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# **In vitro activity of XF-73, a novel antibacterial agent, against antibiotic-sensitive and -resistant Gram-positive and Gram-negative bacterial species**

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IA 52317, USA.

## ABSTRACT

The antibacterial activity of XF-73, a dicationic porphyrin drug, was investigated against a range of Gram-positive and Gram-negative bacteria with known antibiotic resistance profiles, including resistance to cell wall synthesis, protein synthesis, and DNA and RNA synthesis inhibitors as well as cell membrane-active antibiotics. Antibiotic-sensitive strains for each of the bacterial species tested were also included for comparison purposes. XF-73 was active [minimum inhibitory concentration (MIC) 0.25–4 mg/L] against all of the Gram-positive bacteria tested, irrespective of the antibiotic resistance profile of the isolates, suggesting that the mechanism of action of XF-73 is unique compared with all other antibiotic classes tested. Gram-negative activity was lower (MIC 1 mg/L to >64 mg/L). Minimum bactericidal concentration data confirmed that the activity of XF-73 was bactericidal. Time–kill kinetics against healthcare-associated and community-associated methicillin-resistant *Staphylococcus aureus* isolates demonstrated that XF-73 was rapidly bactericidal, with >5 log<sub>10</sub> kill obtained after 15 min at 2× MIC, the earliest time point sampled. The post antibiotic effect (PAE) for XF-73 under conditions where the PAE for vancomycin was <0.4 h was found to be >5.4 h. XF-73 represents a novel broad-spectrum Gram-positive antibacterial drug with potentially beneficial characteristics for the treatment and prevention of Gram-positive bacterial infections.

## 1. Introduction

The impact of antibiotic-resistant bacteria on the treatment of infections continues its relentless march upwards. Meticillin-resistant *Staphylococcus aureus* (MRSA) is the prime example; MRSA is increasingly prevalent throughout the world and is endemic in some hospital and community settings [1–3]. Healthcare-associated MRSA (HA-MRSA) now accounts for 40% of *S. aureus* infections in the UK and 63% of *S. aureus* infections in the USA [1,2].

Community-associated MRSA (CA-MRSA) is also becoming more widespread; in the USA, 59% of skin and soft-tissue infections (SSTIs) presenting to emergency departments are caused by CA-MRSA [3]. The most prevalent CA-MRSA clone in the USA, USA300, which carries the staphylococcal cassette chromosome *mec* (SCC*mec*) type IVa and Panton–Valentine leukocidin genes, now accounts for 61–87% of MRSA isolates from SSTIs in North American emergency departments [4,5] and is also emerging as a serious cause of healthcare-associated infections [6–8].

A further complication is the emergence of multidrug-resistant (MDR) bacteria both in Gram-positive and Gram-negative species. There is a critical need for new antibacterials, particularly those with activity against MDR bacteria.

XF-73 is a dicationic porphyrin drug and previous studies have shown that XF-73 has potent bactericidal activity [minimum bactericidal concentration for 90% of organisms (MBC<sub>90</sub>) = 0.5 mg/L] in vitro against a range of clinically

important *S. aureus* isolates, including methicillin-sensitive *S. aureus* (MSSA), HA-MRSA and CA-MRSA [9]. The drug is being developed to treat *S. aureus* and MRSA infections, and clinical trials are underway to assess the potential of XF-73 for nasal decolonisation of *S. aureus*. Investigations into the mechanism of action of XF-73 against *S. aureus* have demonstrated that the drug exhibits a rapid cell membrane-perturbing activity that is likely to be responsible for inhibition of macromolecular synthesis and death of staphylococci exposed to the drug [10]. During prolonged laboratory subculture (55 passages) in the presence of sub-minimum inhibitory concentrations (sub-MICs) of XF-73, no resistant mutants were generated using five Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) [11] strains of MRSA [9,12].

The purpose of this study was (i) to examine the breadth of in vitro activity of XF-73, using broth microdilution, against a range of antibiotic-sensitive and -resistant Gram-positive and Gram-negative aerobic and anaerobic bacterial species and (ii) to investigate the effect of existing antibiotic resistance mechanisms on the activity of XF-73. Strains in the screening panel were chosen to include examples of bacteria with all known antibacterial resistance mechanisms. Comparator antibiotics were included to confirm the expected MICs for reference strains and to confirm that the MICs for clinical isolates were within the expected ranges, confirming the relevance of selection of these clinical isolates. Time–kill kinetics using *S. aureus* were also investigated to determine the speed of the bactericidal activity of XF-73.

Determination of the post-antibiotic effect (PAE) for *S. aureus* of XF-73 for a

range of different multiples of the MIC was also undertaken and compared with a comparator antibiotic.

## 2. Materials and methods

### 2.1. Bacterial strains

A panel of 101 Gram-positive and Gram-negative organisms, including anaerobes, was selected for susceptibility and cross-resistance testing (Table 1; Supplementary Table 1). The panel comprised 65 Gram-positive isolates (56 aerobic and 9 anaerobic) and 36 Gram-negative isolates (31 aerobic and 5 anaerobic). Examples of isolates resistant to cell wall inhibitors ( $\beta$ -lactams, glycopeptides and cephalosporins), protein synthesis inhibitors (oxazolidinones, macrolides and mupirocin), DNA synthesis inhibitors (fluoroquinolones), RNA synthesis inhibitors (rifampicin) and bacterial cell membrane-active antibiotics (daptomycin) were included in the panel.

The Gram-positive panel (Table 1; Supplementary Table 1) included: MRSA (control and clinical isolates, including a linezolid-resistant isolate); MSSA; vancomycin-intermediate-resistant *S. aureus* (VISA); high-level mupirocin-resistant (MIC > 256 mg/L) and daptomycin-non-susceptible (MIC = 2 mg/L) clones of *S. aureus*; antibiotic-susceptible and meticillin-resistant *Staphylococcus epidermidis*; antibiotic-susceptible and penicillin- and/or macrolide-resistant *Streptococcus* spp.; vancomycin-resistant (*vanA*, *vanB*



and *vanC*) *Enterococcus* spp.; and toxin-positive and ceftriaxone-resistant strains of *Clostridium difficile*.

The Gram-negative panel (Table 1; Supplementary Table 1) included MDR strains of *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Clinical and Laboratory Standards Institute (CLSI) antimicrobial susceptibility control strains were included in the screen to confirm that the expected MICs were obtained for the comparator compounds (vancomycin, linezolid and clindamycin) and, where possible, an antibiotic-sensitive and an antibiotic-resistant isolate was included for all species tested.

For investigation of killing kinetics and determination of the PAE, two NARSA [11] strains were used, comprising one CA-MRSA (NRS-123) and one HA-MRSA (NRS-382).

## 2.2. Antimicrobial agents

XF-73 was obtained from Destiny Pharma Ltd. (Brighton, UK). Vancomycin (batch number 016K1102) and fusidic acid (batch number 065K1404) were obtained from Sigma-Aldrich Ltd. (Gillingham, UK), and mupirocin (batch number B143832) was obtained from Mast Diagnostics (Bootle, UK).

### 2.3. Susceptibility testing

MICs were determined by the broth microdilution method according to approved M7-A7 (aerobes) and M11-A7 (anaerobes) standards of the CLSI [13,14]. MIC values were read after 24 h or 48 h of incubation, depending on the species.

MBC values were determined by subculturing wells showing no visible growth onto pre-labelled and quartered Columbia blood agar plates. Plates were incubated appropriate to the species and the number of surviving bacterial colonies was counted. MBC values were determined for each isolate as the antimicrobial concentration producing a 3 log<sub>10</sub> drop (99.9%) in bacterial colony-forming units (CFU)/mL compared with the original inoculum, based on a 5% error for determination of final inoculum [15].

### 2.4. Media used

Mueller–Hinton (MH) broth (Oxoid Ltd., Basingstoke, UK) was used to prepare the inoculum and according to CLSI requirements [13,14]. For fastidious isolates, MH broth was supplemented with 2.5–5% v/v lysed horse blood. For *Haemophilus influenzae*, plates and inocula were prepared in *Haemophilus* test medium (Trek Diagnostic Systems Ltd., East Grinstead, UK). For anaerobic isolates, supplemented *Brucella* broth was prepared according to CLSI recommendations [13,14].

### 2.5. Determination of bactericidal activity by time–kill

XF-73, vancomycin, fusidic acid and mupirocin were added at final concentrations of 2×, 4× and 8× MIC to exponentially dividing cultures of the test strains. A higher final bacterial concentration of ca.  $10^6$  CFU/mL was utilised to allow an ca. 5 log<sub>10</sub> reduction to be measured. Cultures were incubated at  $37 \pm 1$  °C in a shaking water-bath and samples were removed for determination of surviving bacteria using the spiral plating system (Don Whitley Scientific, Shipley, UK) on Iso-Sensitest agar after 0, 0.25, 0.5, 1, 3, 6 and 8 h of exposure. To remove the antimicrobials prior to performing viable counts, each time point sample was spun and washed twice with sterile Iso-Sensitest broth. A growth control was run under the same conditions. Count plates were incubated for 48 h and the kinetics of bactericidal activity was determined by plotting the concentration of surviving bacteria against time. The absolute limit of detection was  $2 \times 10^1$  CFU/mL.

### 2.6. Determination of the post-antibiotic effect

The PAE is the duration of bacterial growth suppression following abrupt removal of the test antibacterial from the culture medium [16–18]. This duration is related to the antibacterial concentration and exposure time prior to its removal [16] and is also specific for the antibacterial–bacterium system [16,18].

XF-73 was added at a final concentration of 0.5×, 1× and 2× MIC and vancomycin at a final concentration of 2× MIC to exponentially dividing

cultures of the test strains in Iso-Sensitest broth (final concentration ca.  $10^6$  CFU/mL). Following exposure for 30 min, each culture was spun and washed twice with sterile Iso-Sensitest broth and the re-suspended culture was then incubated in a shaking water-bath at  $37 \pm 1$  °C until re-growth was visible with the naked eye or for a period of 8 h. The control culture was diluted 1:100 and 1:1000 to account for antibacterial activity that may occur in the exposed cultures in order to provide a comparable starting inoculum on re-incubation for PAE calculation. The three controls were also spun and washed before sampling all of the cultures, at time point 0. Thereafter, both control and 'exposed' cultures were counted at hourly intervals using the spiral plating method.

Count plates were incubated for 48 h and the PAE was determined by plotting the concentration of surviving bacteria against time. The PAE was calculated as the time taken for the test culture to increase by  $1.0 \log_{10}$  CFU/mL minus the time taken for the control culture to increase by  $1.0 \log_{10}$  CFU/mL.

### 3. Results

#### 3.1. XF-73 spectrum of activity

##### 3.1.1. Gram-positive activity

XF-73 was active (MIC range 0.25–4 mg/L) (Table 1; Supplementary Table 1) against all of the aerobic and anaerobic Gram-positive organisms tested in this study. The MBC for all isolates was achieved at, or for some species

within, 1–2 dilutions of the MIC (Supplementary Table 1), demonstrating that the antibacterial activity was bactericidal. MICs of the comparator antibiotics were as expected for the CLSI antimicrobial susceptibility control strains (Table 1; Supplementary Table 1).

### 3.1.2. *Gram-negative activity*

MICs for XF-73 against the Gram-negative species tested ranged from 1 mg/L to >64 mg/L (Table 1; Supplementary Table 1). MICs of the comparator antibiotics were as expected for the CLSI antimicrobial susceptibility control strains (Table 1; Supplementary Table 1).

### 3.1.3. *Activity of XF-73 against antibiotic-susceptible and antibiotic-resistant isolates*

The antimicrobial activity of XF-73 was independent of the antibiotic resistance status for the Gram-positive bacteria tested. In every case for XF-73, the MICs for antibiotic-resistant strains were similar or identical to those observed for the antibiotic-susceptible strains. XF-73 was active against all 12 of the MRSA clones tested, with MICs in the range 0.5 mg/L to 1 mg/L, the same as that for the 3 methicillin-sensitive isolates tested. The presence of resistance genes against cell wall synthesis inhibitors, protein synthesis inhibitors, RNA and DNA synthesis inhibitors and cell membrane-active antibiotics in the MRSA strains screened also had no effect on the MICs (Table 1; Supplementary Table 1). The range of MICs for XF-73 against penicillin-resistant or macrolide-resistant *Streptococcus* spp. was 0.25–4 mg/L

and for susceptible isolates was 0.5–2 mg/L. XF-73 demonstrated a similar order of activity against vancomycin-resistant enterococci, where the MIC range was 0.5–2 mg/L, compared with 1–2 mg/L for susceptible isolates.

### 3.2. Determination of bactericidal activity by time–kill

The in vitro bactericidal activity of XF-73, vancomycin, fusidic acid and mupirocin at 2×, 4× and 8× MIC against *S. aureus* NRS-382, an HA-MRSA strain, and NRS-123, a CA-MRSA strain, was investigated and the results for 2× MIC are shown in Fig. 1. The results demonstrated a rapid bactericidal activity, with a  $>5 \log_{10}$  reduction obtained even at 2× MIC after the first sampling point (15 min). This compares with a reduction of  $<1 \log_{10}$  for the three comparators. There was an increase in CFUs between 6 h and 8 h for XF-73 at 2× and 4× MIC, but this was due to the higher initial inoculum used for this study. At 8× MIC, no increase in CFUs was observed between 6 h and 8 h (data not shown).

### 3.3. Post-antibiotic effect

Owing to the rapid bactericidal activity of XF-73, the PAE was determined at 0.5×, 1× and 2× MIC with a relatively short exposure time of 30 min, which is one-half the usual 1 h exposure time (Fig. 2). To enable comparison using these conditions, the PAE of vancomycin at 2× MIC was also determined. For NRS-382 at 0.5×, 1× and 2× MIC, a PAE of 3.5, 5 and 5.4 h, respectively, was observed. By comparison, the PAE of vancomycin at 2× MIC was significantly

shorter at 0.4 h (Fig. 2a). For NRS-123, a CA-MRSA strain, the PAE for XF-73 was longer at all three multiples tested (Fig. 2b), being recorded at 5.4, 6.1 and 6.3 h at 0.5 $\times$ , 1 $\times$  and 2 $\times$  MIC, respectively. Again, by comparison, the PAE of vancomycin at 2 $\times$  MIC was 0.3 h.

#### 4. Discussion

Despite the increasing threat of antibiotic drug resistance, very few new antibacterials are emerging and even fewer with novel mechanisms of action. For instance, in the 5-year period between 2002 and 2007, only five new antibiotics were approved by the US Food and Drug Administration (FDA) [19]. Thus, a novel bactericidal agent with potent activity against antibiotic-resistant strains is an important advance.

XF-73 demonstrated excellent bactericidal activity against all Gram-positive strains tested regardless of species, phenotype or genotype, with MICs of 0.25–4 mg/L. The antibacterial activity against Gram-negative species was lower, with MICs of 1 mg/L to >64 mg/L.

The difference in susceptibility to some antimicrobial agents between Gram-positive and Gram-negative bacteria has been attributed to the low permeability of the Gram-negative bacterial outer cell wall to antibacterial compounds [20]. The bactericidal activity of XF-73 appears to involve accumulation of the molecule at, and perturbation of, the cytoplasmic membrane barrier, causing rapid cell death within 5 min [10]. The less

permeable outer cell wall of Gram-negative bacteria may impede accumulation at the cytoplasmic membrane, resulting in a lower killing efficacy. Further studies are underway to characterise fully the antibacterial mechanism of action of XF-73.

MICs of  $\leq 2$  mg/L for XF-73 were, however, obtained for the Gram-negative anaerobes *Porphyromonas asaccharolytica* and *Prevotella intermedia* as well as the Gram-negative aerobe *Neisseria meningitidis*. Moreover, the XF-73 MICs against a  $\beta$ -lactamase-positive clinical isolate and a reduced fluoroquinolone susceptibility clinical isolate of *Moraxella catarrhalis* were also  $\leq 4$  mg/L, demonstrating that XF-73 did have some activity against certain Gram-negative bacteria.

The strains in this screening panel were chosen to include examples of bacteria with all known antibacterial resistance mechanisms. For the *S. aureus* strains tested, the panel included MSSA, MRSA (SCC*mec* types 1–4), VISA and high-level mupirocin-resistant (MIC > 256 mg/L) and daptomycin-non-susceptible (MIC = 2 mg/L) isolates. In all cases, MICs and MBCs for XF-73 were within 1 dilution multiple of one another. Results for the daptomycin-non-susceptible isolate are particularly interesting, as daptomycin, like XF-73 [10] is postulated to act primarily by disruption of the cytoplasmic membrane [21]. The results for XF-73 suggest that the mechanism of action of XF-73 is distinct from that of daptomycin.



The bactericidal activity of XF-73 was not affected by the presence of VanA-, VanB- or VanC-derived vancomycin resistance in the enterococci isolates tested, suggesting that the mechanism of action of XF-73 does not involve inhibition of peptidoglycan synthesis. Similarly, resistance to penicillins, cephalosporins and carbapenems in other Gram-positive isolates tested did not affect the MICs obtained, suggesting that the mechanism of action of XF-73 does not involve inhibition of cell wall synthesis.

Macrolide-resistant isolates of *Streptococcus* (Group C, Group G, *S. agalactiae*, *S. bovis*, *S. constellatus*, *S. mitis*, *S. oralis*, *S. pyogenes* and *S. sanguinis*) all had MIC/MBCs identical to antibiotic-susceptible isolates of the same strains, suggesting that the mechanism of action of XF-73 does not involve inhibition of bacterial protein biosynthesis. This is supported by similar MICs for a high-level gentamicin-resistant isolate of *Enterococcus faecalis* and a CLSI antimicrobial susceptibility control strain. Identical MICs were also obtained for doxycycline-sensitive and -resistant isolates of *Corynebacterium jeikeium* and *S. aureus* as well as tetracycline-sensitive and -resistant isolates of *E. faecalis* and *Streptococcus pneumoniae*, further suggesting that inhibition of protein synthesis is not the mechanism of action of XF-73.

A number of *S. aureus* isolates, an isolate of *S. epidermidis* and *Enterococcus faecium* were resistant to levofloxacin. The MICs obtained for these isolates were identical to the MICs obtained for corresponding levofloxacin-sensitive isolates, suggesting that the mechanism of action of XF-73 does not involve inhibition of DNA supercoiling.

The bacterial time–kill experiments demonstrated that XF-73 exerted a rapid and significant bacterial kill, with a  $>5 \log_{10}$  reduction in viable cell numbers after only 15 min incubation time (Fig. 1), the earliest sampling time point, based on conventional experimental protocols. Bacterial cell numbers were reduced to or below the limit of detection of the assay by this first sampling point. Thus, the actual speed of bacterial kill cannot be stated. Further work needs to be undertaken to obtain data points at even earlier time points to determine what the reduction in viable cell numbers is to obtain a more accurate measure of the rate of bacterial kill.

A long PAE was obtained for XF-73 against both a CA-MRSA strain (5.4, 6.1 and 6.3 h at 0.5 $\times$ , 1 $\times$  and 2 $\times$  MIC, respectively) and an HA-MRSA strain (3.5, 5 and 5.4 h at 0.5 $\times$ , 1 $\times$  and 2 $\times$  MIC, respectively) using an exposure time that was half the standard exposure time (Fig. 2). XF-73 was found to have a PAE 21 times longer than vancomycin for NRS-123 (0.3 h at 2 $\times$  MIC) and 14 times longer for NRS-382 (0.4 h at 2 $\times$  MIC) when tested under the same conditions. A long PAE is beneficial as it provides additional time for the immune system to remove bacteria that might have survived antibiotic treatment before they could eventually re-grow after removal of the drug. A longer PAE can therefore influence the clinical outcome of antimicrobial therapy.

The data presented here, along with previous studies on its antibacterial activity (including remote resistance profile [9,12] and recently confirmed

bacterial biofilm activity [22]), suggest that XF-73 is potentially of value for the prevention and treatment of antibiotic-resistant bacterial infections. Clinical trials are now underway to assess the potential of XF-73 for nasal decolonisation of *S. aureus*.

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### **Competing interests**

DJF is a member of the scientific advisory board of Destiny Pharma Ltd. WR-W and WGL are employees of Destiny Pharma Ltd.

### **Ethical approval**

Not required

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**Fig. 1.** In vitro bactericidal activity of XF-73 and comparators at 2× MIC against (a) *Staphylococcus aureus* NRS-382 (HA-MRSA) and (b) *S. aureus* NRS-123 (CA-MRSA): (a) MIC (MBC) values (mg/L): XF-73 = 0.25 (0.5); vancomycin = 2 (4); fusidic acid = 0.12 (8); and mupirocin = 0.25 (4); and (b) MIC (MBC) values (mg/L): XF-73 = 0.5 (0.5); vancomycin = 1 (2); fusidic acid = 0.12 (4); mupirocin = 0.25 (16). MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; HA-MRSA, healthcare-associated methicillin-resistant *S. aureus*; CA-MRSA, community-associated methicillin-resistant *S. aureus*; CFU, colony-forming units.

**Fig. 2.** Post-antibiotic effect (PAE) of XF-73 at 0.5×, 1× and 2× MIC and vancomycin at 2× MIC for (a) *Staphylococcus aureus* NRS-382 (HA-MRSA) and (b) *S. aureus* NRS-123 (CA-MRSA). Control 1, control culture for XF-73 experiment; Control 2, control culture for vancomycin experiment. MIC, minimum inhibitory concentration; HA-MRSA, healthcare-associated methicillin-resistant *S. aureus*; CA-MRSA, community-associated methicillin-resistant *S. aureus*; CFU, colony-forming units.



**Table 1**

In vitro activity of XF-73 and comparator antibiotics against Gram-positive and Gram-negative aerobes and anaerobes

Species (no. of isolates)	MIC (mg/L)			
	XF-73	Vancomycin	Linezolid	Clindamycin
<i>Corynebacterium jeikeium</i> (2) <sup>a</sup>	0.25	0.5–1	0.25–1	>64
<i>Enterococcus faecalis</i> (5) <sup>b</sup>	0.5–2	0.5 to >64	1–2	16 to >64
<i>Enterococcus faecium</i> (3) <sup>c</sup>	1	1 to >64	1–4	>64
<i>Enterococcus gallinarum</i> (1) <sup>d</sup>	1	8	2	>64
<i>Listeria monocytogenes</i> (1) <sup>e</sup>	0.5	0.5	2	1
Meticillin-sensitive	0.5	0.5	2–4	0.12
<i>Staphylococcus aureus</i> (2) <sup>f</sup>				
Meticillin-resistant <i>S. aureus</i> (10) <sup>g</sup>	0.5–1	0.5–2	1–64	0.12 to >64
Vancomycin-intermediate-resistant <i>S. aureus</i> (1) <sup>h</sup>	1	4	2	>64
Daptomycin-non-susceptible <i>S. aureus</i> (1) <sup>i</sup>	1	NT	2	NT
High-level mupirocin-resistant <i>S. aureus</i> (1) <sup>j</sup>	1	NT	2	NT
<i>Staphylococcus epidermidis</i> (3) <sup>k</sup>	0.25–1	0.5	0.5–1	0.12–0.25
<i>Staphylococcus haemolyticus</i> (1) <sup>e</sup>	0.5	4	4	0.12
<i>Staphylococcus saprophyticus</i> (1) <sup>e</sup>	0.5	0.5	2	0.12
Group C <i>Streptococcus</i> (2) <sup>l</sup>	0.5–2	0.5	1	<0.03–0.06
Group G <i>Streptococcus</i> (2) <sup>l</sup>	0.25–0.5	0.5	1	0.06

<i>Streptococcus agalactiae</i> (2) l	1	0.5	1	<0.03–0.06
<i>Streptococcus bovis</i> (2) <sup>l</sup>	0.5	0.5	1–2	0.12–32
<i>Streptococcus constellatus</i> (2) <sup>l</sup>	0.5	0.5–1	1	<0.03–64
<i>Streptococcus mitis</i> (2) <sup>l</sup>	1	1	2	<0.06–64
<i>Streptococcus oralis</i> (2) <sup>l</sup>	2–4	1	1	<0.03–64
<i>Streptococcus pneumoniae</i> (5) <sup>m</sup>	1–2	0.25–0.5	1–2	<0.06 to >64
<i>Streptococcus pyogenes</i> (3) n	0.5	0.25–0.5	1–2	<0.03–0.12
<i>Streptococcus sanguinis</i> (2) <sup>k</sup>	0.5–1	0.5–1	1	0.06 to >64
<i>Clostridium difficile</i> (2) <sup>o</sup>	1	0.5	0.25	4
<i>Clostridium perfringens</i> (2) <sup>p</sup>	2	0.5	1	0.06–0.25
<i>Eggerthella lenta</i> (1) <sup>q</sup>	0.25	1	1	0.12
<i>Peptostreptococcus magnus</i> (1) <sup>e</sup>	2	0.25	2	0.5
<i>Peptostreptococcus micros</i> (1) <sup>e</sup>	4	0.5	2	0.06
<i>Propionibacterium acnes</i> (2) <sup>r</sup>	0.5	0.5	0.5	NT
<i>Acinetobacter baumannii</i> (1) e	>64	>64	>64	>64
<i>Burkholderia cepacia</i> (1) <sup>e</sup>	>64	>64	>64	>64
<i>Enterobacter cloacae</i> (2) <sup>a</sup>	>64	>64	>64	>64
<i>Escherichia coli</i> (3) <sup>s</sup>	32 to >64	>64	>64	>64
<i>Haemophilus influenzae</i> (3) <sup>t</sup>	16 to >64	64 to >64	8	4–8
<i>Klebsiella pneumoniae</i> (2) <sup>u</sup>	>64	>64	>64	>64
<i>Moraxella catarrhalis</i> (2) <sup>v</sup>	2–4	64	2–4	2
<i>Morganella morganii</i> (2) <sup>a</sup>	>64	>64	>64	16
<i>Neisseria meningitidis</i> (1) <sup>e</sup>	2	64	4	8

<i>Proteus mirabilis</i> (2) <sup>a</sup>	>64	>64	>64	>64
<i>Providencia stuartii</i> (2) <sup>a</sup>	>64	>64	64 to >64	>64
<i>Pseudomonas aeruginosa</i> (2) <sup>v</sup>	>64	>64	>64	>64
<i>Salmonella</i> spp. (2) <sup>a</sup>	>64	>64	>64	>64
<i>Serratia marcescens</i> (2) <sup>a</sup>	>64	>64	>64	>64
<i>Shigella</i> spp. (2) <sup>a</sup>	64 to >64	>64	>64	64
<i>Stenotrophomonas maltophilia</i> (2) <sup>a</sup>	4 to >64	16–64	32 to >64	8 to >64
<i>Bacteroides fragilis</i> (1) <sup>q</sup>	32	16	8	0.5
<i>Bacteroides thetaiotaomicron</i> (1) <sup>q</sup>	16	16	1	1
<i>Fusobacterium necrophorum</i> (1) <sup>e</sup>	64	>64	0.25	0.06
<i>Porphyromonas asaccharolytica</i> (1) <sup>e</sup>	1	32	8	<0.03
<i>Prevotella intermedia</i> (1) <sup>e</sup>	2	64	0.5	<0.03

MIC, minimum inhibitory concentration; MDR, multidrug-resistant; CLSI,

Clinical and Laboratory Standards Institute; NT, not tested.

<sup>a</sup> Antibiotic-susceptible and MDR clinical isolate.

<sup>b</sup> CLSI antimicrobial susceptibility control strain; vancomycin-susceptible, vancomycin-resistant (VanA and VanB) and high-level gentamicin-resistant clinical isolates.

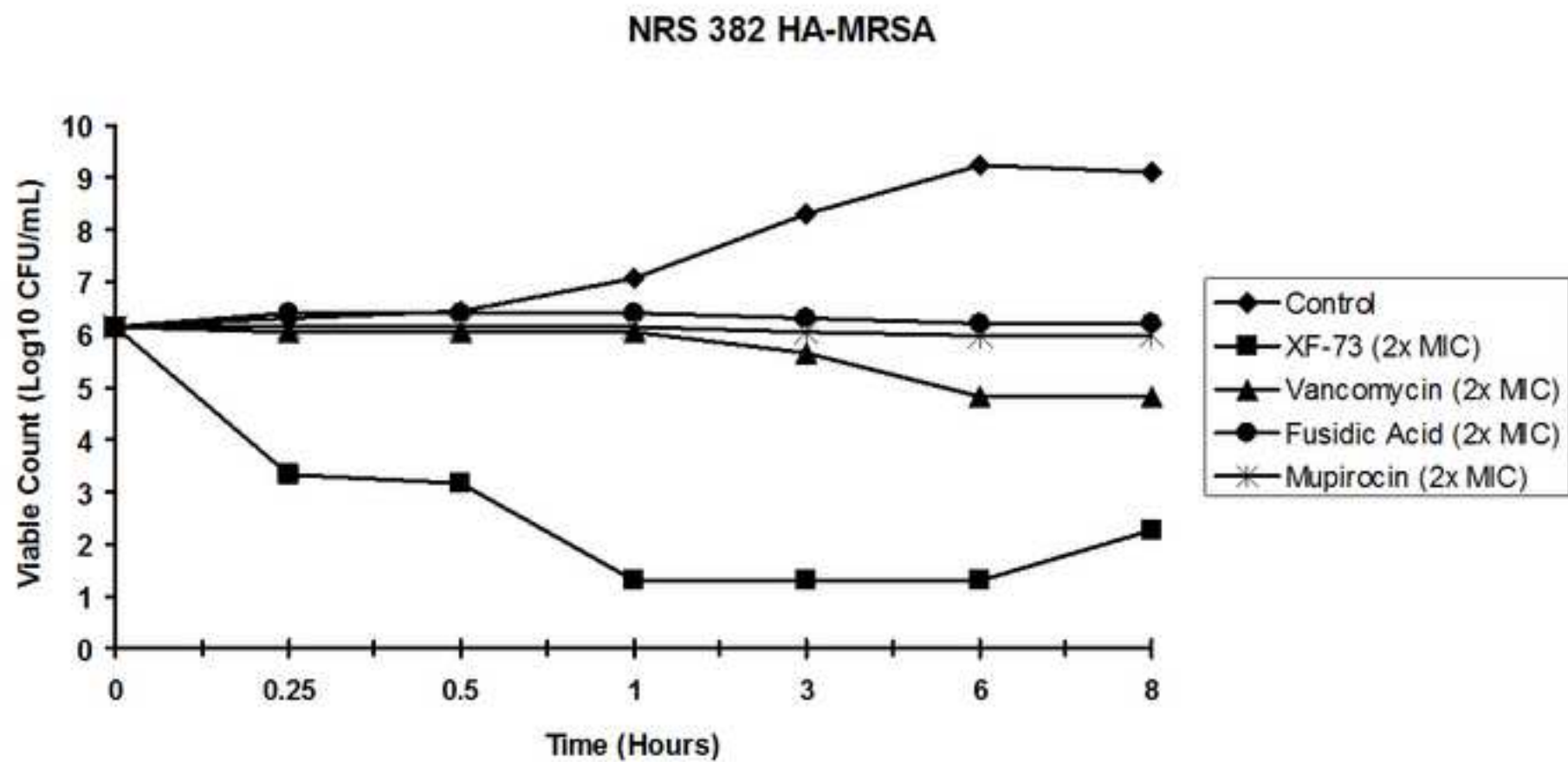
<sup>c</sup> Vancomycin-susceptible and -resistant (VanA and VanB) clinical isolates.

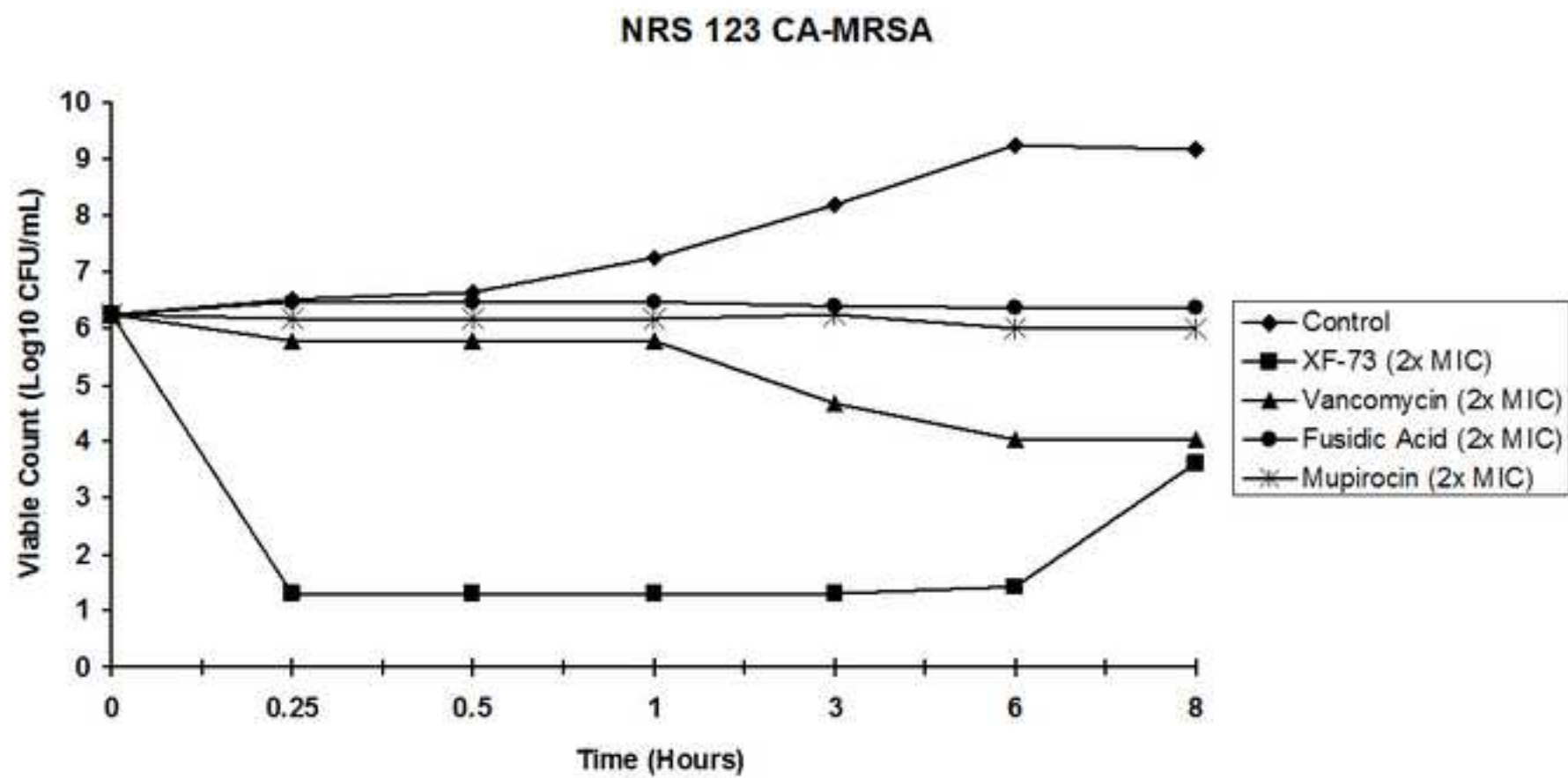
<sup>d</sup> Vancomycin-resistant (VanC) clinical isolate.

<sup>e</sup> Clinical isolate

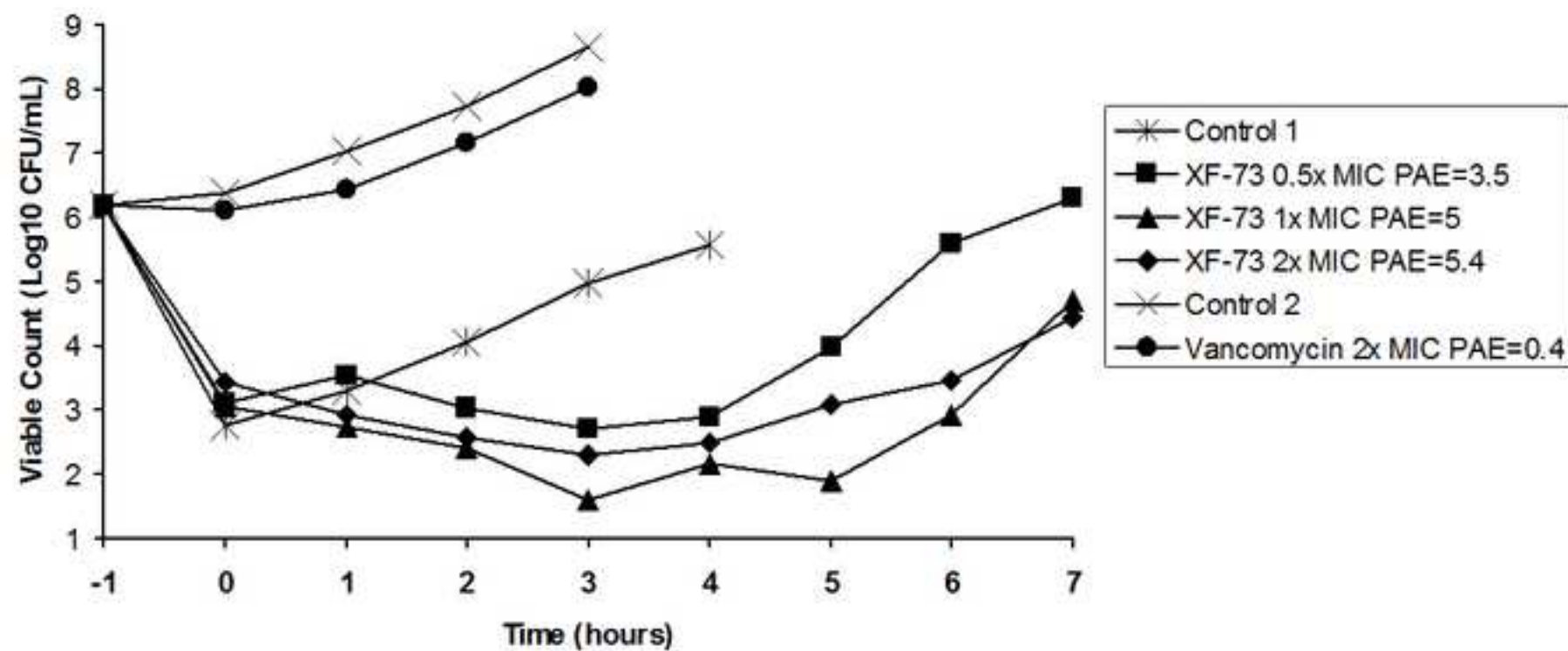
<sup>f</sup> Meticillin-sensitive.

- <sup>g</sup> Meticillin-resistant.
- <sup>h</sup> Vancomycin-intermediate-resistant.
- <sup>i</sup> Daptomycin-non-susceptible.
- <sup>j</sup> High-level mupirocin-resistant (MIC > 256 mg/L) clinical isolate.
- <sup>k</sup> Antibiotic-susceptible; meticillin-resistant and daptomycin-non-susceptible (MIC = 2 mg/L) clinical isolates.
- <sup>l</sup> Antibiotic-susceptible and macrolide-resistant clinical isolates.
- <sup>m</sup> Penicillin-susceptible, -intermediate and -resistant and MDR clinical isolates; CLSI antimicrobial susceptibility control strain.
- <sup>n</sup> Macrolide-resistant (MLS and M-type) and antibiotic-susceptible clinical isolates.
- <sup>o</sup> Toxin-positive and clinical isolates.
- <sup>p</sup> Reference control strain and clinical isolate.
- <sup>q</sup> CLSI antimicrobial susceptibility control strain.
- <sup>r</sup> Erythromycin-resistant strain and clinical isolate.
- <sup>s</sup> MDR clinical isolate; CLSI  $\beta$ -lactamase-positive control strain and CLSI antimicrobial susceptibility control strain.
- <sup>t</sup>  $\beta$ -Lactamase-negative ampicillin-resistant clinical isolate and  $\beta$ -lactamase-negative and -positive clinical isolates.
- <sup>u</sup> CLSI antimicrobial susceptibility control strain and MDR clinical isolate.
- <sup>v</sup>  $\beta$ -Lactamase-positive and reduced fluoroquinolone susceptibility clinical isolates.





# NRS 382 HA-MRSA



# NRS 123 CA-MRSA

