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João Guilherme Neves Maia

Species distribution and in vitro antifungal susceptibility profile of yeast isolates from invasive infections on a Portuguese multicenter survey

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Species distribution and *in vitro* antifungal susceptibility profile of yeast isolates from invasive infections on a Portuguese multicenter survey.

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Running Head: Fungemia in Portugal

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ABSTRACT

Invasive yeast infections (IYI) represent a serious nosocomial problem. This is the first Portuguese multicenter prospective, observational and descriptive study that provides insights on species distribution and susceptibility profile of yeast isolates from fungemia episodes. All Portuguese hospitals were invited to integrate this study during 2012 (a twelve-month period). Ten district hospitals across mainland Portugal contributed by collecting yeast isolates from blood cultures and answering questionnaires concerning patients' data. The susceptibility profile of each isolate, considering eight of the most used antifungals, was determined. Two hundred and forty yeast isolates were recovered and identified. Incidence of fungemia was 0.88/1000 admissions. Fifteen different species were found, with *Candida albicans* as the most prevalent; however, 60% of fungemias were caused by non-*albicans* species. Most isolates were recovered from patients admitted at surgical wards or Intensive Care Units with 57% being males and 32% aged 41 to 60 years old. About 13% of yeast isolates were resistant to fluconazole, 18% to posaconazole and 16% to voriconazole. Regarding echinocandins, the highest percentage of resistance was to caspofungin (17%). Rare cases of strains displaying high MIC values to amphotericin B were also obtained (n=3). Death within 30 days associated with IYI occurred in 25% of the cases with more than half of *C. glabrata* infections being fatal. Studies addressing species distribution and susceptibility profile are extremely valuable as they provide important clues about worldwide IYI tendencies and may help improving empirical treatment guidelines.

INTRODUCTION

Increased incidence of invasive yeast infections (IYI) represents a clinical problem and its impact has significantly risen during the last twenty years (1). Fungemia is an important cause of morbidity and mortality, being related to longer hospital stays and very high economic costs. *Candida albicans* still remains as the leading cause of fungemia worldwide (2). *Candida parapsilosis, Candida glabrata* and *Candida tropicalis* occupy the following places, varying according to the region. Due to the medical relevance of IYI and its very strong association with an unfavorable outcome, epidemiological surveillance studies are urgently needed in order to evaluate species' geographic distribution and changes in susceptibility profiles. In addition, there is a limited number of therapeutic options, with the main class of drugs (azoles) controversially used for prophylaxis, possibly leading to decreased susceptibility and the selection of non-*albicans* species. In recent years we have witnessed several worldwide epidemiological and clinical changes regarding IYI (3-5). The aim of this study was to provide an overview about IYIs in Portugal and to evaluate the susceptibility profile of yeasts isolated from blood cultures.

MATERIALS AND METHODS

Study design Ten hospitals from northern (four), central (two) and southern (four) regions of Portugal accepted to participate in this study, providing isolates collected from patients with fungemia from September 2011 to September 2012. Two hospitals had more than 1000 beds, 3 had between 600 and 1000 beds and 5 had less than 600 beds. All participating hospitals were asked to collect and send the strains to

68 the Microbiology Department of the Faculty of Medicine of the University of Porto,
69 where the study was conducted. In addition, a questionnaire regarding patient's
70 clinical and demographic data was also sent.

71 **Definitions** An episode of fungemia was defined as the first isolation from a
72 blood culture of a yeast strain from a patient with related signs and symptoms.
73 Nosocomial fungemia was defined whenever the yeast isolate was obtained more than
74 48h after hospital admission. Ages were grouped into five categories: less than 20
75 years, 20-40 years, 41-60 years, 61-70 years and more than 70 years old. For each
76 individual patient, the outcome of the fungemia episode was evaluated at 30 days
77 after the first yeast isolation. Death associated with fungemia was defined as death
78 within 30 days after recovery of the first yeast isolate, without any other concomitant
79 cause of death, like intracerebral or gastrointestinal bleeding or pulmonary embolism.

80 **Identification and in vitro antifungal susceptibility testing of yeast isolates**
81 Yeasts were identified using Vitek 2 YST card from bioMérieux (Paris, France). The
82 characterization of *Candida glabrata* sensu stricto*, Candida bracarensis* and *Candida*
83 *nivariensis* was confirmed as previously described by Romeo *et al* (6). *Candida*
84 *parapsilosis* sensu stricto, *Candida orthopsilosis* and *Candida metapsilosis* isolates were
85 identified as previously described by Tavanti *et al* (7). Furthermore, the identities of
86 these species were confirmed by DNA amplification and further sequencing of *ITS1* and
87 *ITS4* regions of rRNA genes. Minimal inhibitory concentrations (MIC) were determined
88 by broth microdilution, following the CLSI guidelines (8). Antifungal powders were
89 obtained from the manufacturers - fluconazole (FLC), voriconazole (VRC) and
90 anidulafungin (AND) [from Pfizer, Groton, C.T., USA], posaconazole (POS) [Schering-

Plough Research Institute, Kenilworth, N.J., USA], caspofungin (CAS) [from Merck, Rahway, N.J., USA], micafungin (MCF) [from Astelas Pharma, UK], flucytosine (5FC) [Roche Laboratory Inc., Nutley, N.J., USA] and deoxycholate amphotericin B (AMB) [Bristol-Myers Squibb, N.Y., USA]. FLC, CAS and MCF were dissolved in water and the other drugs in DMSO. The new species-specific breakpoints for FLC, VRC and echinocandins were applied (8, 9). Isolates of *C. albicans*, *C. tropicalis* and *C. parapsilosis* for which FLC MICs were ≤2mg/L were categorized as susceptible and resistant at MICs >4 mg/L. *C. glabrata* was considered FLC susceptible-dose dependent (S-DD) at MICs ≤32 mg/L and resistant at MICs >32 mg/L. *C. albicans*, *C. tropicalis*, and *C. parapsilosis* were classified as susceptible to VRC at MICs ≤0.125 mg/L, and as resistant at ≥1 mg/L, and for *C. krusei*, susceptible when MICs ≤0.5 mg/L and resistant if >1 mg/L. Isolates of *C. albicans*, *C. tropicalis*, and *C. krusei*, for which AND, CAS and MCF MICs were ≤0.25 mg/L, were classified as susceptible and >0.5 mg/L as resistant. *C. glabrata* was categorized as susceptible when AND or CAS MICs were ≤0.12 mg/L and as resistant when >0.25 mg/L, while for MCF they were ≤0.06 mg/L (susceptible) and >0.12 mg/L (resistant). *C. parapsilosis* and *Candida guilliermondii* were classified as susceptible or resistant when MICs of the three echinocandins were ≤2 mg/L and >4 mg/L, respectively. For POS and 5FC the epidemiological cut-off values were applied to separate the wild type from non-wild type isolates: 0.06 mg/L for *C. albicans*, 0.12 mg/L for *C. tropicalis*, 0.25 mg/L for *Candida lusitaniae* and *C. parapsilosis*, 0.5 mg/L for *C. krusei* and 2 mg/L for *C. glabrata* for POS; and for 5FC it was 0.5 mg/L for *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. parapsilosis* and 32 mg/L for *C. krusei* (9-11). MICs were registered after 24 and 48 hours for *Candida* species and after 48 and 72 hours for *Cryptococcus neoformans*. Since breakpoints for AMB have not yet been established,

yeasts inhibited by ≤1 mg/L were considered to be susceptible according to suggestions by Pfaller *et al* (11). The quality control (QC) strains *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were included in all assays (8).

Statistical analysis A descriptive revision of the collected data was performed using the statistic program SPSS v21.0 (SPSS Software, Chicago, USA). The Chi-square test was used to compare proportions and analyze differences in the species distribution and in antifungal susceptibility profiles.

RESULTS

Patient's data A total of 240 fungemia episodes were reported in the current survey (corresponding to 240 different patients) and included in the study. The mean incidence of fungemia was 0.88 per 1000 admissions, ranging from 0.15 to 2.4. The mean incidence of nosocomial fungemia was 0.74 per 1000 admissions, ranging from 0.14 to 2.1, and corresponding to approximately 86% of all episodes of fungemia. Fifty-seven per cent of patients with fungemia were males and the most affected group was the one aged 41-60 years old (32%) closely followed by the group of patients over 70, together accounting for 62% of cases (Table1). Most patients enrolled in this study were admitted at ICU (39%) and surgical wards (30%). The crude mortality rate associated with fungemia was 25%. The deadliest species was *C. glabrata*, with more than 50% of patients dying within 30 days of fungemia detection. The other main *Candida* species did not differ much from the general value. (Table 1)

Species distribution and identification Fifteen different yeast species were identified, with the five most prevalent being *C. albicans* (40.4%), *C. parapsilosis* (22.9%), *C. glabrata* (13.3%), *C. tropicalis* (6.3%) and *C. krusei* (5%). *C. neoformans* was responsible for eight (3.3%) episodes of fungemia followed by *C. lusitaniae* (2.5%), *Candida guilliermondii* (1.7%), *Candida dubliniensis* (1.3%) and *Candida famata* (1.3%). Single cases due to *Candida sake*, *Candida inconspicua*, *Candida haemulloni*, *Candida kefyr* and *Trichosporon mucoides* were also found, as a whole accounting for 1.7% of all cases. Regarding the molecular identification of the cryptic species, from 55 isolates of *C. parapsilosis* sensu lato, 49 corresponded to *C. parapsilosis* sensu stricto, 4 to *C. orthopsilosis* (3 isolated from Medicine Department and 1 from UCI; 1 belonging to a 52 years old and the other 3 to patients over 70 years old) and 2 to *C. metapsilosis* (both from the Medicine Department; one from a 17 year old patient and the other from a 32 year-old patient). The 32 isolates primarily identified as belonging to the *C. glabrata* group corresponded to *C. glabrata* sensu stricto. There were no significant differences in the species distribution by gender or age (for a *p* value of 0.05).

Species susceptibility profiles Table 2 summarizes susceptibility testing results; endpoints shown were obtained after 24h of incubation and they differ no more than a single dilution from those obtained after 48h. Global antifungal resistance rates were calculated (considering the five main species, except for FLC, where *C. krusei* were excluded on the basis of intrinsic resistance; for the echinocandins the four isolates of *C. guilliermondii* were taken into account; for POS, epidemiological cutoff values were used). Among the group of azoles, the highest resistance rate was observed for POS (18.5%), followed by VRC (15.6%) and FLC (13.1%). Concerning the echinocandins, CAS was the drug with the most resistances (17.2% of isolates), surpassing AND (14.0%)

and MCF (11.6%). Resistance to 5FC was rare. In the matter of simultaneous resistance to antifungals of different classes (excluding strains with intrinsic resistances such as *C. krusei* for FLC and *C. neoformans* to echinocandins), we detected one isolate of *T. mucoides* (resistant to FLC, AND, CAS and MCF), one of *C. krusei* (resistant to AMB and CAS) and one of *C. parapsilosis* (resistant to FLC and CAS). Resistance to drugs within the same class was more frequent, with 8 strains of *C. albicans* and 1 of *C. tropicalis* displaying resistance to the three azoles and 6 strains of *C. parapsilosis* resistant to the three echinocandins (MIC ≥8mg/L). Regarding the *in vitro* activity of AMB, with the exception of three isolates (one each of *C. glabrata*, *C. krusei* and *C. kefyr*) which corresponded to a high MIC value (2 mg/L), all the other strains were susceptible with very low MIC values, ranging from 0.03 to 0.125 mg/L. Susceptibility testing results revealed *C. parapsilosis* as the most resistant species to echinocandins, with only 20% of the isolates susceptible to the 3 antifungals (resistance rate of 31% to MCF, 53% to AND and 51% to CAS). On the other hand, *C. albicans* displayed high susceptibility to these drugs but considerable resistance to azoles (18.6% to FLC, 18.6% to VRC and 16.5% to POS). Almost one third of *C. glabrata* exhibited a high MIC of VRC, with lower values to other azoles (6.25% for POS and FLC) and echinocandins (18.75% for MCF, 9.4% to CAS and 0% to AND). All *C. tropicalis* isolates were susceptible to echinocandins, but there were resistances to azole antifungals (60% to POS, 33% to VRC and 20% to FLC). *C. krusei* was susceptible to the azoles (apart from the expected resistance to FLC), AND and MCF. High MIC values to CAS were found in 5 isolates (41.6%). All the participating hospitals had Intensive Care Units (ICU) and Pediatric Departments and although very distinct geographic areas were covered by these

hospitals, no significant differences in species distribution and the antifungal susceptibility profile were found (for p=0.05).

DISCUSSION

Our study reports an incidence of fungemia of 0.88/1000 admissions (ranging from 0.15 to 2.4), figures comparable to recent data from other European countries (12, 13) albeit somewhat lower than in Brazil (14). A previous study in Portugal, based on a single university hospital, uncovered a similar, though slightly higher, incidence of 2.7/1000 admissions (15). Healthcare-associated fungemia remains the vast majority, representing more than 80% in most works (16, 17). Thus, it is not unsurprising that most of the isolates were recovered from ICU, surgery and internal medicine departments, given that most patients admitted to these wards are in critical health conditions and are submitted to invasive procedures, aggressive antibiotic and immunosuppressive drug regimens and placement of indwelling devices (central and peripheral venous catheters, urinary catheters and invasive ventilation procedures), all previously associated with increased rates of fungal infections (18-21). We report a crude 30-day mortality rate of 25%, with more than half (53%) of *C. glabrata* fungemias being lethal. Costa-de-Oliveira et al. reported, five years ago, even higher values, with a 78% mortality rate for *C. glabrata* fungemia and 46%, 30% and 53% for *C. albicans*, *C. parapsilosis* and *C. tropicalis*, respectively (15). The reasons behind this decrease can only be speculated. In other European studies crude mortality rates due to fungemia ranged from around 30% (12, 16) to 40%(17, 22), but *C. glabrata* did not carry a heavier death burden. *C. albicans* appears as the leading causative agent of fungemia in our study (40%) and in other European studies, accounting for an even

higher proportion of cases (12, 16, 22-24). However, the second position is not occupied by *C. parapsilosis* in most European countries; instead, *C. glabrata* appears repeatedly as the most common non-*albicans* species isolated from blood cultures (2), reaching almost a third of all *Candida* strains in some studies (17, 23). In fact, some authors place *C. parapsilosis* in the fourth (16) or even fifth position (23), behind *C. tropicalis* and *C. krusei*. The causes of this difference in incidences are unknown to us. Curiously, our data parallels those of Spanish and Brazilian works, where *C. parapsilosis* is responsible for 27 and 37% of cases of fungemia (13, 25). Taking into account the recent separation of the cryptic species in the *C. parapsilosis* and *C. glabrata* complexes into species in their own right, we strived to identify these in our studies. *C. orthopsilosis* and *C. metapsilosis* represented 7.3 and 3.6%, respectively, of the *C. parapsilosis* sensu lato isolates, while *C. nivariensis* and *C. bracarensis* were not identified, coinciding with results in other Mediterranean countries (13, 26, 27) and Brazil (25). In our work we applied the new CLSI M27-S4 breakpoints for antifungal susceptibilities (9), which may have altered the final resistance values for the five main species. Comparison with other studies is hindered by the fact that most prior works do not use these breakpoints and a large part of those that do does not present the susceptibility results as MIC distribution in the range of tested antifungal concentrations. Our work does show relatively high resistance rates to azoles and echinocandins, compared to other countries (28). However, it is important to highlight the small size of our sample and point out that more isolates (either through greater participation or by extending the period of the study) would strengthen our results. Mortality due to fungemia remains at unacceptably high values. Studies like the one we present (the first multicenter study carried out in Portugal) are of utmost

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233

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251

TRANSPARENCY DECLARATIONS

No conflicts to declare.

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Table 1 - Distribution of the isolated yeast strains

	No. of isolates (%)						
	C. albicans	*C. parapsilosis*	*C. glabrata*	*C. tropicalis*	*C. krusei*	Other species	Overall
Gender							
Male	59 (61)	26 (47)	21 (66)	11 (73)	4 (33)	16 (55)	137 (57)
Female	38 (39)	29 (53)	11 (34)	4 (27)	8 (67)	13 (45)	103 (43)
Total	97 (40)	55 (23)	32 (13)	15 (6)	12 (5)	29 (12)	240 (100)
Age group (years)							
<20	8 (8)	13(23)	1(3)	-	-	12 (42)	34 (14)
20-40	10 (10)	4(7)	3(9)	2(13)	2(17)	6 (21)	27 (11)
41-60	35 (35)	13(24)	9(29)	7(47)	6(50)	7 (24)	77 (32)
61-70	15 (16)	7(13)	3(9)	3(20)	1(8)	2 (7)	31 (13)
>70	29 (30)	18(32)	16(50)	3(20)	3(25)	2 (7)	71 (30)
Hospital Department							
ICU	42(43)	21(38)	12 (38)	4(27)	5(42)	10 (35)	94(39)
Surgery	22 (23)	18(33)	8(25)	8(53)	3(25)	11 (38)	70(30)
Medicine	28 (29)	13(24)	11(34)	3(20)	4(33)	6 (21)	65(27)
Pediatrics	5 (5)	3 (6)	1(3)	-	-	2 (7)	11 (5)
Outcome							
Death 30 days	23 (24)	12 (22)	17 (53)	3 (20)	-	4 (14)	59 (25)

380
381

382 Table 2 – Distribution of MIC values according to the species

Species (no.isolates)	Drug	R breakpoint or ECV	No. of isolates with MIC (mg/L)														No (%) of Resistance
			0,015	0,03	0,06	0,125	0,25	0,5	1	2	4	8	16	32	64	128	
C. albicans (97)	AND	>0.5	91	2	3						**1**		-	-	-	-	1 (1)
	CAS	>0.5		86	1	1	5	3	**1**				-	-	-	-	1 (1)
	MCF	>0.5	82	1	3	2	4	3		**1**		**1**	-	-	-	-	2 (2.1)
	FLC	>4	-	-	-	8	31	24	15	1		**17**			**1**		18 (18.6)
	VOR	>0.5	-	58	7	11	1	2		**2**	**12**	**4**		-	-	-	18 (18.6)
	POS	>0.06*	-	81		**1**						**4**	**11**	-	-	-	16 (16.5)
	AMB	>1	11	21	54	1	2	4	4				-	-	-	-	0
	5FC	>0.5*	-	-	-	61	18	10	**5**	**2**					**1**	-	8 (8.2)
C. parapsilosis (55)	AND	>4				1	1	4	1	6	13	**19**	**10**	-	-	-	29 (52.7)
	CAS	>4		1	2	1	1	3	12		7	**20**	**8**	-	-	-	28 (50.9)
	MCF	>4		4		8	2	4	9	3	8	**6**	**8**	**3**	-	-	17 (30.9)
	FLC	>4	-	-	-		16	27	4	2	3	**1**	**2**				3 (5.5)
	VOR	>0.5		17		23	8	7						-	-	-	0
	POS	>0.25*			31	12		**9**	**2**	**1**				-	-	-	12 (21.8)
	AMB	>1		42	8	4	1							-	-	-	0
	5FC	>0.5*	-	-	-	49	3	2	**1**								1 (1.8)
C. glabrata (32)	AND	>0.25		19	8	3	2						-	-	-	-	0
	CAS	>0.25		13	9	3	4	**2**			**1**		-	-	-	-	3 (9.4)
	MCF	>0.12		21		5		**2**	**3**		**1**		-	-	-	-	6 (18.75)
	FLC	>32	-	-	-			2	1	8	7	12			**2**		2 (6.25)
	VOR	>0.5*			8	3	9	2	**6**		**3**	**1**		-	-	-	10 (31.25)
	POS	>2*			4	5	5	3	9	4		**2**		-	-	-	2 (6.25)
	AMB	>1		4	9	7	6	3	2	**1**							1 (3.1)
	5FC	>0.5*	-	-	-	22	2	5	**3**								3 (9.4)
C.tropicalis (15)	AND	>0.5		7	3	4	1						-	-	-	-	0
	CAS	>0.5		6	3	2	4						-	-	-	-	0
	MCF	>0.5		3		5	3	4					-	-	-	-	0
	FLC	>4	-	-	-	5	3	3			1	**2**			**1**		3 (20)
	VOR	>0.5		1	3	1	5		**3**		**2**			-	-	-	5 (33.3)
	POS	>0.12*		2	1	3	**4**	**1**	**1**		**2**	**1**		-	-	-	9 (60)

	AMB	>1		12	3												0
	5FC	>0.5*				6	4	3			**1**	**1**					2 (13.3)
C. krusei (12)	AND	>0.5		8		2	2						-	-	-	-	0
	CAS	>0.5				3	2	2	**3**	**1**	**1**		-	-	-	-	5 (41.6)
	MCF	>0.5				3	6	3					-	-	-	-	0
	FLC	>64*											**2**	**7**	**3**		-
	VOR	>1		6	5	1								-	-	-	0
	POS	>0.5*		3	1	1	7							-	-	-	0
	AMB	>1		9	1		1			**1**							1 (8.3)
	5FC	>32*									2	3	6	1			0
Other *spp*. (29)	AND	>2	5	4	1	12	1	1	3	1	**1**						1
	CAS	>2	6	1	4	3	1	10	1	2	**1**						1
	MCF	>2	4	2	5	2	3	2	4	4	**2**	**1**					3
	FLC	>64		5	1	4	3	6	5		1		3		**1**		1
	VOR	>4		15	2	3	6	1	2								0
	POS	>4		17	5	3	2	1			1			-			0
	AMB	>1		4	2	7	7	7	1	**1**							1
	5FC	>32			5	8	5	6	2	2				**1**			1
Overall (240)	AND	NA	96	40	15	22	7	5	4	7	15	19	10				
	CAS	NA	6	107	19	13	17	20	17	3	10	20	8				
	MCF	NA	86	31	8	25	18	18	16	8	11	8	8	**3**			
	FLC	NA		5	1	17	53	62	25	11	12	32	7	**7**	8		
	VOR	NA		97	25	42	29	12	11	2	17	5					
	POS	NA		103	42	25	18	14	12	5	3	7	11				
	AMB	NA	11	92	77	19	17	14	7	3							
	5FC	NA			5	146	32	26	11	4	3	4	6	**2**	1		

383

*ECV value

NA = not applicable; Resistant strains are **boldface**

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ao Departamento de Microbiologia da Faculdade de Medicina da Universidade do Porto.

Normas da Revista

Journal of Clinical Microbiology

JCM
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January 2014, Instructions to Authors, pages 1–21

2014 INSTRUCTIONS TO AUTHORS

SCOPE

The *Journal of Clinical Microbiology* (JCM) is devoted to the dissemination of new knowledge concerning the laboratory diagnosis of human and animal infections. In addition, JCM is an appropriate forum for the publication of information related to the role of the laboratory in both the management of infectious diseases and the elucidation of the epidemiology of infections. Manuscripts which present the results of original scientific investigations are encouraged. The three principal attributes that we require of papers published in JCM are timeliness, relevance to the practice of clinical microbiology, and quality science. Manuscripts that present information that is largely only of relevance to a restricted geographic area are discouraged.

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To protect the privacy of individuals mentioned in clinical studies, in case histories, and as sources of isolates, do not identify them by their initials, even as part of a strain designa-

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Newly determined nucleotide and/or amino acid sequence data must be deposited and GenBank/ENA/DDBJ accession numbers must be included in the manuscript no later than the modification stage of the review process. It is expected that the sequence data will be released to the public no later than the publication (online posting) date of the accepted manuscript. The accession numbers should be included in a separate paragraph at the end of the Materials and Methods section for full-length papers or at the end of the text for Short-Form papers. If conclusions in a manuscript are based on the analysis of sequences and a GenBank/ENA/DDBJ accession number is not provided at the time of the review, authors should provide the annotated sequence data as supplemental material for information only.

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See "Presentation of Nucleic Acid Sequences" for nucleic acid sequence formatting instructions.

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Provide the names of all the authors and/or editors for each reference; names should not be abbreviated with "et al." Since title and byline information that is downloaded from PubMed does not always show accents, italics, or special characters, authors should refer to the PDF files or hard-copy versions of the articles and incorporate the necessary corrections in the submitted manuscript. Abbreviate journal names according to the PubMed Journals Database (National Library of Medicine, National Institutes of Health; available at http://www.ncbi.nlm.nih.gov/nlmcatalog/journals), the primary source for ASM style.

Follow the styles shown in the examples below for print references.

1. **Caserta E, Haemig HAH, Manias DA, Tomsic J, Grundy FJ, Henkin TM, Dunny GM.** 2012. *In vivo* and *in vitro* analyses of regulation of the pheromone-responsive *prgQ* promoter by the PrgX pheromone receptor protein. J. Bacteriol. **194:**3386–3394.
2. **Falagas ME, Kasiakou SK.** 2006. Use of international units when dosing colistin will help decrease confusion related to various formulations of the drug around the world. Antimicrob. Agents Chemother. **50:**2274–2275. (Letter.) {*"Letter" or "Letter to the editor" is allowed but not required at the end of such an entry.*}
3. **Cox CS, Brown BR, Smith JC.** J. Gen. Genet., in press.* {*Article title is optional; journal title is mandatory.*}
4. **da Costa MS, Nobre MF, Rainey FA.** 2001. Genus I. Thermus Brock and Freeze 1969, 295,[AL] emend. Nobre, Trüper and da Costa 1996b, 605, p 404–414. *In* Boone DR, Castenholz RW, Garrity GM (ed), Bergey's manual of systematic bacteriology, 2nd ed, vol 1. Springer, New York, NY.
5. **Stratagene.** 2006. Yeast DNA isolation system: instruction manual. Stratagene, La Jolla, CA. {*Use the company name as the author if none is provided for a company publication.*}
6. **Forman MS, Valsamakis A.** 2011. Specimen collection, transport, and processing: virology, p 1276–1288. *In* Versalovic J, Carroll KC, Jorgensen JH, Funke G, Landry ML, Warnock DW (ed), Manual of clinical microbiology, 10th ed, vol 2. ASM Press, Washington, DC.
7. **Fitzgerald G, Shaw D.** *In* Waters AE (ed), Clinical microbiology, in press. EFH Publishing Co, Boston, MA.* {*Chapter title is optional.*}
8. **García CO, Paira S, Burgos R, Molina J, Molina JF, Calvo C, Vega L, Jara LJ, García-Kutzbach A, Cuellar ML, Espinoza LR.** 1996. Detection of *Salmonella* DNA in synovial membrane and synovial fluid from Latin American patients using the polymerase chain reaction. Arthritis Rheum. **39**(Suppl 9)**:**S185. {*Meeting abstract published in journal supplement.*}
9. **Carlson E.** 2013. Selective penicillin-binding protein imaging probes reveal substructure in bacterial cell division, p 59. Final Program 113th Gen. Meet. Am. Soc. Microbiol. American Society for Microbiology, Washington, DC. {*Abstract title is optional.*}
10. **Rotimi VO, Salako NO, Mohaddas EM, Philip LP.** 2005. Abstr. 45th Intersci. Conf. Antimicrob. Agents Chemother., abstr D-1658. {*Abstract title is optional.*}
11. **Green PN, Hood D, Dow CS.** 1984. Taxonomic status of some methylotrophic bacteria, p 251–254. *In* Crawford RL, Hanson RS (ed), Microbial growth on C_1 compounds. Proceedings of the 4th International Symposium. American Society for Microbiology, Washington, DC.
12. **O'Malley DR.** 1998. Ph.D. thesis. University of California, Los Angeles, CA. {*Title is optional.*}
13. **Odell JC.** April 1970. Process for batch culturing. US patent 484,363,770. {*Include the name of the patented item/process if possible; the patent number is mandatory.*}
14. **Elder BL, Sharp SE.** 2003. Cumitech 39, Competency assessment in the clinical laboratory. Coordinating ed, Sharp SE. ASM Press, Washington, DC.

*A reference to an in-press ASM publication should state the control number (e.g., JCM00123-14) if it is a journal article or the name of the publication if it is a book.

Online-only references must provide essentially the same information that print references do. For online journal articles, posting or revision dates may replace the year of publication; a DOI (preferred) or URL is required for articles with nontraditional page numbers or electronic article identifiers.

1. **Bina XR, Taylor DL, Vikram A, Ante VM, Bina JE.** 2013. *Vibrio cholerae* ToxR downregulates virulence factor production in response to cyclo(Phe-Pro). mBio **4**(5)**:**e00366-13. doi:10.1128/mBio.00366-13.
2. **Winnick S, Lucas DO, Hartman AL, Toll D.** 2005. How do you improve compliance? Pediatrics **115:**e718–e724. doi:10.1542/peds.2004-1133.
3. **Dionne MS, Schneider DS.** 2002. Screening the fruitfly immune system. Genome Biol. **3:**reviews1010-reviews1010.2. doi:10.1186/gb-2002-3-4-reviews1010.
4. **Gregory ST.** 2 September 2009. Chapter 2.5.4, Structural basis for the decoding mechanism. *In* Böck A, et al (ed), *EcoSal—Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, DC. doi:10.1128/ecosal.2.5.4. {*Note that each chapter has its own posting date.*}

Note: a posting or accession date is required for any online reference that is periodically updated or changed.

Citations of ASM Accepts manuscripts should look like the following example.

> **Wang GG, Pasillas MP, Kamps MP.** 15 May 2006. Persistent transactivation by Meis1 replaces Hox function in myeloid leukemogenesis models: evidence for co-occupancy of Meis1-Pbx and Hox-Pbx complexes on promoters of leukemia-associated genes. Mol. Cell. Biol. doi:10.1128/MCB.00586-06.

Other journals may use different styles for their publish-ahead-of-print manuscripts, but citation entries must include the following information: author name(s), posting date, title, journal title, and volume and page numbers and/or DOI. The following is an example:

Zhou FX, Merianos HJ, Brunger AT, Engelman DM. 13 February 2001. Polar residues drive association of polyleucine transmembrane helices. Proc. Natl. Acad. Sci. U. S. A. doi:10.1073/pnas.041593698.

(ii) References cited in the text. References that should be cited in the text include

- Unpublished data
- Manuscripts submitted for publication
- Unpublished conference presentations (e.g., a report or poster that has not appeared in published conference proceedings)
- Personal communications
- Patent applications and patents pending
- Computer software, databases, and websites

These references should be made parenthetically in the text as follows:

. . . similar results (R. B. Layton and C. C. Weathers, unpublished data).
. . . system was used (J. L. McInerney, A. F. Holden, and P. N. Brighton, submitted for publication).
. . . as described previously (M. G. Gordon and F. L. Rattner, presented at the Fourth Symposium on Food Microbiology, Overton, IL, 13 to 15 June 1989). {*For nonpublished abstracts and posters, etc.*}
. . . this new process (V. R. Smoll, 20 June 1999, Australian Patent Office). {*For non-U.S. patent applications, give the date of publication of the application.*}
. . . available in the GenBank database (http://www.ncbi.nlm.nih.gov/Genbank/index.html).
. . . using ABC software (version 2.2; Department of Microbiology, State University [http://www.state.micro.edu]).

URLs for companies that produce any of the products mentioned in your study or for products being sold may not be included in the article. However, company URLs that permit access to scientific data related to the study or to shareware used in the study are permitted.

(iii) Citations in abstracts. Because the abstract must be able to stand apart from the article, references cited in it should be clear without recourse to the References section. Use an abbreviated form of citation, omitting the article title, as follows.

(P. S. Satheshkumar, A. S. Weisberg, and B. Moss, J. Virol. 87:10700–10709, 2013, doi:10.1128/JVI.01258-13)
(J. H. Coggin, Jr., p. 93–114, *in* D. O. Fleming and D. L. Hunt, ed., *Biological Safety. Principles and Practices*, 4th ed., 2006)
". . . in a recent report by D. A. Hopwood [mBio 4(5): e00612-13, 2013, doi:10.1128/mBio00612-13] . . . ."

This style should also be used for Addenda in Proof.

(iv) References related to supplemental material. If references must be cited in the supplemental material, list them in a **separate** References section within the supplemental material and cite them by those numbers; do not simply include citations of numbers from the reference list of the associated article. If the same reference(s) is to be cited in both the article itself and the supplemental material, then that reference would be listed in both References sections.

Short-Form Papers

The Short-Form format is intended for the presentation of brief observations that do not warrant full-length papers. However, Short-Form papers should contain firm data; observations alone are not acceptable. Submit Short-Form papers in the same way as full-length papers. They receive the same review, they are not published more rapidly than full-length papers, and they are not considered preliminary communications.

The title, running title (not to exceed 54 characters and spaces), byline, and correspondent footnote should be prepared as for a full-length paper. Each Short-Form paper must have an abstract of no more than 50 words. Do not use section headings in the body of the Short Form; combine methods, results, and discussion in a single section. Paragraph lead-ins are permissible. The text should be kept to a minimum and if possible should not exceed 1,000 words; the number of figures and tables should also be kept to a minimum. Materials and methods should be described in the text, not in figure legends or table footnotes. Present acknowledgments as in full-length papers. The References section is identical to that of full-length papers.

Minireviews

Minireviews are expected to be focused discussions of defined topics relevant to clinical microbiologists. In general, they are to be submitted only following invitation by the editor in chief of JCM. Unsolicited Minireviews are discouraged. A topical outline should be provided to the editor in chief for approval prior to submission of the completed Minireview manuscript in the eJP online manuscript submission and peer review system.

Minireviews are not expected to be comprehensive reviews of the literature but rather focused discussions of specific topics. A standard title page should be provided. This is followed by an abstract of 100 words or less and then the text of the Minireview, which should not exceed 12 double-spaced manuscript pages in length, exclusive of tables, figures, photographs, and references. Up to three tables, figures, or photographs, total, may be included. References should be limited to no more than 30. Minireviews will be reviewed by two JCM editors, with the aim of expedited processing. In general, it is hoped that, barring the necessity of major revisions, accepted Minireviews will appear in print within 3 months of their submission and online ahead of print 6 to 8 weeks earlier.

Author bio. A short biographical sketch and photograph of the **one** author most responsible for the minireview should be submitted along with the initial version of the manuscript. These will be published at the end of the article.

- The text limit is 150 words and should include WHO you are (your name), WHERE you received your education, WHAT positions you have held and at WHICH institutions, WHERE you are now (your current institution), WHY you have this interest, and HOW LONG you have been in this area, as well as a brief review of your scholarly interests and record of publication. In addition, please list pertinent significant awards you have received.
- The photo should be a recent black-and-white head shot of passport size. It will be reduced to approximately 1.125 inches wide by 1.375 inches high. The photo must meet the production criteria for regular figures and should be checked for production quality by using Rapid Inspector, provided at the following URL: http://rapidinspector.cadmus.com/RapidInspector/zmw/index.jsp.
- To submit, upload the text and photo with your manuscript in the submission and review system. Include the biographical text immediately **after** the References section of your manuscript, in the same file. It should be labeled with the heading "Biosketch." Upload the head shot photograph in the submission system as a "Minireview Bio Photo"; **include the author's name or enough of it for identification in the photo's file name.**

Contact the scientific editor if you have questions about what to write. Contact the production editor if you have questions about submitting your files.

Commentaries

Commentaries are invited communications concerning topics relevant to the readership of JCM and are intended to engender discussion. Reviews of the literature, methods and other how-to papers, and responses targeted at a specific published paper are not appropriate. Commentaries are subject to review.

The length may not exceed four printed pages, and the format is like that of a Minireview (see above) except that the abstract is limited to 75 words.

Point-Counterpoint

Point-Counterpoint is a feature of JCM in which two experts present opposing views on a contemporary issue in the laboratory diagnosis of infectious diseases. This feature will be the lead article in the issue of JCM in which it appears. Participation as an author of a Point-Counterpoint feature is by invitation only.

A JCM editor will write a brief introductory piece of approximately 200 words outlining why a specific issue is important and then present the issue in the form of a question. The two experts will then each write a commentary, no more than 1,000 words in length, in which they present evidence in support of either the pro or con view. One table or one figure may be included. Since these discussions will be evidence based, authors may also cite up to 10 references. Unpublished or in-press data which reflect the current practice in their laboratory may be used but should not be the sole basis for their position.

Authors should send commentaries directly back to the JCM editor within 30 days of receipt of the introductory statement. Following receipt of both the pro and con commentaries, the editor will review the submissions and may return them to the author(s) with comments and/or suggested revisions. If revisions are required, the author(s) will have 14 days to craft a revised commentary, which will be sent directly back to the editor. Upon receipt of final commentaries, the JCM editor will write a brief summary consisting of no more than six one-sentence bullet points, outlining where the experts agree (no more than three points) and disagree (no more than three points). The JCM editor will then upload the introduction, both commentaries, and the summary in eJP.

Case Reports

While a full-length article or a Short-Form paper may contain a case report section when the report is incidental to the rest of the paper, a specific Case Report format must be used when the report constitutes the entire article.

A Case Report must include an abstract of no more than 50 words. The text starts with presentation of the case under the section heading "Case Report"; there is no introductory text before the Case Report heading. After the case is presented, the rest of the text follows in a separate section after a ruled line to separate the sections. No separate head is used for this short discussion section, but paragraph lead-ins are permitted. The total number of tables and figures (combined) must not exceed 3. For an example of a correctly formatted Case Report, see J. Clin. Microbiol. **39:**1678–1679, 2001.

Photo Quiz

A Photo Quiz submission should present the findings of some relevant, interesting, and new observation pertinent to the practice of clinical microbiology in which a photograph is particularly useful in conveying important information **and** where the observation can serve as the basis for both a question and an answer. The photograph may be of a micrograph, some other laboratory material, a clinical lesion, or the results of an imaging study.

A Photo Quiz consists of two parts: (i) a case presentation featuring a photograph depicting some unusual and/or informative finding in clinical microbiology and (ii) an answer to the quiz. The case presentation and the answer must be submitted as two separate articles. Note that authors and affiliations are listed below the title.

Photo Quiz case presentation. The text in the Photo Quiz case presentation should be limited to 200 to 300 words. The header for the case presentation should read "Photo Quiz."

Please include a photograph about 39 picas (6.5 inches) wide and 28 picas (4.625 inches) high. Since photos appearing with published Photo Quizzes appear on the cover of the journal, a high-resolution TIFF or EPS file is preferred. A short legend for the photo must be provided, and the photo must be cited in the case presentation. Refer to a recently published Photo Quiz for correct formatting.

Answer to Photo Quiz. The text of the answer to the Photo Quiz should also be limited to 200 to 300 words. The header to the answer should read "Answer to Photo Quiz." Four to six references may be cited at the end of the Photo Quiz answer.

Submission. The Photo Quiz case presentation should be submitted in the "Photo Quiz" manuscript category. The Photo Quiz answer should be submitted in the "Photo Quiz Answer" manuscript category.

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 publish-ahead-of-print manuscripts) 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 papers 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.

Fast-Track Communications

The Fast-Track route is intended for accelerated review of short communications that are of significant interest to clinical microbiologists. Manuscripts are limited to 750 words, one figure, one table, and 10 or fewer references. The format should be the same as that of a New-Data Letter (see "Letters to the Editor," above). Fast-Track articles should be submitted via the eJP online manuscript submission and peer review system.

A Fast-Track submission is subject to approval as such by the editor in chief. If approved for the Fast-Track route, the manuscript will be assigned to an appropriate JCM editor and reviewed, according to the same standards applied for traditional manuscripts, within 1 week. If accepted, the manuscript will be scheduled for the next available issue and edited. An acceptance letter and copyright agreement will be mailed to the corresponding author. Proofs will be made available electronically as for regular articles.

A Fast-Track submission that is not approved for the Fast-Track route will be handled as a New-Data Letter according to normal procedures.

Errata

The Erratum section provides a means of correcting errors that occurred during the writing, typing, editing, or publication (e.g., a misspelling, a dropped word or line, or mislabeling in a figure) of a published article. Submit Errata via the eJP online manuscript 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

The Author Correction section provides 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 conclusions 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 agreement must be supplied as supplemental material for information only (scanned PDF files).

Retractions

Retractions are reserved for major errors or breaches of ethics 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 Publication 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 agreement signed by all of the authors must be supplied as supplemental material for information only (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.

ILLUSTRATIONS AND TABLES

Illustrations

Image manipulation. Digital images submitted for publication may be inspected by ASM production specialists for any manipulations or electronic enhancements that may be considered 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. Investigation 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 necessary 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 enhancement, 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 compiling 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, but the cost of printing in color must be borne by the author. Suggestions about how to reduce costs and 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 figure to assist review. At the modification stage, production-quality digital files must be provided. The legends will be copy-edited and typeset for final publication 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 (preferred) or CMYK color mode. See "Color illustrations." Halftone images (those with various densities or shades) must be grayscale, not bitmap. JCM accepts TIFF or EPS files but discourages PowerPoint for either black-and-white or color images.

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 converted 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 composition system requirements, you must verify that your PowerPoint files can be converted to PDF without any errors.

We strongly recommend that before returning their modified 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 render the image unusable for production.

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

Minimum resolution. It is extremely important that a high enough 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 resolutions 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;** that is, the image uploaded should be 100% of its print dimensions so that no reduction or enlargement is necessary. 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, illustrations 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 number well outside the boundaries of the image itself. (Numbering 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 uploading 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, where a nonproportional (monospace) font is required. All fonts other than these must be converted to paths (or outlines) in the application with which they were created. For proper font use in PowerPoint images, refer to the Cadmus digital art website, http://art.cadmus.com/da/instructions/ppt_disclaimer.jsp.

Color illustrations. Color costs must be borne by the author. See "Publication Fees." All figures submitted in color will be processed as color. Adherence to the following guidelines will help to minimize costs and to ensure color reproduction that is as accurate as possible.

The final online version is considered the version of record for JCM and all other ASM journals. To maximize online reproduction, 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 scientific data, and it can display a wider range of colors (especially bright fluorescent hues) than the CMYK (cyan, magenta, yellow, black) color space used by print devices that put ink (or toner) on paper. For the print version (and reprints), ASM's print provider will automatically create CMYK versions of color illustrations from the supplied RGB versions. Color in the print journal 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 mathematical formulas, diagrams, and other drawings as finished products not requiring additional artwork or typesetting. All elements, including letters, numbers, and symbols, must be easily readable, and both axes of a graph must be labeled. Keep in mind that the journal is published both in print and online and that the same electronic files submitted by the authors are used to produce both.

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 without moiré patterns. Various pattern backgrounds are preferable 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 areas.

(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 appropriate Système International d'Unités (SI) symbols (μ 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, 2011); an abbreviated list is available at http://old.iupac.org/reports/1993/homann/index.html.

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 of 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 sequence 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 reported in a legend only if the discussion 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.

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 currently an acceptable format. Excel files must be either embedded 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.

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.

NOMENCLATURE

Chemical and Biochemical Nomenclature

The recognized authority for the names of chemical compounds is *Chemical Abstracts* (CAS; http://www.cas.org/) and its indexes. *The Merck Index*, 15th ed. (RSC Books, Cambridge, UK, 2013), is also an excellent source. For biochemical terminology, including abbreviations and symbols, consult *Biochemical Nomenclature 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* and the *Archives of Biochemistry and Biophysics*.

Do not express molecular weight in daltons; molecular weight is a unitless ratio. Molecular mass is expressed in daltons.

For enzymes, use the recommended (trivial) name assigned by the Nomenclature Committee of the International Union of Biochemistry (IUB) as described in *Enzyme Nomenclature* (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 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/projekte/strenda/guidelines/).

For nomenclature of restriction enzymes, DNA methyltransferases, homing endonucleases, and their genes, refer to the article by Roberts et al. (Nucleic Acids Res. **31:**1805–1812, 2003).

Drugs

Whenever possible, use generic names of drugs; the use of trade names is not permitted.

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 taxa (kingdoms, phyla, classes, orders, families, genera, species, and subspecies) are printed in italics and should be italicized in the manuscript; strain designations and numbers are not. Vernacular (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.pasteur.fr/ip/portal/action/WebdriveActionEvent/oid/01s-000036-089). 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.

For guidelines regarding new names and descriptions of new genera and species, see the articles by Tindall (Int. J. Syst. Bacteriol. **49:**1309–1312, 1999) and Stackebrandt et al. (Int. J. Syst. Evol. Microbiol. **52:**1043–1047, 2002). To validate new names and/or combinations, authors must submit three copies of their published article to the *International Journal of Systematic and Evolutionary Microbiology*.

It is recommended that a strain be deposited in at least two recognized culture collections in different countries when that strain is necessary for the description of a new taxon (Int. J. Syst. Evol. Microbiol. **50:**2239–2244, 2000).

Since the classification of fungi is not complete, it is the responsibility of the author to determine the accepted binomial 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 generally advisable to include a worker's initials or a descriptive symbol 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 italicized genotypic and phenotypic 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. Use the recommendations of Demerec et al. (Genetics **54:**61–64, 1966) as a guide to the use of these terms. If your manuscript contains information including genetic nomenclature, please refer to the Instructions to Authors of the *Journal of Bacteriology*.

"Mutant" versus "mutation." Keep in mind the distinction between a mutation (an alteration of the primary sequence 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.

Tetracycline resistance determinants. The nomenclature for tetracycline resistance determinants is based on the proposal 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 article also gives the correct format for genes, proteins, and determinants in this family.

Locus tags. Locus tags are systematic, unique identifiers that are assigned to each gene in GenBank. All genes mentioned 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, authors 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.

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.

Eukaryotes. FlyBase (http://flybase.org/) is the genetic nomenclature authority for *Drosophila melanogaster*. WormBase (http://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.aspergillus.org.uk/indexhome.htm?secure/sequence_info/nomenclature.htm 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, respectively. For more information about the genetic nomenclature of eukaryotes, see the Instructions to Authors for *Eukaryotic Cell* and *Molecular and Cellular Biology*.

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 Results 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 show*s* 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 for 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 Système International d'Unités (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. (oligodeoxythymidylic acid, etc.)
UV (ultraviolet)
PFU (plaque-forming units)
CFU (colony-forming units)
MIC (minimal inhibitory concentration)
Tris (tris[hydroxymethyl] aminomethane)
DEAE (diethylaminoethyl)
EDTA (ethylenediaminetetraacetic acid)
EGTA (ethylene glycol-bis[β-aminoethyl ether]-*N*,*N*,*N*′,*N*′-tetraacetic acid)
HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)
PCR (polymerase chain reaction)
AIDS (acquired immunodeficiency 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)
tr (trace)
vol (volume)
vs (versus)
wk (week)
wt (weight)
yr (year)

Drugs. Should an author decide to abbreviate the names of antimicrobial agents in a manuscript, the following standard abbreviations are strongly recommended.

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) and cefuroxime (sodium) (CXM)
cephalexin (LEX)
cephalothin (CEF)
cephapirin (HAP)
cephradine (RAD)
chloramphenicol (CHL)
cinoxacin (CIN)
ciprofloxacin (CIP)
clarithromycin (CLR)
clinafloxacin (CLX)
clindamycin (CLI)
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)
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)
tobramycin (TOB)
trimethoprim (TMP)
trimethoprim-sulfamethoxazole (SXT)
trovafloxacin (TVA)
vancomycin (VAN)

β-Lactamase inhibitors. Use the indicated abbreviations for the following β-lactamase inhibitors.

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

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)

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)

Reporting Numerical Data

Standard metric units are used for reporting length, weight, and volume. For these units and for molarity, use the prefixes m, μ, 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 mμ or μμ. Use μg/ml or μg/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 μg or 10 min. For example, "pmol/min" is preferable to "nmol/10 min," and "μmol/g" is preferable to "nmol/μg." It is also preferable that an unambiguous form, such as exponential notation, be used; for example, "$\mu mol\ g^{-1}\ min^{-1}$" is preferable to "μmol/g/min." Always report numerical data in the appropriate SI units.

Representation of data as accurate to more than two significant 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 article by Olsen (Infect. Immun. **71:**6689–6692, 2003).

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

Statistics

Statistical analysis of data is a crucial component of scientific publication. Authors who are unsure of proper statistical analysis should have their manuscripts checked by a qualified statistician.

The following is a list of important items that must be considered before manuscript submission. Deficiencies in any of these areas may delay review and/or publication.

(i) Statistical analyses were performed on all quantitative data regardless of how significant the differences look in the tables or figures.

(ii) Data were appropriately analyzed as parametric (normally distributed) or nonparametric data.

(iii) Parametric and nonparametric data are presented appropriately. Means and standard deviations or standard errors are appropriate means of presenting data analyzed by parametric analyses (i.e., *t* test and analysis of variance [ANOVA]), but only medians and surrounding levels (quartiles, quintiles, and 10th and 90th percentiles, etc.) are appropriate for nonparametric statistics (Mann-Whitney test and Kruskal-Wallis test, etc.). Means have no meaning in nonparametric analyses.

(iv) For any data in which there are more than two comparisons (i.e., between one control and more than one experimental group), an analysis must be done for multigroup comparisons. Such an analysis would usually be an ANOVA for parametric data or a Kruskal-Wallis test for nonparametric data. *t* tests cannot be used when more than two groups are being compared (except as indicated below). Failure to use multigroup tests generates type 1 errors: concluding that two data sets within the overall data set being compared are different when in fact they are not. Exception: some statisticians argue that two-group comparisons can be used on multigroup data if the expected outcomes are appropriately anticipated before the experiment. For example, data generated by individually testing two unrelated factors for their effects on a target with only a single, untreated target as a control could be appropriately analyzed by *t* tests instead of ANOVA.

(v) For all appropriate multigroup comparisons, two *P* values must be generated and provided in the manuscript. The main *P* value applies to the overall data set and indicates that within that data set at least two groups differ from each other. The overall *P* value does not indicate which two groups are different. The main *P* value and the overall *P* value should be computed by using a *post hoc* test. For ANOVA, these *post hoc* tests are usually Dunnett's test (used to compare multiple experimental groups to a single control), the Fisher protected least significant difference (PLSD) test, the Tukey-Kramer test, and the Games-Howell test. Others may be used. Note that each *post hoc* test has certain underlying assumptions that may not be applicable to the data under analysis. For a Kruskal-Wallis nonparametric ANOVA, the Dunn procedure is appropriate to generate *P* values for two-group comparisons.

(vi) Data presented as endpoints (i.e., LD_{50} and ID_{50}, etc.) contain both the calculated value and a confidence interval with a statistical significance associated with it (95%, 99%, or similar confidence interval), calculated by logit or probit analysis. Simple LD_{50} values, such as Reed-Muench calculations, may not be used alone.

(vii) When samples are taken multiple times from one experimental entity (i.e., multiple serum samples from one animal, gross pathology scores measured for the same animal over time or growth curves, etc.), one cannot use analyses such as *t* tests, ANOVA, or the Mann-Whitney test, etc., because these tests assume that each measure is independent. An entity with a high score on day 1 is more likely to have a high score on day 2 than is an entity with a low score. It is likely that some expert statistical help will be needed for these situations, usually involving regression analysis or survival analysis, etc.

(viii) Statistical significance and biological significance are not the same. There is nothing magical about a *P* value of 0.05. When results from large sample sizes are compared, a *P* value of <0.05 will often be obtained, as *P* value is a function of both sample size and effect size. If sample sizes are large, then more-rigorous (i.e., smaller) *P* values may be desirable. If sample sizes are small, *P* values of >0.05 may still be important. There should be both statistical and biological significance to the results and conclusions in the manuscript.

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

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 introduced is placed in square brackets directly preceding the part of the name that describes the labeled entity. Note that configuration symbols and modifiers precede the isotopic symbol. The following examples illustrate correct usage:

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