific epithet (e.g., *Escherichia coli*), must be used for all microorganisms. Names of categories at or above the genus level may be used alone, but specific and subspecific epithets may not. A specific epithet must be preceded by a generic name, written out in full the first time it is used in a paper. Thereafter, the generic name should be abbreviated to the initial capital letter (e.g., *E. coli*), provided there can be no confusion with other genera used in the paper. Names of all bacterial taxa (king-doms, phyla, classes, orders, families, genera, species, and sub-species) are printed in italics and should be italicized in the manuscript; strain designations and numbers are not. Ver-nacular (common) names should be in lowercase roman type (e.g., streptococcus, brucella). For *Salmonella*, genus, species, and subspecies names should be rendered in standard form:

Salmonella enterica at first use, *S. enterica* thereafter; *Salmonella enterica* subsp. *arizonae* at first use, *S. enterica* subsp. *arizonae* thereafter. Names of serovars should be in roman type with the first letter capitalized: *Salmonella enterica* serovar Typhimurium. After the first use, the serovar may also be given without a species name: *Salmonella* Typhimurium, *S.* Typhimurium, or *Salmonella* serovar Typhimurium. For other information regarding serovar designations, see *Antigenic Formulae of the Salmonella Serovars*, 9th ed. (P. A. D. Grimont and F.-X. Weill, WHO Collaborating Centre for Reference and Research on Salmonella, Institut Pasteur, Paris, France, 2007; see http://www.scacm.org/free/Antigenic%20Formulae %20of%20the%20Salmonella%20Serovars%202007%209th% 20edition.pdf). For a summary of the current standards for *Salmonella* nomenclature and the Kaufmann-White criteria, see the article by Brenner et al. (J Clin Microbiol **38:**2465–2467, 2000), the opinion of the Judicial Commission of the International Committee on Systematics of Prokaryotes (Int J Syst Evol Microbiol **55:**519 –520, 2005), and the article by Tindall et al. (Int J Syst Evol Microbiol **55:**521–524, 2005).

The spelling of bacterial names should follow the *Approved Lists of Bacterial Names (Amended) & Index of the Bacterial and Yeast Nomenclatural Changes* (V. B. D. Skerman et al., ed., American Society for Microbiology, Washington, DC, 1989) and the validation lists and notification lists published in the *International Journal of Systematic and Evolutionary Microbiology* (formerly the *International Journal of Systematic Bacteriology*) since January 1989. In addition, two sites on the World Wide Web list current approved bacterial names: Prokaryotic Nomenclature Up-to-Date (http://www.dsmz.de/bacterial-diversity /prokaryotic-nomenclature-up-to-date.html) and List of Prokaryotic Names with Standing in Nomenclature (http://www .bacterio.net/). If there is reason to use a name that does not have standing in nomenclature, the name should be enclosed in quotation marks in the title and at its first use in the abstract and the text and an appropriate statement concerning the nomenclatural status of the name should be made in the text. "*Candidatus*" species should always be set in quotation marks.

Since the classification of fungi is not complete, it is the responsibility of the author to determine the accepted bino-mial for a given organism. Sources for these names include *The Yeasts: a Taxonomic Study*, 5th ed. (C. P. Kurtzman, J. W. Fell, and T. Boekhout, ed., Elsevier Science, Amsterdam, Netherlands, 2011), and *Dictionary of the Fungi*, 10th ed. (P. M. Kirk, P. F. Cannon, D. W. Minter, and J. A. Stalpers, ed., CABI International, Wallingford, Oxfordshire, United Kingdom, 2008); see also http: //www.speciesfungorum.org/Names/Fundic.asp.

Names used for viruses should be those approved by the International Committee on Taxonomy of Viruses (ICTV) and reported on the ICTV Virus Taxonomy website (http: //www.ictvonline.org/index.asp). In addition, the recommendations of the ICTV regarding the use of species names should generally be followed: when the entire species is discussed as a taxonomic entity, the species name, as with other taxa, is italic and has the first letter and any proper nouns capitalized (e.g., *Tobacco mosaic virus*, *Murray Valley encephalitis virus*). When the behavior or manipulation of individual viruses is discussed, the vernacular (e.g., tobacco mosaic virus, Murray Valley encephalitis virus) should be used. If desired, synonyms may be added parenthetically when the name is first mentioned. Approved generic (or group) and family names may also be used.

Microorganisms, viruses, and plasmids should be given designations consisting of letters and serial numbers. It is gener-ally advisable to include a worker's initials or a descriptive sym-bol of locale or laboratory, etc., in the designation. Each new strain, mutant, isolate, or derivative should be given a new (serial) designation. This designation should be distinct from those of the genotype and phenotype, and genotypic and phe-notypic symbols should not be included. Plasmids are named with a lowercase "p" followed by the designation in uppercase letters and numbers. To avoid the use of the same designation as that of a widely used strain or plasmid, check the designation against a publication database such as Medline.

Genetic Nomenclature

To facilitate accurate communication, **it is important that standard genetic nomenclature be used whenever possible and that deviations or proposals for new naming systems be endorsed by an appropriate authoritative body**. Review and/or publication of submitted manuscripts that contain new or nonstandard nomenclature may be delayed by the editor or the Journals Department so that they may be reviewed.

Bacteria. The genetic properties of bacteria are described in terms of phenotypes and genotypes. The phenotype describes the observable properties of an organism. The genotype refers to the genetic constitution of an organism, usually in reference to some standard wild type. The guidelines that follow are based on the recommendations of Demerec et al. (Genetics **54:**61–76, 1966).

(i) Phenotype designations must be used when mutant loci have not been identified or mapped. They can also be used to identify the protein product of a gene, e.g., the OmpA protein. Phenotype designations generally consist of three-letter sym-bols; these are not italicized, and the first letter of the symbol is capitalized. It is preferable to use Roman or Arabic numerals (instead of letters) to identify a series of related phenotypes. Thus, a series of nucleic acid polymerase mutants might be designated Pol1, Pol2, and Pol3, etc. Wild-type characteristics can be designated with a superscript plus (Pol), and, when necessary for clarity, negative superscripts (Pol) can be used to designate mutant characteristics. Lowercase superscript let-ters may be used to further delineate phenotypes (e.g., Str^r for streptomycin resistance). Phenotype designations should be defined.

(ii) Genotype designations are also indicated by three-letter locus symbols. In contrast to phenotype designations, these are lowercase italic (e.g., *ara his rps*). If several loci govern related functions, these are distinguished by italicized capital letters following the locus symbol (e.g., *araA araB araC*). Promoter, terminator, and operator sites should be indicated as described by Bachmann and Low (Microbiol Rev **44:**1–56, 1980): e.g., *lacZp*, *lacAt*, and *lacZo*.

(iii) Wild-type alleles are indicated with a superscript plus (*ara his*). A superscript minus is not used to indicate a mutant locus; thus, one refers to an *ara* mutant rather than an *ara* strain.

(iv) Mutation sites are designated by placing serial isolation numbers (allele numbers) after the locus symbol (e.g., *araA1 araA2*). If only a single such locus exists or if it is not known in which of several related loci the mutation has occurred, a hyphen is used instead of the capital letter (e.g., *ara-23*). It is essential in papers reporting the isolation of new mutants that allele numbers be given to the mutations. For *Escherichia coli*, there is a registry of such numbers: *E. coli* Genetic Stock Center (http://cgsc.biology.yale.edu/). For the genus *Salmonella*, the registry is *Salmonella* Genetic Stock Center (http://people .ucalgary.ca/~kesander/). For the genus *Bacillus*, the registry is *Bacillus* Genetic Stock Center (http://www.bgsc.org/).

(v) The use of superscripts with genotypes (other than to indicate wild-type alleles) should be avoided. Designations indicating amber mutations (Am), temperature-sensitive mutations (Ts), constitutive mutations (Con), cold-sensitive mutations (Cs), production of a hybrid protein (Hyb), and other important phenotypic properties should follow the allele number [e.g., *araA230*(Am) *hisD21*(Ts)]. All other such designations of phenotype must be defined at the first occurrence. If superscripts must be used, they must be approved by the editor and defined at the first occurrence in the text.

Subscripts may be used in two situations. Subscripts may be used to distinguish between genes (having the same name) from different organisms or strains; e.g., $his_{E.\ coli}$ or $his_{K\text{-}12}$ for the *his* gene of *E. coli* or strain K-12, respectively, may be used to distinguish this gene from the *his* gene in another species or strain. An abbreviation may also be used if it is explained. Sim-ilarly, a subscript is also used to distinguish between genetic elements that have the same name. For example, the promoters of the *gln* operon can be designated $glnAp_1$ and $glnAp_2$. This form departs slightly from that recommended by Bachmann and Low (e.g., *desC1p*).

(vi) Deletions are indicated by the symbol placed before the deleted gene or region, e.g., *trpA432*, (*aroP-aceE*)*419*, or (*hisQ-hisJo*)*1256*. Similarly, other symbols can be used (with appropriate definition). Thus, a fusion of the *ara* and *lac* operons can be shown as (*ara-lac*)*95*. Likewise, (*araB - lacZ*)*96* indicates that the fusion results in a truncated *araB* gene fused to an intact *lacZ* gene, and (*malE-lacZ*)*97*(Hyb) shows that a hybrid protein is synthesized. An inversion is shown as IN(*rrnD-rrnE*)*1*. An insertion of an *E. coli his* gene into plasmid pSC101 at zero kilobases (0 kb) is shown as pSC101 (0kb::K-12*hisB*)*4*. An alternative designation of an insertion can be used in simple cases, e.g., *galT236*::Tn*5*. The number *236* refers to the locus of the insertion, and if the strain carries an additional *gal* mutation, it is listed separately. Addi-tional examples, which utilize a slightly different format, can be found in the papers by Campbell et al. and Novick et al. cited below. It is important in reporting the construction of strains in which a mobile element was inserted and subsequently de-leted that this fact be noted in the strain table. This can be done by listing the genotype of the strain used as an intermediate in a table footnote or by making a direct or parenthetical remark in the genotype, e.g., (F), Mu *c*ts, or *mal*:: Mu *c*ts::*lac*. In setting parenthetical remarks within the genotype or dividing the genotype into constituent elements, parentheses and brackets are used without special meaning; brackets are used outside pa-rentheses. To indicate the presence of an episome, parentheses (or brackets) are used (, F). Reference to an integrated episome is indicated as described above for inserted elements, and an exog-enote is shown as, for example, W3110/F 8(*gal*).

For information about genetic maps of locus symbols in current use, consult Berlyn (Microbiol Mol Biol Rev **62:**814 – 984, 1998) for *E. coli* K-12, Sanderson and Roth (Microbiol Rev **52:**485–532, 1988) for *Salmonella* serovar Typhimurium, Holloway et al. (Microbiol Rev **43:**73–102, 1979) for the genus *Pseudomonas*, Piggot and Hoch (Microbiol Rev **49:**158 –179, 1985) for *Bacillus subtilis*, Perkins et al. (Microbiol Rev **46:** 426 –570, 1982) for *Neurospora crassa*, and Mortimer and Schild (Microbiol Rev **49:**181–213, 1985) for *Saccharomyces cerevisiae*. For yeasts, *Chlamydomonas* spp., and several fungal species, symbols such as those given in the *Handbook of Microbiology*, 2nd ed. (A. I. Laskin and H. A. Lechevalier, ed., CRC Press, Inc., Cleveland, OH, 1988) should be used.

Conventions for naming genes. It is recommended that (entirely) new genes be given names that are mnemonics of their function, avoiding names that are already assigned and earlier or alternative gene names, irrespective of the bacterium for which such assignments have been made. Similarly, it is recommended that, whenever possible, orthologous genes present in different organisms receive the same name. When homology is not apparent or the function of a new gene has not

been established, a provisional name may be given by one of the following methods. (i) The gene may be named on the basis of its map location in the style *yaaA*, analogous to the style used for recording transposon insertions (*zef*) as discussed below. A list of such names in use for *E. coli* has been published by Rudd (Microbiol Mol Biol Rev **62:**985–1019, 1998). (ii) A provisional name may be given in the style described by Demerec et al. (e.g., *usg*, gene upstream of *folC*). Such names should be unique, and names such as *orf* or *genX* should not be used. For reference, the *E. coli* Genetic Stock Center's database includes an updated listing of *E. coli* gene names and gene products. It is accessible on the Internet (http://cgsc.biology.yale.edu/index.php). A list can also be found in the work of Riley (Microbiol Rev **57:**862–952, 1993). For the genes of other bacteria, consult the references given above.

For prokaryotes, gene names should not begin with prefixes indicating the genus and species from which the gene is derived. (However, subscripts may be used where necessary to distinguish between genes from different organisms or strains as described in section v of "Bacteria," above.) For eukaryotes, such prefixes may be used for clarity when discussing genes with the same name from two different organisms (e.g., Sc*URA3* versus Ca*URA3*); the prefixes are not considered part of the gene name proper and are not italicized.

Locus tags. Locus tags are systematic, unique identifiers that are assigned to each gene in GenBank. All genes men-tioned in a manuscript should be traceable to their sequences by the reader, and locus tags may be used for this purpose in manuscripts to identify uncharacterized genes. In addition, au-thors should check GenBank to make sure that they are using the correct, up-to-date format for locus tags (e.g., uppercase versus lowercase letters and the presence or absence of an underscore, etc.). Locus tag formats vary between different organisms and also may be updated for a given organism, so it is important to check GenBank at the time of manuscript preparation.

"Mutant" versus "mutation." Keep in mind the distinc-tion between a mutation (an alteration of the primary se-quence of the genetic material) and a mutant (a strain carrying one or more mutations). One may speak about the mapping of a mutation, but one cannot map a mutant. Likewise, a mutant has no genetic locus, only a phenotype.

"Homology" versus "similarity." For use of terms that describe relationships between genes, consult the articles by Theissen (Nature **415:**741, 2002) and Fitch (Trends Genet **16:**227–231, 2000). "Homology" implies a relationship between genes that have a common evolutionary origin; partial homology is not recognized. When sequence comparisons are discussed, it is more appropriate to use the term "percent sequence similarity" or "percent sequence identity," as appropriate.

Strain designations. Do not use a genotype as a name (e.g., ". . . subsequent use of *leuC6* for transduction . . ."). If a strain designation has not been chosen, select an appropriate word combination (e.g., "another strain containing the *leuC6* muta-tion").

Viruses. The genetic nomenclature for viruses differs from that for bacteria. In most instances, viruses have no phenotype, since they have no metabolism outside host cells. Therefore, distinctions between phenotype and genotype cannot be made. Superscripts are used to indicate hybrid genomes. Genetic symbols may be one, two, or three letters. For example, a mu-tant strain of might be designated *A*am11 *int*2 *red*114 *c*I857; this strain carries mutations in genes *c*I, *int*, and *red* and an amber-suppressible (Am) mutation in gene *A*. A strain desig-nated att^{434} imm^{21} would represent a hybrid of phage that carries the immunity region (*imm*) of phage 21 and the attach-ment (*att*) region of phage 434. Host DNA insertions into vi-ruses should be delineated by square brackets, and the genetic symbols and designations for such inserted DNA should con-form to those used for the host genome. Genetic symbols for phage can be found in reports by Szybalski and Szybalski (Gene **7:**217–270, 1979) and Echols and Murialdo (Microbiol Rev **42:**577–591, 1978).

Eukaryotes. FlyBase (http://flybase.org/) is the genetic nomenclature authority for *Drosophila melanogaster*. WormBase (http://www.wormbase.org/#01-23-6) is the genetic nomenclature authority for *Caenorhabditis elegans*. When naming genes for *Aspergillus* species, the nomenclature guidelines posted at http://www.aspergillusgenome.org/Nomenclature.shtml should be followed, and the *Aspergillus* Genome Database (http://www.aspgd.org/) should be searched to ensure that any new name is not already in use. The *Saccharomyces* Genome Database (http://www.yeastgenome.org/) and the *Candida* Genome Database (http://www.candidagenome.org/) are authorities for *Saccharomyces cerevisiae* and *Candida albicans* genetic nomenclature, respec-tively. For information about the genetic nomenclature of other eukaryotes, see the Instructions to Authors for *Molecular and Cellular Biology.*

Transposable elements, plasmids, and restriction enzymes. Nomenclature of transposable elements (insertion sequences, transposons, and phage Mu, etc.) should follow the recommendations of Campbell et al. (Gene **5:**197–206, 1979), with the modifications given in section vi of "Bacteria," above. The Internet site where insertion sequences of eubacteria and archaea are described and new sequences can be recorded is https://www-is.biotoul.fr.

The system of designating transposon insertions at sites where there are no known loci, e.g., *zef-123*::Tn*5*, has been described by Chumley et al. (Genetics **91:**639 – 655, 1979). The nomenclature recommendations of Novick et al. (Bacteriol Rev **40:**168 –189, 1976) for plasmids and plasmid-specified activities, of Low (Bacteriol Rev **36:**587– 607, 1972) for F factors, and of Roberts et al. (Nucleic Acids Res **31:**1805–1812, 2003) for restriction enzymes, DNA methyltransferases, homing endonucleases, and their genes should be used whenever possi-ble. The nomenclature for recombinant DNA molecules, constructed *in vitro*, follows the nomenclature for insertions in general. DNA inserted into recombinant DNA molecules should be described by using the gene symbols and conven-tions for the organism from which the DNA was obtained.

Tetracycline resistance determinants. The nomenclature for tetracycline resistance determinants is based on the pro-posal of Levy et al. (Antimicrob Agents Chemother **43:**1523– 1524, 1999). The style for such determinants is, e.g., Tet B; the

space helps distinguish the determinant designation from that for phenotypes and proteins (TetB). The above-referenced ar-ticle also gives the correct format for genes, proteins, and de-terminants in this family.

ABBREVIATIONS AND CONVENTIONS

Verb Tense

ASM strongly recommends that for clarity you use the **past** tense to narrate particular events in the past, including the procedures, observations, and data of the study that you are reporting. Use the present tense for your own general conclusions, the conclusions of previous researchers, and generally accepted facts. Thus, most of the abstract, Materials and Methods, and Re-sults will be in the past tense, and most of the introduction and some of the Discussion will be in the present tense.

Be aware that it may be necessary to vary the tense in a single sentence. For example, it is correct to say "White (30) demonstrat*ed* that XYZ cells *grow* at pH 6.8," "Figure 2 shows that ABC cells fail*ed* to grow at room temperature," and "Air *was* removed from the chamber and the mice *died*, which *proves* that mice *require* air." In reporting statistics and calculations, it is correct to say "The values for the ABC cells *are* statistically significant, indicating that the drug inhibit*ed* . . . ."

For an in-depth discussion of tense in scientific writing, see *How To Write and Publish a Scientific Paper*, 7th ed.

Abbreviations

General. Abbreviations should be used as an aid to the reader, rather than as a convenience to the author, and therefore their **use should be limited**. Abbreviations other than those recommended by the IUPAC-IUB (*Biochemical Nomenclature and Related Documents*, 1992) should be used only when a case can be made for necessity, such as in tables and figures.

It is often possible to use pronouns or to paraphrase a long word after its first use (e.g., "the drug" or "the substrate"). Standard chemical symbols and trivial names or their symbols (folate, Ala, and Leu, etc.) may also be used.

Define each abbreviation and introduce it in parentheses the first time it is used; e.g., "cultures were grown in Eagle minimal essential medium (MEM)." Generally, eliminate abbreviations that are not used at least three times in the text (including tables and figure legends).

Not requiring introduction. In addition to abbreviations for Syste`me International d'Unite´s (SI) units of measurement, other common units (e.g., bp, kb, and Da), and chemical symbols for the elements, the following should be used without definition in the title, abstract, text, figure legends, and tables:

DNA (deoxyribonucleic acid)
cDNA (complementary DNA)
RNA (ribonucleic acid)
cRNA (complementary RNA)
RNase (ribonuclease)
DNase (deoxyribonuclease)
rRNA (ribosomal RNA)
mRNA (messenger RNA)
tRNA (transfer RNA)
AMP, ADP, ATP, dAMP, ddATP, and GTP, etc. (for the respective 5 phosphates of adenosine and other nucleosides) (add 2 -, 3 -, or 5 - when needed for contrast)
ATPase and dGTPase, etc. (adenosine triphosphatase and deoxyguanosine triphosphatase, etc.)
NAD (nicotinamide adenine dinucleotide)
NAD (nicotinamide adenine dinucleotide, oxidized)
NADH (nicotinamide adenine dinucleotide, reduced)
NADP (nicotinamide adenine dinucleotide phosphate)
NADPH (nicotinamide adenine dinucleotide phosphate, reduced)
NADP (nicotinamide adenine dinucleotide phosphate, oxidized)
poly(A) and poly(dT), etc. (polyadenylic acid and polydeoxythymidylic acid, etc.)
oligo(dT), etc. (oligodeoxy-thymidylic acid, etc.)
UV (ultraviolet)
PFU (plaque-forming units)
CFU (colony-forming units)
MIC (minimal inhibitory concentration)
Tris (tris[hydroxymethyl] aminomethane)
DEAE (diethylaminoethyl)
EDTA (ethylenediamine-tetraacetic acid)
EGTA (ethylene glycol-bis[-aminoethyl ether]-*N*,*N*,*N* ,*N* -tetraacetic acid)
HEPES (*N*-2-hydroxyethyl-piperazine-*N* -2-ethanesulfonic acid)
PCR (polymerase chain reaction)
AIDS (acquired immuno-deficiency syndrome)

Abbreviations for cell lines (e.g., HeLa) also need not be defined.

The following abbreviations should be used without definition in tables:

amt (amount)
approx (approximately)
avg (average)
concn (concentration)
diam (diameter)
expt (experiment)
exptl (experimental)
ht (height)
mo (month)
mol wt (molecular weight) no. (number)
prepn (preparation)
SD (standard deviation)
SE (standard error)
SEM (standard error of the mean) sp act (specific activity)
sp gr (specific gravity)
temp (temperature) vol (volume)
vs (versus)
wk (week)
wt (weight)
yr (year)

Drugs and pharmaceutical agents. Should an author decide to abbreviate the names of antimicrobial agents in a man-uscript, the following standard abbreviations are strongly rec-ommended.

(i) Antibacterial agents. Use the indicated abbreviations for the following antibacterial agents.

amikacin (AMK)
amoxicillin (AMX)
amoxicillin-clavulanic acid (AMC)
ampicillin (AMP)
ampicillin-sulbactam (SAM)
azithromycin (AZM)
azlocillin (AZL)
aztreonam (ATM)
carbenicillin (CAR)
cefaclor (CEC)
cefadroxil (CFR)
cefamandole (FAM)
cefazolin (CFZ)
cefdinir (CDR)
cefditoren (CDN)
cefepime (FEP)
cefetamet (FET)
cefixime (CFM)
cefmetazole (CMZ)
cefonicid (CID)
cefoperazone (CFP)
cefotaxime (CTX)
cefotetan (CTT)
cefoxitin (FOX)
cefpodoxime (CPD)
cefprozil (CPR)
ceftazidime (CAZ)
ceftibuten (CTB)
ceftizoxime (ZOX)
ceftriaxone (CRO)
cefuroxime (axetil or sodium) (CXM)
cephalexin (LEX)
cephalothin (CEF)
cephapirin (HAP)
cephradine (RAD)
chloramphenicol (CHL)
cinoxacin (CIN)
ciprofloxacin (CIP)

clarithromycin (CLR)
clinafloxacin (CLX)
clindamycin (CLI)
colistin (CST)
daptomycin (DAP)
dicloxacillin (DCX)
dirithromycin (DTM)
doxycycline (DOX)
enoxacin (ENX)
erythromycin (ERY)
fleroxacin (FLE)
fosfomycin (FOF)
gatifloxacin (GAT)
gentamicin (GEN)
grepafloxacin (GRX)
imipenem (IPM)
kanamycin (KAN)
levofloxacin (LVX)
linezolid (LZD)
lomefloxacin (LOM)
loracarbef (LOR)
meropenem (MEM)
methicillin (MET)
mezlocillin (MEZ)
minocycline (MIN)
moxalactam (MOX)
moxifloxacin (MXF)
nafcillin (NAF)
nalidixic acid (NAL)
netilmicin (NET)
nitrofurantoin (NIT)
norfloxacin (NOR)
ofloxacin (OFX)
oxacillin (OXA)
penicillin (PEN)
piperacillin (PIP)
piperacillin-tazobactam (TZP)
polymyxin B (PMB)
quinupristin-dalfopristin (Synercid) (Q-D)
rifabutin (RFB)
rifampin (RIF)
rifapentine (RFP)
sparfloxacin (SPX)
spectinomycin (SPT)
streptomycin (STR)
teicoplanin (TEC)
telithromycin (TEL)
tetracycline (TET)
ticarcillin (TIC)
ticarcillin-clavulanic acid (TIM)
tigecycline (TGC)
tobramycin (TOB)
trimethoprim (TMP)
trimethoprim-sulfamethoxazole (SXT)
trovafloxacin (TVA)
vancomycin (VAN)

(ii) -Lactamase inhibitors. Use the indicated abbrevia-tions for the following -lactamase inhibitors.

clavulanic acid (CLA) tazobactam (TZB) sulbactam (SUL)

(iii) Antifungal agents. Use the indicated abbreviations for the following antifungal agents.

amphotericin B (AMB)
clotrimazole (CLT)
flucytosine (5FC)
fluconazole (FLC)
itraconazole (ITC)
ketoconazole (KTC)
nystatin (NYT)
terbinafine (TRB)
voriconazole (VRC)

(iv) Antiviral agents. Use the indicated abbreviations for the following antiviral agents.

acyclovir (ACV)
cidofovir (CDV)
famciclovir (FCV)
foscarnet (FOS)
ganciclovir (GCV)
penciclovir (PCV)
valacyclovir (VCV)
zidovudine (AZT)

The use of "nonstandard" abbreviations to designate names of antibiotics and other pharmaceutical agents generally will not be accepted, because the use of different abbreviations for a single agent has often caused confusion. If, on occasion, a nonstandardized abbreviation for a drug or pharmaceutical substance is used, it will be accepted under the following con-ditions: (i) it must be defined at the first use in the text, (ii) it must be unambiguous in meaning, and (iii) it must contribute to ease of assimilation by readers.

Chemical or generic names of drugs should be used; the use of trade names is not permitted. Avoid the ambiguous term "generation" when classes of drugs are described. When code names or corporate proprietary numbers are to be used, either the chemical structure of the compound or a published litera-ture reference illustrating the chemical structure, if known, must be provided at the first occurrence of the code name or number. For compounds not identified by generic nomencla-ture, all previous or concurrent identification numbers or ap-pellations should be listed in the manuscript.

Pharmacodynamic terminology. Pharmacodynamic indi-ces (PDIs) must be introduced at their first occurrence in the text and follow guidelines set forth by Mouton et al. (J Anti-microb Chemother **55:**601– 607, 2005). In Materials and Methods, it should be clearly stated how the PDIs were derived. The most common indices used are the following: AUC/MIC ratio (the area under the concentration-time curve over 24 h in steady state divided by the MIC), AUIC (the area under the inhibitory curve; note that the AUC/MIC ratio is not equal to the AUIC), $\%T_{MIC}$ (the cumulative percentage of a 24-h period that the drug concentration exceeds the MIC un-der steady-state pharmacokinetic conditions), C_{max}/MIC ratio (the peak level divided by the MIC), PTA (probability of target attainment), and CFR (cumulative fraction of response). Clear distinction should be made between $\%T_{MIC}$, which is ex-pressed as a percentage of the dosing interval, and T_{MIC}, ex-pressed in hours. It is strongly recommended that the prefix *f* be used with an index (e.g., *f*AUC) if the free, unbound fraction of the drug is meant.

-Lactamases

Studies performed to characterize a -lactamase or the in-teraction of a compound with a -lactamase (i.e., as a sub-strate, inhibitor, or inducer) should follow the guidelines set forth by Bush and Sykes (Antimicrob Agents Chemother **30:** 6 –10, 1986). Assays that measure the hydrolysis of -lactam antibiotics must be appropriate for the substrate examined (e.g., iodometric methods are not appropriate quantitative as-says for substrates whose products are unknown). Reproduc-ibility of results must be shown. When referring to -lactama-ses, please use the functional designations defined by Bush and Jacoby (Antimicrob Agents Chemother **54:**969 –976, 2010). Alternatively, if the amino acid sequence for the enzyme is known, the -lactamases may be described by molecular class as initiated by Ambler (Philos Trans R Soc Lond B Biol Sci **289:**321–331, 1980).

A database of defining amino acid alterations for many -lactamases is maintained at the Internet address http://www.lahey.org/studies/. The managers of that site should be con-sulted about the name of a potentially novel -lactamase se-quence before a new designation or number is proposed for publication.

In Vitro Susceptibility Tests

Tabulate results of determinations of minimal inhibitory and bactericidal concentrations according to the range of con-centrations of each antimicrobial agent required to inhibit or kill the members of a species or of each group of microorgan-isms tested, as well as the corresponding concentrations re-quired to inhibit 50 and 90% of the strains (MIC_{50} and MIC_{90},

respectively) and those required to kill 50 and 90% of the strains (MBC_{50} and MBC_{90}, respectively). The MIC_{50} and MIC_{90} reported should be the actual concentrations tested that inhibited 50 and 90%, respectively, of the strains. They should not be values calculated from the actual data obtained. When only six to nine isolates of a species are tested, tabulate only the MIC range of each antimicrobial agent tested.

If more than a single drug is studied, insert a column labeled "Test agent" between the columns listing the organisms and the columns containing the numerical data and record data for each agent in the same isolate order. Cumulative displays of MICs or MBCs in tables or figures are acceptable only under unusual circumstances.

The percentage of strains susceptible and/or resistant to an antibiotic at its breakpoint concentration may be given only if an appropriate breakpoint has been approved, as by the Clin-ical and Laboratory Standards Institute (http://clsi.org/). In the absence of approved breakpoints, authors cannot assign breakpoints or use breakpoints from related antibiotics. An exploratory analysis tabulating the percentage of strains inhib-ited over a range of concentrations is acceptable.

Bactericidal tests must be performed with a sufficient in-oculum (5 10^5 CFU/ml) and subculture volume (0.01 ml) to ensure accurate determination of the 99.9% killing endpoint, as described by Pearson et al. (Antimicrob Agents Chemother **18:**699 –708, 1980) and Taylor et al. (Antimi-crob Agents Chemother **23:**142–150, 1983). Inoculum size and subculture volume are also critical to studies of combi-nations of antimicrobial agents.

Synergy is defined in two-dimensional or checkerboard tests when the fractional inhibitory concentration (FIC) or fractional bactericidal concentration (FBC) index ( ) is #0.5. In killing curves, synergy is defined as a $2-$\log_{10}$ decrease in CFU per milliliter between the combination and its most active constituent after 24 h, and the number of surviving organisms in the presence of the combination must be $2 $\log_{10}$ CFU/ml below the starting inoculum. At least one of the drugs must be present in a concentration which does not affect the growth curve of the test organism when used alone. Antagonism is defined by a FIC or FBC of 4.0.

When standard twofold-dilution schemes are used to deter-mine checkerboard interactions, the inherent variability of the method casts doubt on the significance of interactions repre-sented by FICs or FBCs of 0.5 but #4. Therefore, such inter-actions, if labeled at all, should be termed "indifferent." Alterna-tively, indices in this range may be described as "nonsynergistic" or "nonantagonistic," as appropriate. The technically imprecise term "additive" should be avoided, as it is too easily misun-derstood. See reports by W. R. Greco et al. (Pharmacol Rev **47:**331–385, 1995), F. C. Odds (J Antimicrob Chemother **52:**1, 2003), and M. D. Johnson et al. (Antimicrob Agents Chemother **48:**693–715, 2004) for further discussion of these issues.

For killing curve tests, the minimal, accurately countable number of CFU per milliliter must be stated and the method used for determining this number must be described. In the absence of any drug and with a sample size of 1 ml, this number is 30 (1.5 in $\log_{10}$) CFU. If procedures for drug inactivation or removal have not been performed, the author must state how drug carryover effects were eliminated or quantified. For drugs showing an inoculum effect, mere dilution below the MIC ob-tained in standard tests is not sufficient.

Clinical Trials

(i) **Registration.** AAC requires the prospective registration (i.e., before the first patient is enrolled) of a clinical trial in a public trials registry in accordance with guidelines established by the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html). The ICMJE defines a clinical trial as "any research project that prospectively assigns people or a group of peo-ple to an intervention, with or without concurrent compar-ison or control groups, to study the cause-and-effect rela-tionship between a health-related intervention *and* a health outcome."

AAC does not require registration in a particular registry, but the registry chosen must meet the following criteria, in agreement with ICMJE recommendations. It must be (a) accessible to the public free of charge, (b) open to all registrants, (c) managed by a not-for-profit organization, (d) monitored by a mechanism to ensure validity of registration data, and (e) searchable electroni-cally. A registration with missing fields or uninformative termi-nology will be deemed inadequate.

The registry and the trial registration number must be in-cluded at the end of the abstract. If a registration number is available, the authors should state this number the first time a trial acronym is used to refer to the trial being reported or to other trials mentioned in the manuscript.

(ii) Criteria for enrollment. The methods used to find and enroll patients and the criteria for enrollment in a clinical trial should be stated. In addition, the time period (month/year to month/year) of the enrollment should be specified. It should be indicated, if appropriate, that written informed consent was obtained and that the trial was approved by the pertinent com-mittee on human subjects.

(iii) Method of randomization. Randomized, double-blind studies are preferred. Comparisons using historical con-trols are usually regarded as questionable unless the differences in outcome between the groups are dramatic and almost cer-tainly the result of the new intervention. The rationale for the choice of the control group should be explained. The sample size should be justified, and the method of randomization should be stated.

(iv) Criteria for determining whether a case is evaluable. The minimum criteria for evaluability should be stated explic-itly. For example, it should be stated that the minimum crite-rion for evaluability was *a* or the combination of *b* and *c* rather than *a*, *b*, and *c* without designating which were the minimum criteria. The criteria for evaluability are usually different from those for enrollment.

(v) Reasons for nonevaluability. State the number of pa-tients in each group who were excluded from evaluation and the reason(s) for each exclusion.

(vi) Criteria for assessment. Define each outcome for each category of assessment (e.g., "clinical outcomes were classified as cure, improvement, and failure; microbiological outcomes were classified as eradication, persistence, and relapse"). The frequency and timing of such assessments in relation to treatment should be stated. Specify any changes made in the study regimen(s) during the trial; the results for regimens with and without such modifi-cation generally should be stated separately. The criteria (ques-tionnaires, results of specific laboratory tests) for evaluation of adverse effects should be stated, as should the period encom-passed in the assessment and the time of assessment in relation to the time of treatment (e.g., daily during treatment). Some authors prefer to consider superinfections as failures of treatment, whereas others prefer to consider them separately or even as ad-verse effects. In any event, the manuscript should state the num-ber of superinfections with each regimen and should differentiate between superinfections and colonization. The duration of fol-low-up should be mentioned.

(vii) Statistical analyses. The type of statistical test should be stated, and when appropriate, the reason for the choice of test should be given. References should be given for statistical procedures other than the *t* test, chi-square test, and Wilcoxon rank sum test. The comparability of the treatment groups at the baseline should be evaluated statistically.

For a review of some common errors associated with statistical analyses and reports, plus guidelines on how to avoid them, see the articles by Olsen (Infect Immun **71:**6689 – 6692, 2003; Infect Immun **82:**916 –920, 2014).

For a review of basic statistical considerations for virology experiments, see the article by Richardson and Overbaugh (J Virol **79:**669 – 676, 2005).

(viii) Beta error. For trials which show no statistically sig-nificant difference between regimens, calculate the probability ( ) of a type II error and the power of the study (1) to detect a specified clinically meaningful difference in efficacy between the regimens. For further details, see the article by Freiman et al. (N Engl J Med **299:**690 – 694, 1978). Alterna-tively, or in addition, indicate the magnitude of difference be-tween the regimens that could have been detected at a statisti-cally significant level with the number of evaluable patients studied.

For further details, see the editorial on guidelines for clinical trials (Antimicrob Agents Chemother **33:**1829 –1830, 1989).

Reporting Numerical Data

Standard metric units are used for reporting length, weight, and volume. For these units and for molarity, use the prefixes m, , n, and p for 10^{3}, 10^{6}, 10^{9}, and 10^{12}, respectively. Likewise, use the prefix k for 10^{3}. Avoid compound prefixes such as m or . Use g/ml or g/g in place of the ambiguous ppm. Units of temperature are presented as follows: 37°C or 324 K.

When fractions are used to express units such as enzymatic activities, it is preferable to use whole units, such as g or min, in the denominator instead of fractional or multiple units, such as g or 10 min. For example, "pmol/min" is preferable to "nmol/10 min," and " mol/g" is preferable to "nmol/ g." It is also preferable that an unambiguous form, such as exponential notation, be used; for example, " mol g^{1} min^{1}," is prefera-ble to " mol/g/min." Always report numerical data in the ap-propriate SI units.

Representation of data as accurate to more than two signif-icant figures must be justified by presentation of appropriate statistical analyses.

For a review of some common errors associated with statistical analyses and reports, plus guidelines on how to avoid them, see the articles by Olsen (Infect Immun **71:**6689 – 6692, 2003; Infect Immun **82**:916 –920, 2014).

For a review of basic statistical considerations for virology experiments, see the article by Richardson and Overbaugh (J Virol **79:**669 – 676, 2005).

Isotopically Labeled Compounds

For simple molecules, labeling is indicated in the chemical formula (e.g., $^{14}CO_2$, $^{3}H_2O$, and $H_2{}^{35}SO_4$). Brackets are not used when the isotopic symbol is attached to the name of a compound that in its natural state does not contain the element (e.g., ^{32}S-ATP) or to a word that is not a specific chemical name (e.g., ^{131}I-labeled protein, ^{14}C-amino acids, and ^{3}H-ligands).

For specific chemicals, the symbol for the isotope intro-duced is placed in square brackets directly preceding the part of the name that describes the labeled entity. Note that configu-ration symbols and modifiers precede the isotopic symbol. The following examples illustrate correct usage:

[^{14}C]urea	[-^{32}P]ATP
L-[*methyl*-^{14}C]methionine	UDP-[U-^{14}C]glucose
[2,3-^{3}H]serine	*E. coli* [^{32}P]DNA
[-^{14}C]lysine	fructose 1,6-[1-^{32}P]bisphosphate