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# In Vitro Characterization of E-101 Solution, a Myeloperoxidase-Based Antimicrobial Agent

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## ABSTRACT

**Background:** E-101 Solution (E-101) is a novel drug product containing porcine myeloperoxidase (MPO), glucose oxidase (GO), and their respective substrates, and represents a new class of broad-spectrum antimicrobial for the prevention and treatment of localized infections. Its unique mode of action relies on the local production of singlet oxygen. **Methods:** A total of 530 susceptible and multiresistant Gram-positive (GP) and Gram-negative (GN) clinical isolates were selected to demonstrate the broad-spectrum activity of E-101. Minimal inhibitory concentration/minimal bactericidal concentration (MIC/MBC), kill rate, drug interaction, and resistance studies were conducted using a modification of the Clinical and Laboratory Standards Institute (CLSI) broth microdilution methods. **Results:** E-101 was highly active against all isolates tested and yielded lower MICs than the comparators mupirocin and gentamicin. The MIC range was 0.008 to 1.0 µg MPO/mL against GP strains including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Staphylococcus aureus* (VRSA), and vancomycin-resistant *Enterococcus* (VRE). E-101 also showed potent activity against GN strains (range ≤0.004-0.5 µg MPO/mL) including *Acinetobacter baumannii* (range 0.06-0.12 µg MPO/mL) and *Pseudomonas aeruginosa* (range 0.03-0.12 µg MPO/mL). E-101 was most active against *S. aureus* with a MIC/MBC ratio of 1 (MBC range 0.015-0.03 µg MPO/mL). Time-kill studies showed rapid (30 minutes-4 hours) bactericidal activity against *S. aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *P. aeruginosa*. The rate and extent of kill were concentration- and time-dependent. No antagonism or synergy was observed with 16 checkerboard antimicrobial combinations for *S. aureus*, *Enterococcus faecium*, and *P. aeruginosa*. Parental MICs for *S. aureus*, *E. faecalis*, and *P. aeruginosa* remained unchanged after sub-MIC passages for 21 days. Elevated MICs for *E. coli* were not stable. **Conclusion:** E-101 Solution (Exoxemis, Inc., Little Rock, AR) demonstrated potent, rapid, and broad-spectrum antibacterial activity against drug-susceptible and drug-resistant organisms, with a low propensity for the development of resistance.

## INTRODUCTION

E-101 Solution contains 2 highly purified therapeutic enzymes, porcine myeloperoxidase (MPO) and glucose oxidase (GO) from *Aspergillus niger*, and is prepared from 2 different aqueous solutions, an enzyme solution and a substrate solution, which are packaged in separate vials. The enzyme solution contains the enzymes MPO and GO, with selected amino acids formulated in a phosphate buffer. The substrate solution contains glucose (dextrose, USP) in the same phosphate buffer. The enzyme solution and substrate solution are combined in appropriate proportions prior to use. The mechanism of action of E-101 involves the selective binding of MPO to the surface of target microorganisms, the in situ generation of hydrogen peroxide by GO from glucose, the MPO-catalyzed oxidation of chloride ion by hydrogen peroxide to generate hypochlorous acid, and the nonenzymatic oxidation of additional hydrogen peroxide by hypochlorous acid to yield singlet oxygen (Figure 1).<sup>1,2</sup> The half-life of singlet oxygen, a potent oxygenating agent, is about 1 microsecond and restricts its sphere of reactivity to about 0.1 to 0.2 µm, approximately the width of a bacterial cell wall,<sup>3</sup> thus avoiding bystander damage. Additionally, the hydrogen peroxide and hypochlorous acid produced is sufficient for singlet oxygen generation, but not substantial enough to cause host tissue toxicity. The objectives of these studies were to demonstrate the broad-spectrum and potent activity of E-101 Solution, a new topical/local agent for the prevention and treatment of infections. E-101 Solution contains MPO and GO, but for ease of presentation for this poster, only MPO content is provided; the MPO to GO ratio was the same in all experiments. Additional information describing the potent and in vivo activity of E-101 Solution can be found in posters F1-3956 and F1-3957.

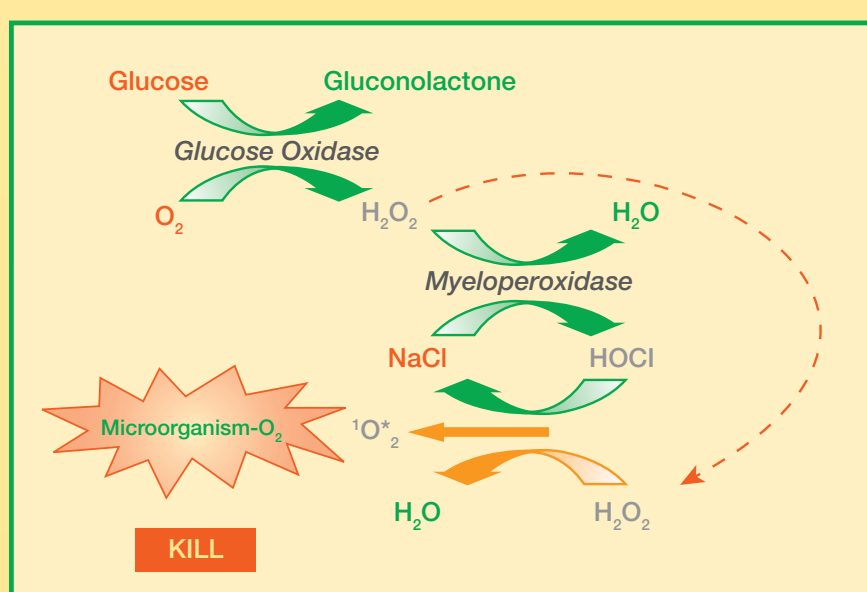


Figure 1. Mechanism of action of E-101 Solution generating singlet oxygen

## METHODS

**MIC Determination:** MICs were determined using a modification of the standard broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) recommendations.<sup>4</sup> Serial 2-fold dilutions of E-101 enzyme component were prepared in 2x Mueller Hinton Broth. Organisms were suspended in 2x substrate component to give a final inoculum of 0.5 x 10<sup>5</sup> CFU/mL.

**MBC Determination:** MBCs were determined by plating out aliquots with no visible growth of bacteria from the MIC microdilution plates according to CLSI recommendations.<sup>5</sup>

**Bactericidal Activity:** Time-kill assays were performed by the modified CLSI broth microdilution method.<sup>5</sup> The bactericidal activity of E-101 was evaluated by determining viable cell counts at defined concentrations and time points. Results are expressed as µg MPO/mL for comparison purposes to standard antibiotics. For clinical applications, MPO is expressed as guaiacol units (GU) mL. The conversion of µg to GU of MPO is based on 0.375 GU/µg of MPO.

## RESULTS

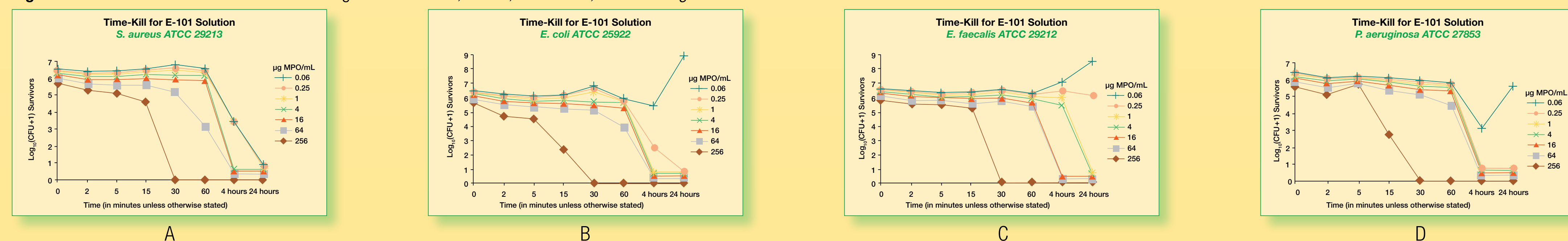
**Table 1.** In vitro antimicrobial activity of E-101 Solution and comparator antibiotics against clinical isolates of Gram-positive and Gram-negative pathogens<sup>a</sup>

Gram-positive Organism (Number of Isolates Tested)	E-101 MIC (µg MPO/mL)		Mupirocin MIC (µg/mL)	
	MIC <sub>50</sub>	Range	MIC <sub>50</sub>	Range
<i>Enterococcus faecalis</i> (33)	0.5	0.12–0.5	>32	8–>32
<i>Enterococcus faecium</i> (22)	0.12	0.06–0.12	0.25	0.06–0.5
<i>Staphylococcus aureus</i> (109)	0.03	0.008–0.06	0.12	≤0.03–>32
<i>Staphylococcus epidermidis</i> (31)	0.03	0.015–0.06	>32	≤0.03–>32
<i>Streptococcus agalactiae</i> (34)	0.5	0.12–1	0.5	0.12–2
<i>Streptococcus Group C</i> (8)	NA <sup>b</sup>	0.5–1	NA	0.06–0.5
<i>Streptococcus Group F</i> (2)	NA	1–1	NA	0.25–0.25
<i>Streptococcus Group G</i> (18)	0.5	0.25–1	0.12	0.06–0.5
<i>Streptococcus pyogenes</i> (33)	0.5	0.12–0.5	0.25	0.06–0.5

Gram-negative Organism (Number of Isolates Tested)	E-101 MIC (µg MPO/mL)		Gentamicin MIC (µg/mL)	
	MIC <sub>50</sub>	Range	MIC <sub>50</sub>	Range
<i>Citrobacter freundii</i> (20)	0.12	0.03–0.12	4	2–>32
<i>Enterobacter cloacae</i> (21)	0.12	0.06–0.12	8	1–>32
<i>Escherichia coli</i> (52)	0.25	0.12–0.5	>32	2–>32
<i>Klebsiella pneumoniae</i> (31)	0.25	0.06–0.25	>32	2–>32
<i>Proteus mirabilis</i> (24)	0.06	0.03–0.06	8	2–>32
<i>Acinetobacter baumannii</i> (23)	0.12	0.06–0.12	>32	0.5–>32
<i>Pseudomonas aeruginosa</i> (53)	0.06	0.03–0.12	16	1–>32
<i>Aeromonas hydrophila</i> (5)	NA	0.015–0.03	NA	1–4
<i>Pasteurella multocida</i> (5)	NA	≤0.004	NA	2–16

<sup>a</sup>Includes MSSA, MRSA, VISA/VRSA, PVL + *S. aureus*, VSE, VRE, ceftazidime-resistant and susceptible Gram-negative rods. <sup>b</sup>NA = not applicable, total number of isolates <10.

**Figure 2.** Time-kill results of E-101 Solution against *S. aureus*, *E. coli*, *E. faecalis*, and *P. aeruginosa*



**Blood Interference Determination:** Time-kill studies were performed using a suspension-neutralization method<sup>6</sup> in the presence of 3% human and 3%, 6%, 12%, and 24% rat blood. The bactericidal activity of E-101 was evaluated by determining viable cell counts at defined concentrations and time points. Results are expressed as µg MPO/mL.

**Synergy Determination:** Drug interaction studies were performed by a modified checkerboard titration method using 96-well microdilution plates.<sup>7</sup> Three antibiotic-resistant strains were selected for testing. A total of 16 checkerboard antimicrobial combinations were tested for each organism. The fractionary inhibitory concentration indexes (FICIs) were interpreted as outlined by Odds.<sup>8</sup>

**Resistance Determination:** Resistance selection studies were performed using a serial passage method.<sup>9</sup> MICs were performed using the modified CLSI method.

**Table 2.** MIC and MBC of E-101 Solution against clinical isolates associated with skin and skin structure infections

Organism (Number of Isolates Tested)	E-101 MIC (µg MPO/mL)			E-101 MBC (µg MPO/mL)		
	MIC <sub>50</sub>	MIC <sub>90</sub>	Range <sup>a</sup>	MBC <sub>50</sub>	MBC <sub>90</sub>	Range <sup>a</sup>
<i>E. faecalis</i> (7)	NA <sup>b</sup>	NA	0.12–0.5	NA	NA	0.5–2
<i>S. aureus</i> (16)	0.015	0.03	0.015–0.03	0.015	0.03	0.015–0.03
<i>S. agalactiae</i> (13)	0.5	0.5	0.12–0.5	0.5	0.5	0.12–1
<i>S. pyogenes</i> (20)	0.5	0.5	0.12–0.5	0.5	1	0.12–1
<i>E. coli</i> (5)	NA	NA	0.12–0.25	NA	NA	0.12–1
<i>P. aeruginosa</i> (5)	NA	NA	0.03–0.06	NA	NA	0.06–0.5

<sup>a</sup>Range of MIC/MBC for all strains tested. <sup>b</sup>NA = not applicable, total number of isolates <10.

**Table 4.** In vitro resistance selection studies

Organism/Strain	E-101 MIC (µg MPO/mL) for Strains at Serial Passage		
	Day 1	Day 21	3-Day Stability <sup>a</sup>
<i>S. aureus</i> 29213 and 1288199	0.015/0.015	0.03/0.03	NT
<i>E. faecalis</i> 29212 and 51299	0.25/0.25	0.5/0.25	NT
<i>E. coli</i> 25922	0.25	0.25	NT
<i>E. coli</i> 1337451	0.12	8	0.5
<i>E. coli</i> 1337019	0.25	8	0.5
<i>E. coli</i> 1075701 parent strain	0.5	8	1
<i>E. coli</i> 1075701 putative mutant <sup>b</sup>	0.5	8	1
<i>P. aeruginosa</i> 27853 and 1077561	0.06/0.06	0.03/0.06	NT

NT = not tested. <sup>a</sup>MIC after 3 passages on drug-free agar plates to determine the stability of increased MICs. <sup>b</sup>Represents repeat testing after an original 21 passages, resulting in an elevated MIC of 4 µg MPO/mL.

**Table 3.** FICI and interpretation of E-101 Solution in combination with selected antimicrobial agents<sup>a</sup>

Organisms	CFZ	CTX	CIP	DOX	GEN	VAN	CEF	IMI
<i>S. aureus</i>	2 NI	2 NI	0.625 NI	0.563 NI	2 NI	0.75 NI	NT	NT
<i>E. coli</i>	1 NI	2 NI	0.75 NI	0.625 NI	0.625 NI	NT	NT	NT
<i>P. aeruginosa</i>	NT	NT	2 NI	0.53 NI	1 NI	NT	0.75 NI	1 NI

<sup>a</sup>FICI interpretation: <0.5 = synergy; >0.5-4 = no interaction (NI); >4 = antagonism.

Abbreviations: CFZ, cefazolin; CTX, ceftriaxone; CIP, ciprofloxacin; DOX, doxycycline; GEN, gentamicin; VAN, vancomycin; CEF, ceftazidime; IMI, imipenem; NT, not tested.

**Table 5.** Effect of whole blood on the activity of E-101 Solution

Blood Source and %	E-101 (µg MPO/mL)	Log <sub>10</sub> Reduction: <i>Staphylococcus aureus</i>			
		≤5 minutes	15 minutes	30 minutes	60 minutes
Rat 24%	1600	7.10	7.10	NT	NT
Rat 12%	800	7.10	7.10	NT	NT
Rat 6%	400	7.10	7.10	NT	NT
Rat 3%	200	7.15	7.15	7.15	7.15
Human 3%	200	7.15	7.15	7.15	7.15
Rat 3%	100	7.15	7.15	7.15	7.15
Human 3%	100	5.57	6.35	6.65	7.15
Rat 3%	50	5.16	5.43	5.93	7.05
Human 3%	50	4.57	4.67	5.81	6.40

NT = not tested

## RESULTS

- E-101 Solution demonstrated broad-spectrum activity against both Gram-positive and Gram-negative organisms (Table 1). No differences in susceptibility were noted between susceptible or resistant phenotypes. Overall, E-101 was more active than comparator antibiotics.
- The potent bactericidal activity of E-101 Solution was confirmed by MBC testing (Table 2). E-101 Solution was most active against *S. aureus* with a MIC to MBC ratio of 1.
- Drug interaction studies showed no interactions between E-101 Solution with any of the antimicrobial combinations tested (Table 3). No antagonism or synergy was observed.
- No stable resistance after 21 days of exposure to E-101 Solution was observed for *S. aureus*, *E. faecalis*, *E. coli*, and *P. aeruginosa* (Table 4).
- Time-kill studies (Figure 2) showed that E-101 Solution is bactericidal against *S. aureus*, *E. coli*, *E. faecalis*, and *P. aeruginosa* in a concentration-dependent manner. The extent of kill increased with longer exposure time.
- The interference of whole blood diminished the activity of E-101 Solution. However, the activity was restored by increasing the enzyme concentrations of the formulation (Table 5). Similar results were seen against *P. aeruginosa* (data not shown).

## CONCLUSIONS

- E-101 Solution is a potent, broad-spectrum, and rapid bactericidal product against clinical and reference organisms including multidrug-resistant strains.
- The microbicidal activity of E-101 Solution is concentration- and time-dependent in the presence and absence of blood.
- The low propensity of E-101 Solution to select for resistance and lack of drug-drug interactions makes it a promising new local/topical anti-infective for the treatment and prevention of infections in a wide variety of wounds and provides an important adjunct to current infection control practices.

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