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P2795 Antimicrobial activity of a new endolysin from the *Acinetobacter baumannii* Ab105-1ø bacteriophage in combination with colistin against the ESKAPE group of Gram-negative bacteria

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Background:

Endolysins are bacteriophage enzymes that can hydrolyze peptidoglycan and thus lyse the bacterial cell wall. They are therefore potentially valuable antimicrobial agents in the fight against multiresistant bacteria. However, in Gram-negative bacteria the outer membrane constitutes an effective barrier to the action of endolysins. Our research group has previously identified endolysin 1Φ in the prophage Ab105Φ1 in *Acinetobacter baumannii* strain Ab105 GEIH-2010. In the present study we examined the effect of combining endolysin activity (unable to overcome the outer-membrane) and colistin activity (which permeabilizes the outer membrane) in order to reduce the MIC of colistin.

Materials/methods:

The lytic activity of endolysin 1Φ was assayed by turbidity test in several strains of Gram-negative bacteria (75 isolates from different STs clones): *A. baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The outer membrane was permeabilized with EDTA (0.5 mM) in Tris buffer (20 mM; pH 8.5). The lytic activity was measured as a decrease in the optical density (OD_{600}) of a culture diluted in Tris-HCl buffer 20mM, pH 8.5 and NaCl 150mM, in the presence of 25 µg/ml of endolysin 1Φ.

The combined activity of endolysin 1Φ and colistin was assayed in colistin susceptible strains, in which the endolysin displays highest and lowest activity. The combined activity was determined (as MICs) by the broth microdilution checkerboard (CB) method. When the MIC for colistin was reduced to ¼ of the initial value, a time-kill curve assay was conducted using the corresponding concentrations of colistin and endolysin.

Results:

Endolysin 1Φ is active against all three species assayed, although highest in *A. baumnannii* (Figure 1). The MIC of colistin for all strains, except *K. pneumoniae* KP16, was reduced in the presence of endolysin 1Φ. The time-kill curves showed an almost 3-log reduction after 6 hours in all strains assayed, except *K. pneumoniae* KP17 (Fig. 2).

Conclusions:

The broad spectrum of activity of endolysin 1Φ against ESKAPE Gram-negative bacteria and the reduction in the MIC of colistin when used in combination with the endolysin indicate that this enzyme is a promising candidate as

a potential antimicrobial agent.

Fig. 1

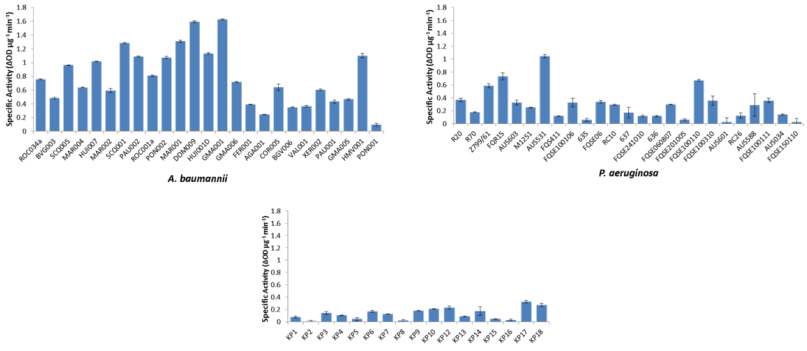

Fig. 2

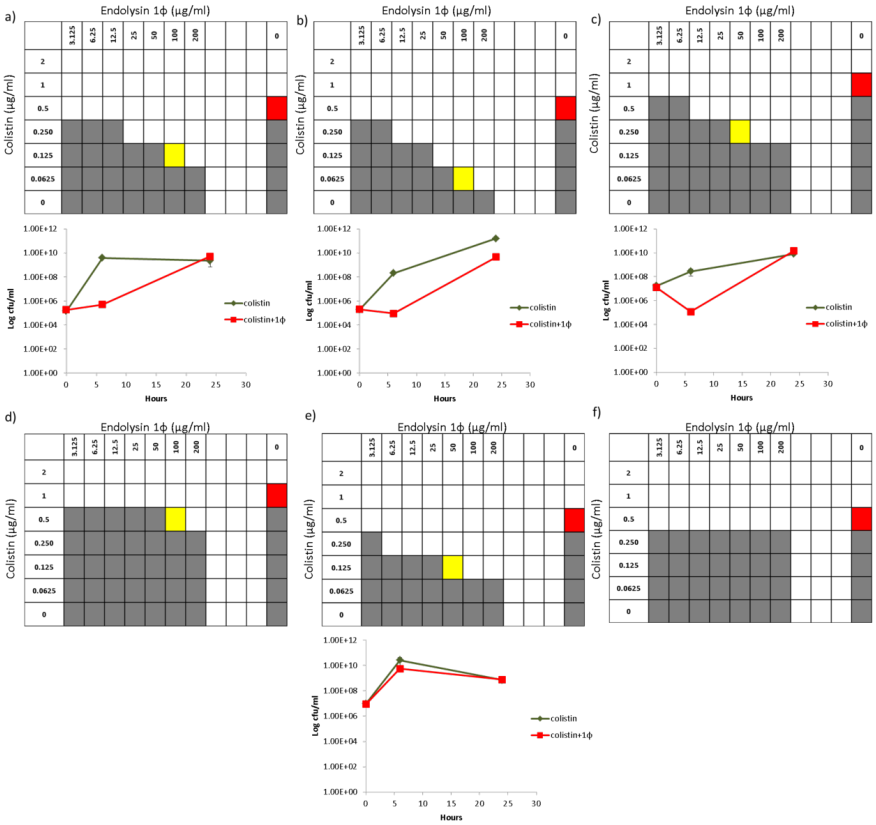

Figure 1. The specific activity of the endolysin 1Φ protein was measured by the turbidity test in clinical isolates of three Gram-negative bacteria: *A. baumannii*, *P. aeruginosa* and *K. pneumoniae*. Figure 2. Colistin activity in combination with endolysin 1Φ measured by MIC assay and time-kill curves in *A. baumannii* strains GMA001 (a) and PON001 (b); *P. aeruginosa* strains AUS531 (c) and FQRC15 (d); *K. pneumoniae* strains KP17 (e) and P16 (f). Time-kill curves were only constructed for strains in which the MIC value (red square) was reduced by a quarter in the presence of endolysin 1Φ (yellow square).