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Bactericidal activity of bacteriophage endolysin HY-133 against *Staphylococcus aureus* in comparison to other antibiotics as determined by minimum bactericidal concentrations and time-kill analysis

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) decolonization is expensive and time consuming, and new agents are necessary due to increasing resistance rates. The administration of bacteriophages or particularly their endolysins may offer an alternative treatment strategy and could provide a solution to overcome the selection pressure due to classical antibiotics. Here, the bactericidal activity was characterized for the recombinant chimeric bacteriophage endolysin HY-133 in comparison to other antimicrobials. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined for 2 reference strains, 24 clinical MRSA and methicillin-susceptible *S. aureus* (MSSA) isolates, as well as 6 isolates with high-level mupirocin resistance. Additionally, HY-133 activity against bacteria in stationary or exponential growth phase was compared in 12 isolates. Time-kill curves were performed with 2 representative isolates to investigate the pharmacodynamics until 48-h incubation time. All experiments were performed in comparison to daptomycin and mupirocin. The $MIC_{50/90}$ and $MBC_{50/90}$ values were in the range 0.12–0.5 mg/L for all 3 growth conditions comparable to daptomycin with 0.5/0.5 mg/L, respectively. The MBC was almost always equal the MIC and without considerable differences between MSSA and MRSA. Time-kill curves revealed a rapid bactericidal effect of HY-133 within the first 2 h, similar to daptomycin. Even with low concentrations, the recombinant endolysin HY-133 was highly active against all tested MSSA and MRSA isolates including mupirocin-resistant isolates. The application of this alternative agent may offer a future strategy for MRSA/MSSA decolonization and, potentially, for treatment purposes.

1. Introduction

Carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with an increased morbidity, mortality, and an enormous socioeconomic burden to healthcare systems (Hübner et al., 2014; Köck et al., 2010). Source of infection and principle habitat of MRSA are the colonization of the nasal cavity (Kaspar et al., 2016; von Eiff et al., 2001).

Nasal decolonization is regarded as a standard measure for hospitalized patients, and several agents for decolonization of MRSA are available, the most widely used being mupirocin (Humphreys et al., 2016; McConeghy et al., 2009). However, this topical antibiotic agent necessitates several administrations per day over 5 to 7 days and, it is not seldom that decolonization fails (Ammerlaan et al., 2009, 2011; Meyer et al., 2012; Nelson et al., 2012). Moreover, resistance against mupirocin, based on several resistance mechanisms, is on the rise (Hetem and Bonten, 2013; Thomas et al., 2010). Therefore, new alternative decolonization therapy options are warranted.

The application of bacteriophage endolysins could represent a future strategy for improved MRSA decolonization (Pastagia et al., 2013). A previous study revealed highly specific activity and low minimum inhibitory concentrations (MICs) of the bacteriophage endolysin PRF-119, a precursor of HY-133, against *S. aureus* (Idelevich et al., 2011). The optimized recombinant chimeric bacteriophage endolysin HY-133 (HYpharm, Bernried, Germany) contains similar to the precursor PRF-119 two distinct functional modules of an enzymatic active domain, namely, the cysteine- and histidine-dependent aminopeptidase/hydrolase (CHAP) domain from the endolysin of phage K, and a cell wall binding domain from the bacteriocin lysostaphin, but is optimized for better stability by shortening the link between the 2 domains. For HY-133, *in vitro* activity against African clonal lineages of the *S. aureus* complex including *S. schweitzeri* has been demonstrated (Idelevich et al., 2016).

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Here, we analyzed the *in vitro* activity of HY-133 towards a representative set of MRSA and methicillin-susceptible *S. aureus* (MSSA) isolates as well as mupirocin-resistant *S. aureus* (MupRSA) isolates. The bactericidal effect was investigated by the determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) under different growth conditions in comparison to mupirocin and daptomycin. Additionally, the rapidity of bactericidal action was characterized by time-kill curve analyses.

2. Materials and methods

The antistaphylococcal activity of the chimeric bacteriophage endolysin HY-133 (HYpharm GmbH, Bernried, Germany) was evaluated by the broth microdilution method in accordance to the Clinical Laboratory Standard Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute (CLSI), 2015, 2017). Mupirocin (AppliChem, Darmstadt, Germany) and daptomycin (biorbyt, Cambridge, United Kingdom) were tested by the same method as comparators.

MIC and MBC were determined for MSSA reference strain ATCC 29213 and MRSA reference strain ATCC 43300 as well as 12 MRSA and 12 MSSA clinical isolates (Idelevich et al., 2011). All isolates were analyzed per direct colony suspension method, as recommended by CLSI for staphylococci (Clinical and Laboratory Standards Institute (CLSI), 2017). The growth medium was cation-adjusted Mueller–Hinton broth (CA-MHB) (BD, Heidelberg, Germany), and the inoculum was standardized to achieve approximately 5×10^5 colony-forming units (CFU) per milliliter, controlled by counting CFU (NCCLS, 1999). The MIC was determined after 18-h incubation at 35 ± 2 °C and the MBC by CFU counting of serial dilutions of at least 2 wells after the MIC until a bactericidal concentration was reached (with the exception of testing of mupirocin; here the well with a mupirocin concentration of 32 mg/L was verified to meet its bacteriostatic mode of action). For CFU determination, we used tryptic soy agar with 10 μL liquid per agar plate for the MBC and 100 μL for the growth control and performed this in triplicate per dilution step. The MIC was determined visually as the first dilution step with a complete growth inhibition. The MBC was defined as the concentration in the first well of the ascending concentration series for which the cell count is reduced by at least 3 $\log_{10}$ steps compared to the initial inoculum (99.9% reduction of start inoculum) (NCCLS, 1999). A possible antibiotic carryover effect was tested several times in accordance to the CLSI M26A guideline (NCCLS, 1999) and was never detected. All MIC and MBC values were determined in triplicate, and the median was calculated.

Additionally, we investigated the activity of the bacteriophage endolysin under different bacterial growth conditions for the 2 reference strains as well as 5 MSSA and 5 MRSA clinical isolates, including inoculation of HY-133 after 16 to 18 h of incubation in tryptic growth (BD, Heidelberg, Germany).

Furthermore, we analyzed the bactericidal activity of the endolysin HY-133 per MIC and MBC on 6 MupRSA isolates with a mupirocin MIC ≥1024 mg/L. Five of the isolates were part of our own clinical strain collection from 2011 and 2015, and 1 isolate was obtained from the National Reference Centre for Staphylococci and Enterococci, Robert-Koch Institute, Wernigerode, from 2011. Verified MIC and MBC of mupirocin were ≥32 mg/L, and the high-level resistance was also confirmed by gradient diffusion test with ≥1024 mg/L (Etest, bioMérieux, Marcy l'Etoile, France) and in-house PCR for *ileS-2* (Anthony et al., 1999).

Finally, we tested the bactericidal activity of the endolysin HY-133 by time-kill methodology in accordance to the CLSI guideline M26-A (NCCLS, 1999). The CA-MHB was preincubated overnight, and afterwards, the start concentrations were adjusted to 5×10^5 CFU per milliliter from an exponential bacteria growth phase with 3-h incubation. Experiments were validated by growth and sterility controls. The bactericidal activity was determined for 1-, 2-, 4-, and 16-fold MIC of HY-133 as well as daptomycin and mupirocin. The killing kinetics of 1 representative MSSA and MRSA isolate was determined by plating of serial dilutions at time points 0 h, 1 h, 2 h, 4 h, 6 h, 8 h, 24 h, and 48 h. Exemplarily, the 16-fold MIC was also tested for the first 10 min every 2 min, followed by 5-min time intervals for the first hour. The time-kill curves were perfomed in duplicate.

3. Results

3.1. MIC and MBC determination

For HY-133 and the standard broth microdilution method with direct colony suspension, the $MIC_{50/90}$ values were 0.25/0.5 mg/L for the 12 MSSA isolates and 0.12/0.25 mg/L for the 12 MRSA isolates. The MICs of the MSSA reference strain ATCC 29213 and the MRSA reference strain ATCC 43300 were 0.25 and 0.5 mg/L. The $MBC_{50/90}$ values for the 12 clinical MSSA isolates and the 2 reference strains were equal to their respective $MIC_{50/90}$ values. Only the MBC_{50} value for the 12 MRSA isolates was 0.25 mg/L, in contrast to the MIC_{50} value with 0.12 mg/L (Tables 1 and 2).

Applying HY-133 under exponential growth conditions, the $MICs_{50/90}$ and $MBCs_{50/90}$ values were equal for all clinical MSSA and MRSA isolates tested as well as for the 2 reference strains with 0.5/0.5 mg/L, respectively.

Applying HY-133 to the cultures in stationary growth phase, $MIC_{50/90}$ values of 0.25/0.25 mg/L were revealed for all clinical MSSA and MRSA isolates ($n = 24$), 0.12/0.12 mg/L for the MSSA reference strain, and 0.5/0.5 mg/L for the MRSA reference strain. Again, the $MBC_{50/90}$ values were equal to the $MIC_{50/90}$ values. Altogether, the $MIC_{50/90}$ and $MBC_{50/90}$ values of HY-133 were always in a range between 0.12 and 0.5 mg/L for all 3 inoculation methods. This was comparable to the $MIC_{50/90}$ and $MBC_{50/90}$ values of daptomycin with 0.5/0.5 mg/L and 0.5/0.5 mg/L for each of the 3 growth conditions.

The clinical isolates and the reference strains showed bacteriostatic activities for mupirocin with $MIC_{50/90}$ values of 0.25/0.25 mg/L and $MBC_{50/90}$ values of >32/>32 mg/L for all growth conditions, except for the exponential growth phase approach of the clinical isolates with $MBC_{50/90}$ values of ≤32/>32 mg/L.

Furthermore, HY-133 demonstrated high bactericidal activity against all 6 MupRSA isolates. Two isolates revealed MIC values of 0.12 mg/L; 3 isolates, 0.25 mg/L; and 1 isolate, 0.5 mg/L. The MBC values were always equal to the MIC.

3.2. Time-kill curves

In the time-kill curve experiments, HY-133 demonstrated a high bactericidal activity against MSSA and MRSA with a maximum effect within 2 h, similar to daptomycin. Already low concentrations of HY-133 with a 2-fold MIC of 0.5 mg/L eradicated *S. aureus*, and a bactericidal concentration was achieved for the 2-, 4-, and 16-fold MIC with 0.5, 1.0, and 4.0 mg/L after 1 h (Fig. 1). For daptomycin, the same effect was reached for the 4-fold MIC with 2.0 mg/L within 2 h and for the 16-fold MIC with 8.0 mg/L within 1 h. Applying 16-fold HY-133 MIC, bactericidal effect was observed already within 4 min for MRSA and within 8 min for MSSA (Fig. 2).

Longer incubation times showed *in vitro* regrowth effects for HY-133, daptomycin, and mupirocin to different extents. The regrowth effect was significantly more distinct for HY-133 in contrast to daptomycin. Regrowth occurred for all tested concentrations and began mostly after the maximum effect within 2 and 6 h. Only the 16-fold MIC with 4.0 mg/L eradicated all bacteria for at least 24 h, but regrowth was detected after 48-h incubation time.

For daptomycin, the regrowth effect was lower for the 1-, 2-, and 4-fold MIC with 0.5, 1.0, and 2.0 mg/L and occurred between 6-h and 24-h incubation time. The 16-fold MIC concentration with 8.0 mg/L showed a complete killing for MSSA after 2 h and for MRSA after 4 h without bacteria regrowth even after 48-h incubation.

In contrast, mupirocin showed only bacteriostatic effect, which was confirmed by the time-kill curves. The 1-fold MIC with 0.25 mg/L could not inhibit the bacterial growth, but the 2-, 4-, and 16-fold MIC with 0.5,

Table 1
MIC and MBC values of HY-133 in comparison to mupirocin and daptomycin applying *S. aureus* reference strains.

Reference strain and approach	HY-133			Daptomycin			Mupirocin		
	MIC (mg/L)	MBC (mg/L)	MBC/MIC	MIC (mg/L)	MBC (mg/L)	MBC/MIC	MIC (mg/L)	MBC (mg/L)	MBC/MIC
S. aureus ATCC 29213 (MSSA):									
- Direct suspension method	0.25	0.25	1.0	0.5	0.5	1.0	0.25	>32	>128
- 3-h incubation[a]	0.5	0.5	1.0	0.5	0.5	1.0	0.25	>32	≤128
- 16–18-h incubation[b]	0.12	0.12	1.0	0.5	0.5	1.0	0.25	>32	>128
S. aureus ATCC 43300 (MRSA):									
- Direct suspension method	0.5	0.5	1.0	0.5	0.5	1.0	0.25	>32	>128
- 3-h incubation[a]	0.5	0.5	1.0	0.5	0.5	1.0	0.25	>32	>128
- 16–18-h incubation[b]	0.5	0.5	1.0	0.5	0.5	1.0	0.25	>32	>128

[a] Reflecting exponential growth phase condition.
[b] Reflecting stationary growth phase condition.

1.0, and 4.0 mg/L mupirocin demonstrated slightly decreasing bacterial concentrations. Bactericidity was reached for the 4- and 16-fold MIC after at least 24 h. Regrowth was less marked and only detectable for MSSA and MRSA for the 1- and 2-fold MIC after 24-h and 48-h incubation time, respectively, as well as for MRSA for the 4-fold MIC after 48 h.

4. Discussion

In 2011, a high *in vitro* activity of the precursor molecule PRF-119 was demonstrated against clinical *S. aureus* isolates, while against most of the clinical coagulase-negative staphylococci (CoNS), no effect was observed (Idelevich et al., 2011). Recently, high activity of HY-133 was shown against African MSSA and MRSA isolates as well as MRSA isolates with ceftaroline/ceftobiprole resistance and *S. aureus* isolates with borderline oxacillin resistance (Idelevich et al., 2016).

The present study revealed a high bactericidal activity of HY-133 against MSSA and MRSA isolates, with an effect beginning within the first minutes of application and also in very low concentrations. There were no considerable differences in the activity against MSSA or MRSA strains.

Based on the claim of Cooper et al. for a standardization of results in the phage and endolysin research and development (Cooper et al., 2016), we performed the tests in accordance to the CLSI guidelines for bacteria including standard reference strains. For comparability, the study investigated the HY-133 activity in comparison to mupirocin and daptomycin determining MIC/MBCs values as well as time-kill curves.

While therapeutic dosing of bacteriophages is complicated (Abedon, 2011, 2016), the recombinant endolysins could be easily applied in defined concentrations. The strong bactericidal activity of HY-133 against several clinical *S. aureus* isolates with $MIC_{50/90}$ and $MBC_{50/90}$ ranges between 0.12 and 0.5 mg/L was comparable to the activities of daptomycin and mupirocin with $MIC_{50/90}$ values of 0.5/0.5 mg/L and 0.25/0.25 mg/L, respectively.

MIC/MBC values for HY-133 determined in this study were lower than reported by Vipra et al., who studied the bacteriophage derived chimeric protein P128 with a range of 2–64 mg/L (Vipra et al., 2012). The same holds true in comparison to *CF*-301, published by Gilmer and Schuch and exhibiting a range of 2–8 mg/L (Gilmer et al., 2013; Schuch et al., 2014). P128 is a somewhat similar phage endolysin that is composed of the phage tail-associated muralytic enzyme of phage K, localized to the C-terminal CHAP domain, and the *Staphylococcus*-specific cell-wall targeting domain SH3b (Paul et al., 2011). *CF*-301, also referred to as PlySs2, is a bacteriophage lysin derived from a *Streptococcus suis* phage and has an N-terminal cysteine-histidine aminopeptidase catalytic domain and a C-terminal SH3b binding domain (Gilmer et al., 2013).

The killing effect of HY-133 was shown to be indepedent of the bacterial growth phase. There was no considerable difference between the direct suspension method and the inoculation after 3 or 18 h of bacterial growth, respectively. Thus, this endolysin could be administered independently of the bacterial growth phase, as for most antibiotics (Barry et al., 1983).

A few probe runs showed a paradoxical phenomenon of having survivors in higher concentrations above the MBC, which was already seen

Table 2
$MIC_{50/90}$ and $MBC_{50/90}$ values of HY-133 for clinical isolates and in contrast to daptomycin and mupirocin.

Antimicrobial agent, approach, and number of *S. aureus* isolates tested (in brackets)	MIC data (mg/L)			MBC data (mg/L)			MBC/MIC data		
	MIC_{50}	MIC_{90}	Range	MBC_{50}	MBC_{90}	Range	MBC_{50}/MIC_{50}	MBC_{90}/MIC_{90}	Range
HY-133:									
- Direct suspension method ($n = 24$)	0.25	0.5	0.12–0.5	0.25	0.5	0.12–0.5	1.0	1.0	1.0–2.0
- MSSA isolates ($n = 12$)	0.25	0.5	0.12–0.5	0.25	0.5	0.12–0.5	1.0	1.0	1.0
- MRSA isolates ($n = 12$)	0.125	0.25	0.12–0.5	0.25	0.5	0.12–0.5	1.0	1.0	1.0–2.0
- 3-h incubation[a] ($n = 10$)	0.5	0.5	0.2–0.5	0.5	0.5	0.25–1.0	1.0	1.0	1.0
- 16–18-h incubation[b] ($n = 10$)	0.25	0.25	0.12–0.5	0.25	0.25	0.12–0.5	1.0	1.0	1.0
Daptomycin:									
- Direct suspension method ($n = 24$)	0.5	0.5	0.25–1.0	0.5	0.5	0.25–1.0	1.0	1.0	1.0–2.0
- MSSA isolates ($n = 12$)	0.5	0.5	0.25–0.5	0.5	0.5	0.25–0.5	1.0	1.0	1.0
- MRSA isolates ($n = 12$)	0.5	0.5	0.25–1.0	0.5	0.5	0.25–1.0	1.0	1.0	1.0–2.0
- 3-h incubation[a] ($n = 10$)	0.5	0.5	0.25–0.5	0.5	0.5	0.25–0.5	1.0	1.0	1.0
- 16–18-h incubation[b] ($n = 10$)	0.5	0.5	0.25–0.5	0.5	0.5	0.25–0.5	1.0	1.0	1.0–2.0
Mupirocin:									
- Direct suspension method ($n = 24$)	0.25	0.25	0.12–0.25	>32	>32	>32	>128	>256	>128- > 256
- MSSA isolates ($n = 12$)	0.25	0.25	0.12–0.25	>32	>32	>32	>128	>256	>128- > 256
- MRSA isolates ($n = 12$)	0.25	0.25	0.12–0.25	>32	>32	>32	>128	>128	>128- > 256
- 3-h incubation[a] ($n = 10$)	0.25	0.25	0.25	≤32	>32	≤32- > 32	≤128	>128	≤128- > 128
- 16–18-h incubation[b] ($n = 10$)	0.25	0.25	0.25	>32	>32	>32	>128	>128	>128

[a] Reflecting exponential growth phase condition.
[b] Reflecting stationary growth phase condition.

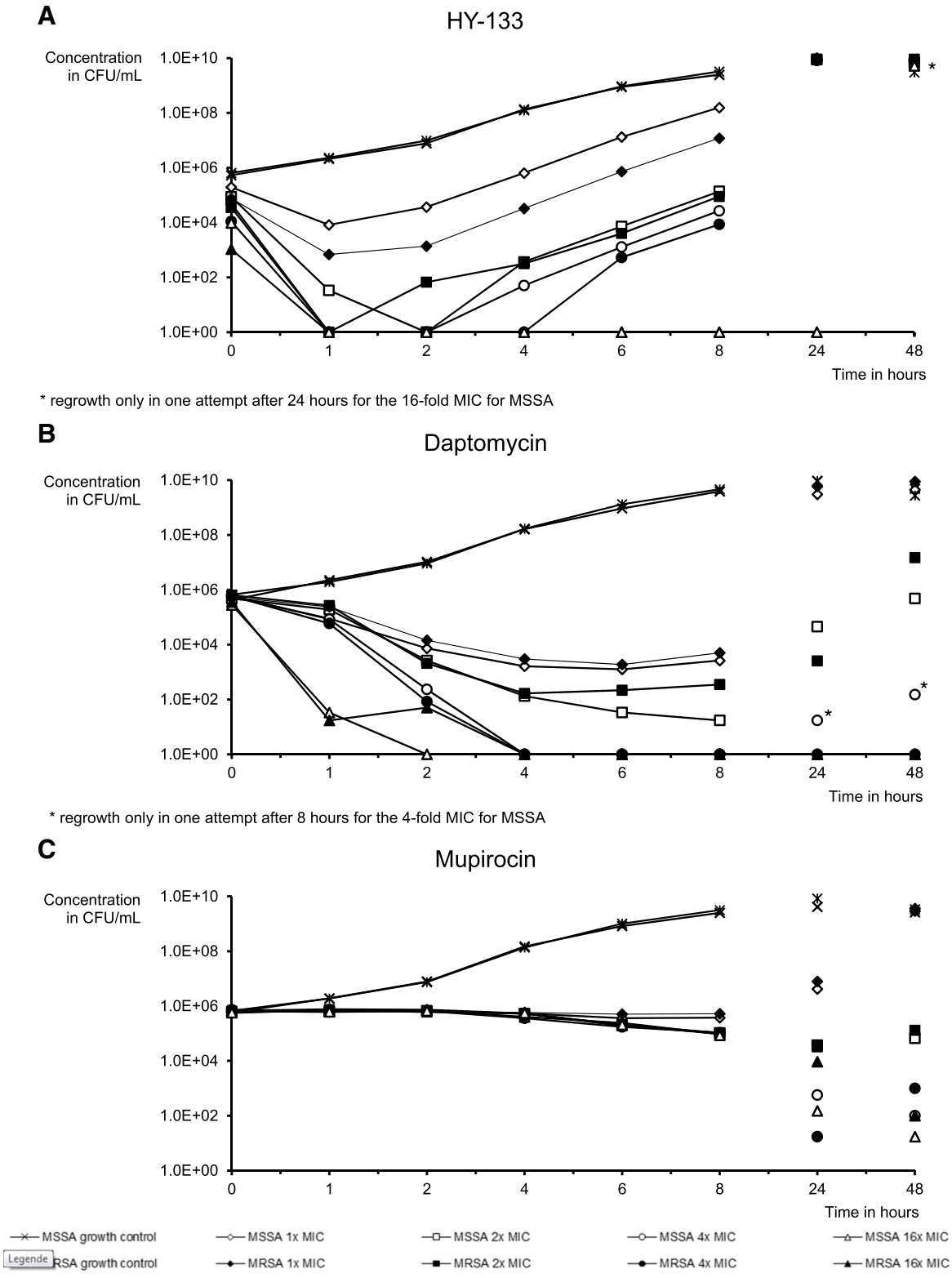

Fig. 1. Time-kill curve analyses of the antimicrobial activity of HY-133 against MSSA and MRSA isolates (A) in comparison to daptomycin (B) and mupirocin (C).

by Eagle 70 years ago (Eagle, 1948; Eagle and Musselman, 1948). This persister phenomenon for bacteriophage endolysins is consistent with the description of Idelevich et al. (2011). In that study, the trailing end-points (reduced point-like growth with concentrations higher than MIC) were seen in MIC determination at some tests and were not reproducible in repeated tests. This study did not observe trailing but observed surviving cells above the MIC; however, infrequently and, if at all, then only in some wells and independently from the endolysin concentration. Similar to Idelevich et al., those survivor cells were exemplarily susceptible to endolysin by subculturing and retesting. Because this cells could survive prolonged exposure despite being genetically susceptible (Balaban et al., 2013), they represent persister

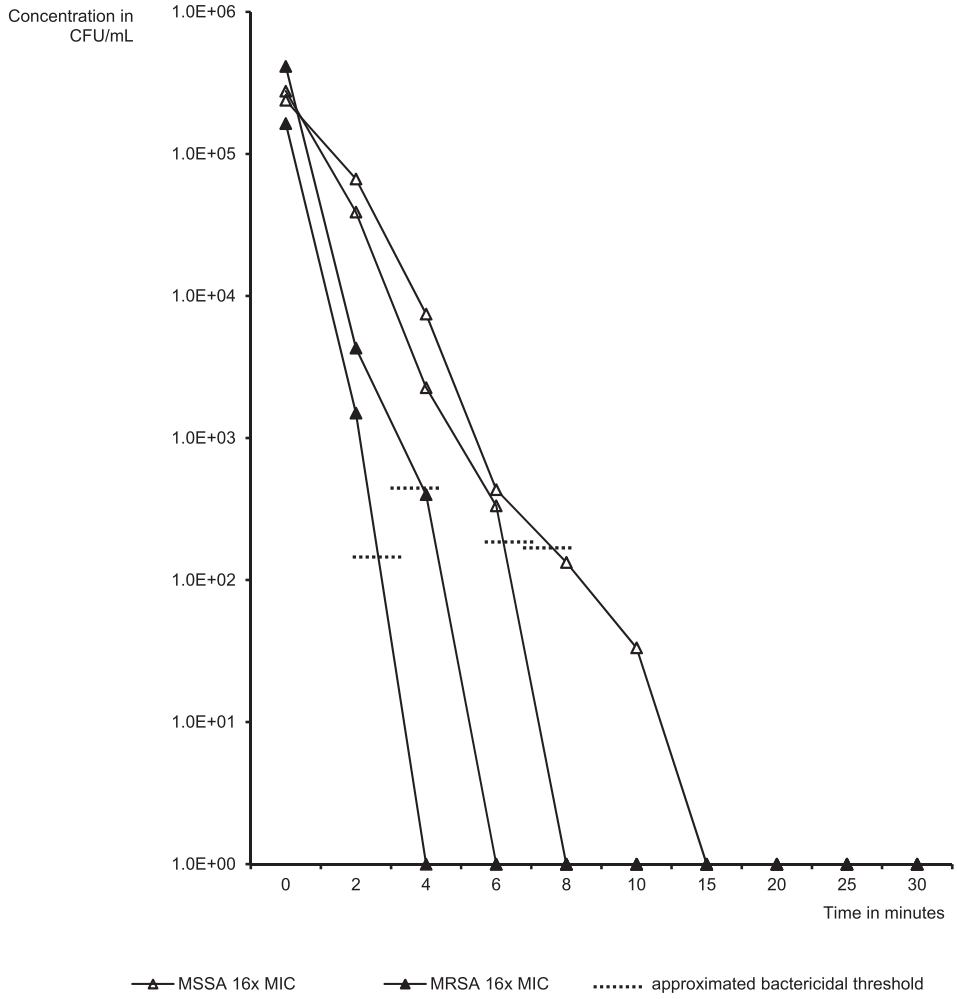

Fig. 2. Detailed time-kill curve analysis of the antimicrobial activity of HY-133 (16-fold MIC) against MSSA and MRSA strains within the first 30 min.

cells. An actual study demonstrated the positive effect of prophage induction against persister cells of *S. aureus* (Sandvik et al., 2015), thus, more research is warranted to clarify the interaction of persister cells and bacteriophage endolysins.

The determination of MIC and MBC values has some pharmacokinetic and pharmacodynamic limitations (Mueller et al., 2004). Therefore, time-kill curves were performed to receive more dynamic insight information. Here, HY-133 demonstrated a rapid and impressive bactericidal activity within the first hour, and the effect was even faster than that of daptomycin. Exemplarily, the initial killing occurred for the 16-fold MIC within the first 15 min. The observed dose-dependent regrowth effects remain unclear, in particular whether this phenomenon represents an artifact of the *in vitro* testing approach. Assay-associated factors such as hiding and surviving of a tiny subset of those bacterial cells adherent on the surfaces of the used microtiter plates could play a role; however, future studies are needed to analyze this phenomenon and its impact on *in vitro* testing procedures.

In methodologically similar studies, *CF*-301 or P128 showed in the majority of cases an entire eradication for the 1-fold MIC up to 6 or 24 h, respectively (Schuch et al., 2014; Vipra et al., 2012), but as mentioned above, the starting concentration of the MIC values were higher, and the observation period in these studies was limited to only 6 or 24 h and thus only partially combarable with our study. Multiple application, increased concentrations, or further improved endolysin stability could probably improve the performance of HY-133. Otherwise, a combination of HY-133 with classical antibiotics could be a potential option also against persister and chronic infections. The synergistic effect was seen previously for other phage endolysins like P128 or *CF*-301 (Nair et al., 2016; Poonacha et al., 2017; Schuch et al., 2014).

Mupirocin revelead only bacteriostatic activity in contrast to HY-133 and daptomycin, but without significant regrowth for 2-fold and higher MIC concentrations and with only 1 initial application up to 24 h and partial up to 48 h. Therefore, it could be speculated that a combination therapy of HY-133 plus mupirocin could enhance the effectiveness of nasal decolonization in terms of reduction of the overall therapy time as well as resistance prevention. HY-133 showed no resistance against tested mupirocin-resistant *S. aureus* isolates. Thus, HY-133 could overcome the rising resistance against current topical antibacterials and antiseptics like, for example, mupirocin or fusidic acid (Humphreys et al., 2016; Williamson et al., 2017).

A recent nasal MRSA decolonization study adiministering a combination of 2 bacteriophages revealed very good activity *in vitro* but unfortunately not in *in vivo* and in an *ex vivo* animal pig model (Verstappen et al., 2016). In contrast, ClyS, another engineered *Staphylococcus*-specific phage lysin, could succesfully be applied in a mouse model (Pastagia et al., 2011). Clinicial phase I/II studies were implemented for nasal MRSA decolonization for P128 (ClinicalTrials.gov NCT01746654) and as additional therapeutic agent for *S. aureus* bloodstream infections/endocarditis for *CF*-301 (ClinicalTrials.gov NCT02439359 and NCT03163446).

A major advantage of phage endolysins is their high specificity towards the target bacteria and, consequently, a minimization of antimicrobial effects on the remaining nasal microbiom, which is in contrast to mupirocin or decolonizing desinfectants. The PRF-119 precursor showed no susceptibility against typical clinical CoNS (Idelevich et al.,

2011), which is in contrast to a current study with P128 (Poonacha et al., 2017). As definite proof, further studies with CoNS are warranted for HY-133, and they should also be extended to analyze potential effects against biofilm formation. Biofilm activity was demonstrated for the bacteriophage K (Kelly et al., 2012; Lungren et al., 2013), P128, and *CF*-301 (Nair et al., 2016; Poonacha et al., 2017; Schuch et al., 2017).

5. Conclusion

As demonstrated by growth curve analyses and MIC and MBC testing, the recombinant endolysin HY-133 demonstrated an exceptionally fast onset of action highly active against all tested *S. aureus* isolates including methicillin- and mupirocin-resistant isolates. Therefore, the use of this chimeric lysin may offer an alternative strategy for MRSA decolonization. Future studies should focus on the regrowth phenomenon, putative synergistic effects in combination with other antimicrobial substances, and the application of HY-133 as possible preventive and therapeutic agent.

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Transparency declarations

S.M. is an employee of HYpharm GmbH and worked on developing HY-133. The other authors have no conflicts of interest to declare.

References

Abedon S. Phage therapy pharmacology: calculating phage dosing. Adv Appl Microbiol 2011;77:1–40. https://doi.org/10.1016/B978-0-12-387044-5.00001-7.

Abedon ST. Commentary: phage therapy of staphylococcal chronic osteomyelitis in experimental animal model. Front Microbiol 2016;7, 1251. https://doi.org/10.3389/fmicb.2016.01251.

Ammerlaan HSM, Kluytmans JAJW, Berkhout H, Buiting A, de Brauwer EIGB, van den Broek PJ, et al. Eradication of carriage with methicillin-resistant *Staphylococcus aureus*: determinants of treatment failure. J Antimicrob Chemother 2011;66:2418–24. https://doi.org/10.1093/jac/dkr250.

Ammerlaan HSM, Kluytmans JAJW, Wertheim HFL, Nouwen JL, Bonten MJM. Eradication of methicillin-resistant *Staphylococcus aureus* carriage: a systematic review. Clin Infect Dis 2009;48:922–30. https://doi.org/10.1086/597291.

Anthony RM, Connor AM, Power EG, French GL. Use of the polymerase chain reaction for rapid detection of high-level mupirocin resistance in staphylococci. Eur J Clin Microbiol Infect Dis 1999;18:30–4.

Balaban NQ, Gerdes K, Lewis K, McKinney JD. A problem of persistence: still more questions than answers? Nat Rev Microbiol 2013;11:587–91.

Barry AL, Badal RE, Hawkinson RW. Influence of inoculum growth phase on microdilution susceptibility tests. J Clin Microbiol 1983;18:645–51.

Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard–tenth edition. CLSI document M07-A10. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. CLSI supplement M100. 27th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.

Cooper CJ, Khan Mirzaei M, Nilsson AS. Adapting drug approval pathways for bacteriophage-based therapeutics. Front Microbiol 2016;7, 1209. https://doi.org/10.3389/fmicb.2016.01209.

Eagle H. A paradoxical zone phenomenon in the bactericidal action of penicillin *in Vitro*. Science 1948;107:44–5. https://doi.org/10.1126/science.107.2767.44.

Eagle H, Musselman AD. The rate of bactericidal action of penicillin *in vitro* as a function of its concentration, and its paradoxically reduced activity at high concentrations against certain organisms. J Exp Med 1948;88:99–131.

von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. N Engl J Med 2001;344:11–6. https://doi.org/10.1056/NEJM200101043440102.

Gilmer DB, Schmitz JE, Euler CW, Fischetti VA. Novel bacteriophage lysin with broad lytic activity protects against mixed infection by *Streptococcus pyogenes* and methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2013;57:2743–50. https://doi.org/10.1128/AAC.02526-12.

Hetem DJ, Bonten MJM. Clinical relevance of mupirocin resistance in *Staphylococcus aureus*. J Hosp Infect 2013;85:249–56. https://doi.org/10.1016/j.jhin.2013.09.006.

Hübner C, Hübner N-O, Hopert K, Maletzki S, Flessa S. Analysis of MRSA-attributed costs of hospitalized patients in Germany. Eur J Clin Microbiol Infect Dis 2014;33:1817–22. https://doi.org/10.1007/s10096-014-2131-x.

Humphreys H, Becker K, Dohmen PM, Petrosillo N, Spencer M, van Rijen M, et al. *Staphylococcus aureus* and surgical site infections: benefits of screening and decolonization before surgery. J Hosp Infect 2016;94:295–304. https://doi.org/10.1016/j.jhin.2016.06.011.

Idelevich EA, Schaumburg F, Knaack D, Scherzinger AS, Mutter W, Peters G, et al. The recombinant bacteriophage endolysin HY-133 exhibits in vitro activity against different African clonal lineages of the *Staphylococcus aureus* complex, including *Staphylococcus schweitzeri*. Antimicrob Agents Chemother 2016;60:2551–3. https://doi.org/10.1128/AAC.02859-15.

Idelevich EA, von Eiff C, Friedrich AW, Iannelli D, Xia G, Peters G, et al. *In vitro* activity against *Staphylococcus aureus* of a novel antimicrobial agent, PRF-119, a recombinant chimeric bacteriophage endolysin. Antimicrob Agents Chemother 2011;55:4416–9. https://doi.org/10.1128/AAC.00217-11.

Kaspar U, Kriegeskorte A, Schubert T, Peters G, Rudack C, Pieper DH, et al. The culturome of the human nose habitats reveals individual bacterial fingerprint patterns. Environ Microbiol 2016;18:2130–42. https://doi.org/10.1111/1462-2920.12891.

Kelly D, McAuliffe O, Ross RP, Coffey A. Prevention of *Staphylococcus aureus* biofilm formation and reduction in established biofilm density using a combination of phage K and modified derivatives. Lett Appl Microbiol 2012;54:286–91. https://doi.org/10.1111/j.1472-765X.2012.03205.x.

Köck R, Becker K, Cookson B, van Gemert-Pijnen JE, Harbarth S, Kluytmans J, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA): burden of disease and control challenges in Europe. Euro Surveill 2010;15:19688.

Lungren MP, Christensen D, Kankotia R, Falk I, Paxton BE, Kim CY, et al. Bacteriophage K for reduction of *Staphylococcus aureus*biofilm on central venous catheter material. Bacteriophage 2013;3, e26825.

McConeghy KW, Mikolich DJ, LaPlante KL. Agents for the decolonization of methicillin-resistant *Staphylococcus aureus*. Pharmacotherapy 2009;29:263–80. https://doi.org/10.1592/phco.29.3.263.

Meyer V, Kerk N, Mellmann A, Friedrich A, Luger TA, Goerge T. MRSA eradication in dermatologic outpatients—theory and practice. J Dtsch Dermatol Ges 2012;10:186–96. https://doi.org/10.1111/j.1610-0387.2011.07861.x.

Mueller M, de la Peña A, Derendorf H. Issues in pharmacokinetics and pharmacodynamics of anti-infective agents: kill curves versus MIC. Antimicrob Agents Chemother 2004;48:369–77.

Nair S, Desai S, Poonacha N, Vipra A, Sharma U. Antibiofilm activity and synergistic inhibition of *Staphylococcus aureus* biofilms by bactericidal protein P128 in combination with antibiotics. Antimicrob Agents Chemother 2016;60:7280–9. https://doi.org/10.1128/AAC.01118-16.

NCCLS. Methods for determining bactericidal activity of antimicrobial agents; approved guideline. NCCLS document M-26A. Wayne, PA, USA: NCCLS; 1999.

Nelson RE, Jones M, Rubin MA. Decolonization with mupirocin and subsequent risk of methicillin-resistant *Staphylococcus aureus* carriage in Veterans Affairs hospitals. Infect Dis Ther 2012;1(1). https://doi.org/10.1007/s40121-012-0001-3.

Pastagia M, Euler C, Chahales P, Fuentes-Duculan J, Krueger JG, Fischetti VA. A novel chimeric Lysin shows superiority to mupirocin for skin decolonization of methicillin-resistant and -sensitive *Staphylococcus aureus* strains. Antimicrob Agents Chemother 2011;55:738–44. https://doi.org/10.1128/AAC.00890-10.

Pastagia M, Schuch R, Fischetti VA, Huang DB. Lysins: the arrival of pathogen-directed anti-infectives. J Med Microbiol 2013;62:1506–16. https://doi.org/10.1099/jmm.0.061028-0.

Paul VD, Rajagopalan SS, Sundarrajan S, George SE, Asrani JY, Pillai R, et al. A novel bacteriophage tail-associated muralytic enzyme (TAME) from phage K and its development into a potent antistaphylococcal protein. BMC Microbiol 2011;11:226. https://doi.org/10.1186/1471-2180-11-226.

Poonacha N, Nair S, Desai S, Tuppad D, Hiremath D, Mohan T, et al. Efficient killing of planktonic and biofilm-embedded coagulase-negative staphylococci by bactericidal protein P128. Antimicrob Agents Chemother 2017;61. https://doi.org/10.1128/AAC.00457-17.

Sandvik EL, Fazen CH, Henry TC, Mok WWK, Brynildsen MP. Non-monotonic survival of *Staphylococcus aureus* with respect to ciprofloxacin concentration arises from prophage-dependent killing of persisters. Pharmaceuticals 2015;8:778–92. https://doi.org/10.3390/ph8040778.

Schuch R, Khan BK, Raz A, Rotolo JA, Wittekind M. Bacteriophage Lysin CF-301, a potent antistaphylococcal biofilm agent. Antimicrob Agents Chemother 2017;61:e02666-16. https://doi.org/10.1128/AAC.02666-16. (pii: e02666-16).

Schuch R, Lee HM, Schneider BC, Sauve KL, Law C, Khan BK, et al. Combination therapy with lysin CF-301 and antibiotic is superior to antibiotic alone for treating methicillin-resistant *Staphylococcus aureus*-induced murine bacteremia. J Infect Dis 2014;209:1469–78. https://doi.org/10.1093/infdis/jit637.

Thomas CM, Hothersall J, Willis CL, Simpson TJ. Resistance to and synthesis of the antibiotic mupirocin. Nat Rev Microbiol 2010;8:281–9. https://doi.org/10.1038/nrmicro2278.

Verstappen KM, Tulinski P, Duim B, Fluit AC, Carney J, van Nes A, et al. The effectiveness of bacteriophages against methicillin-resistant *Staphylococcus aureus* ST398 nasal colonization in pigs. PLoS One 2016;11, e0160242. https://doi.org/10.1371/journal.pone.0160242.

Vipra AA, Desai SN, Roy P, Patil R, Raj JM, Narasimhaswamy N, et al. Antistaphylococcal activity of bacteriophage derived chimeric protein P128. BMC Microbiol 2012;12:41. https://doi.org/10.1186/1471-2180-12-41.

Williamson DA, Carter GP, Howden BP. Current and emerging topical antibacterials and antiseptics: agents, action, and resistance patterns. Clin Microbiol Rev 2017;30:827–60. https://doi.org/10.1128/CMR.00112-16.