E-101, a novel first in class topical anti-infective, maintains a high degree of potency *in vitro* against problematic resistant clinical pathogens (ESKAPE pathogens)

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ABSTRACT

Objective: E-101, a topical anti-infective which utilizes myeloperoxidase (MPO) for the generation of reactive oxygen species to kill bacteria, is being developed for the prevention of surgical site infections. Infections caused by antibiotic resistant pathogens have become common and are increasingly difficult to treat. This study evaluates the activity of E-101 against highly resistant "ESKAPE" pathogens (vancomycin resistant *E. faecium* [VRE], methicillin resistant *S. aureus* [MRSA], extended spectrum b-lactamase [ESBL]/carbapenemase producing *K. pneumoniae*, multi-drug resistant [MDR] *A. baumannii* and *P. aeruginosa*, and AmpC cephalosporinase producing *E. cloacae*).

Methods: E-101 activity was evaluated using a modified broth microdilution method based on CLSI M7. Modifications included serial dilution of enzyme (MPO) and inocula delivery in solution containing enzyme substrate. E-101 MICs represent mg/L of MPO. Comparators (currently marketed agents and phenotypic markers) were tested in accordance with CLSI M7 and M100. 103 non-duplicate clinical isolates were selected based on resistance phenotype for evaluation to include the "ESKAPE" phenotypes noted above.

Results: Against VRE, E-101 had an MIC50/MIC90 of 0.06/0.12 mg/L. Against *S. aureus* consisting of linezolid resistant isolates, daptomycin non-susceptible isolates, hospital and community acquired MRSA, VISA, and VRSA, E-101 had an MIC50/MIC90 of ≤0.008/0.015 mg/L with MICs not exceeding 0.06 mg/L. E-101 had an MIC50 and MIC90 0.12 mg/L against ESBL *E. coli*, ESBL *K. pneumoniae*, and KPC *K. pneumoniae*, with an MIC50 and MIC90 of 0.06 mg/L against *E. cloacae*/*C. freundii* with derepressed AmpC. Against MDR *P. aeruginosa*, E-101 had an MIC50/MIC90 of 0.03/0.06 mg/L, with an MIC50 and MIC90 of 0.03 mg/L against MDR *A. baumannii*. E-101 activity against this subset of purely resistant isolates was equivalent to that observed for E-101 during recent surveillance where isolates with these phenotypes were infrequently or not encountered.

Conclusions: E-101 was potent *in vitro* against ESKAPE pathogens, which constitute clinically important pathogens with problematic resistance (multi-drug resistance, emerging resistance to commonly utilized agents). This attribute highlights the utility of E-101 for the treatment of surgical site infections where resistant organisms are likely to be encountered, and potential for the treatment of other superficial infections caused by resistant organisms.

BACKGROUND

•E-101 is a novel myeloperoxidase (MPO)-mediated antimicrobial. Once activated, E-101 generates combustive oxidative products which damage bacterial cells if MPO, halide and a source of hydrogen peroxide are present (Figure 1)

•E-101 is currently undergoing clinical development for the prevention of surgical site infections in Europe and the US

•"ESKAPE" pathogens (Boucher et al, CID 2009;48:1) include pathogens with problematic resistance:
- *E. faecium* (VRE)
- *S. aureus* (MRSA and MDR)
- *K. pneumoniae* (ESBL, KPC)
- *A. baumannii* (MDR)
- *P. aeruginosa* (MDR)
- *E. cloacae* (AmpC)

•This study evaluates the *in vitro* activity of E-101 against "ESKAPE" pathogens, including those with emerging resistances

METHODS

•Clinical isolates included those pre-selected for a particular resistance phenotype based on test history and genetically characterized isolates were selected from both the Eurofins and NARSA repositories (Table 1).

•Susceptibility of isolates to E-101 was determined using a modified broth microdilution method based on CLSI M7 guidelines. Modifications included diluting E-101 enzyme solution containing MPO in 2x cation-adjusted Mueller-Hinton broth in the panel, and delivering the inoculum at 2x final concentration in 2x substrate solution to achieve a final concentration of 1x E-101, 1x substrate solution, and 5 x 105 CFU/mL. Immediately post-inoculation, E-101 begins to generate reactive oxygen species. MICs are reported based on mg/L MPO in E-101.

•Isolates were concurrently tested against relevant comparators in accordance with CLSI M7

RESULTS

TABLE 1. Evaluated ESKAPE pathogens

***S. aureus* (N=44)**
- 10 hospital-acquired MRSA (HA-MRSA)[1]
  - 1 USA600 (MDR US HA-MRSA clone)
  - 9 USA100 (prevalent US HA-MRSA clone)
- 10 community-acquired MRSA (CA-MRSA)[1]
  - 10 USA300 (prevalent US CA-MRSA clone)
- 7 daptomycin non-susceptible (DaptoNS) isolates
- 9 linezolid resistant (LZD R) isolates
- 8 vancomycin non-susceptible (VAN NS) isolates
  - 3 vancomycin resistant (VRSA)
  - 5 vancomycin intermediate (VISA)

***Enterococcus* spp. (n=9)**
- 5 *E. faecalis* (vancomycin resistant)
- 4 *E. faecium* (vancomycin resistant)

***Enterobacteriaceae* spp. (N=30)**
- 10 ESBL phenotype confirmed[2]
  - 5 *E. coli*
  - 5 *K. pneumoniae*
- 10 KPC positive *K. pneumoniae*[3]
- 10 derepressed AmpC[4]
  - 5 *Citrobacter spp.*
  - 5 *Enterobacter spp.*

MDR[5] *P. aeruginosa* (N=10)

MDR[5] *Acinetobacter* spp. (N=10)

[1]CA vs HA MRSA designations based on USA type reported in NARSA repository
[2]phenotypically positive per CLSI M100 in prior testing - >two-fold reduction in ceftazidime/cefotaxime MIC when combined with clavulanic acid
[3]positive for KPC-2/KPC-3 by PCR
[4]phenotypically positive based on prior testing (cefoxitinR, ceftazidimeR/cefotaximeR not inhibited by clavulanic acid, carbapenem susceptible; Livermore, JAC 2001;48:S59)
[5]MDR based on prior testing; resistance to > 3 different antimicrobial classes

FIGURE 1. Generation of reactive oxygen species from MPO; mechanism of action of E-101

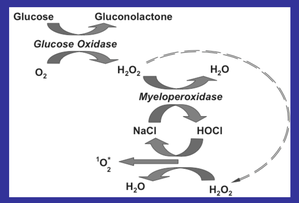
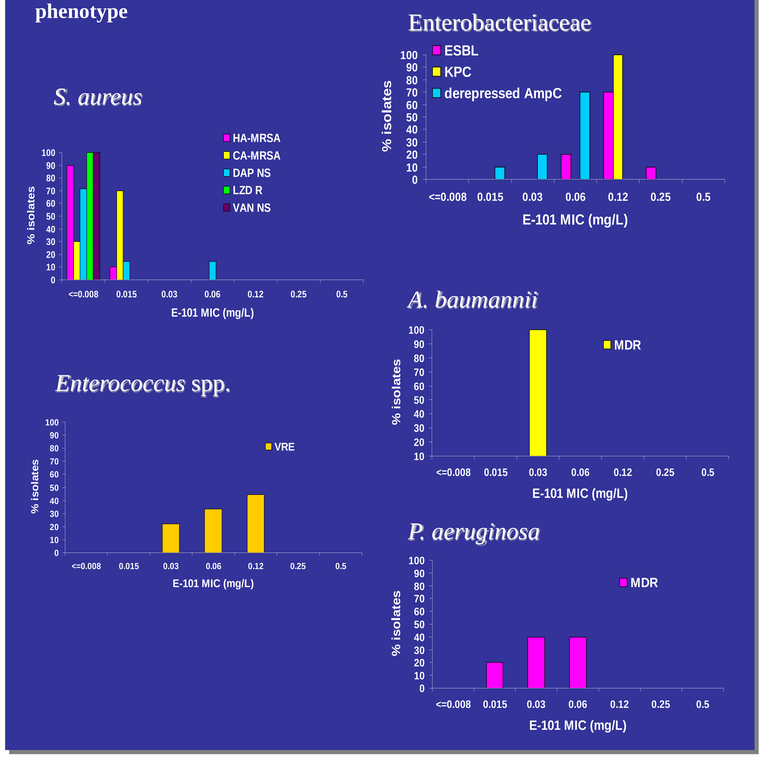


TABLE 2. Activity profile of E-101 against evaluated ESKAPE pathogens

Organism	Phenotype	N	MIC (mg/L) range	mode	MIC_{50}	MIC_{90}
S. aureus	overall	44	<=0.008-0.06	<=0.008	<=0.008	0.015
	HA-MRSA	10	<=0.008-0.015	<=0.008	<=0.008	<=0.008
	CA-MRSA	10	<=0.008-0.015	0.015	0.015	0.015
	DAP NS	7	<=0.008-0.06	<=0.008	NA	NA
	LZD R	9	<=0.008-0.015	<=0.008	NA	NA
	VAN NS	11	<=0.008-<=0.008	<=0.008	<=0.008	<=0.008
Enterococcus spp.	VRE	9	0.03-0.12	0.12	NA	NA
Enterobacteriaceae	overall	30	0.015-0.25	0.12	0.12	0.12
	ESBL	10	0.06-0.25	0.12	0.12	0.12
	KPC	10	0.12-0.12	0.12	0.12	0.12
	derepressed AmpC	10	0.015-0.06	0.06	0.06	0.06
A. baumannii	MDR	10	0.03-0.03	0.03	0.03	0.03
P. aeruginosa	MDR	10	0.015-0.06	0.03	0.03	0.06

FIGURE 2. E-101 MIC distributions against ESKAPE pathogens by phenotype



S. aureus
•E-101 maintained potent MICs (0.015 mg/L or less excluding one daptomycin non-susceptible isolate) overall against *S. aureus*, including those resistant to current anti-Gram positive agents

•E-101 was active against prevalent HA-MRSA and CA-MRSA clones

Enterococci
•E-101 maintained potency against vancomycin resistant enterococci, with MICs in the 0.03-0.12 mg/L range.

•E-101 MICs were 2-4 fold lower against vancomycin resistant *E. faecium* (0.03-0.06 mg/L) relative to vancomycin resistant *E. faecalis* (0.06-0.12 mg/L).

Enterobacteriaceae
•Against various species and types of beta-lactamase producing Enterobacteriaceae, E-101 maintained potent MICs, including the recently emerged KPC producing *K. pneumoniae*.

A. baumannii
•E-101 had MICs of 0.03 mg/L against all evaluated multi-drug resistant *A. baumannii* isolates.

P. aeruginosa
•Against multi-drug resistant *P. aeruginosa*, E-101 was highly active with an MIC_{50} of 0.03 mg/L and an MIC_{90} of 0.06 mg/L .

CONCLUSIONS

•The emergence and spread of resistance combined with the increasing prevalence of multi-drug resistance among ESKAPE pathogens has left relatively few effective therapeutic options for the treatment of drug resistant infections

•These developments highlight the need for new agents active against these resistant organisms (further illustrated by the recent emergence and spread of KPC and NDM-1 carbapenemases)

•E-101, currently undergoing evaluation for topical prevention of surgical infections, is a novel agent with multiple mechanisms of action that maintains its activity against ESKAPE pathogens with challenging resistance phenotypes

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