**Supplementary Table S1.** Results of bacterial identification by Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) shown as matched pattern, score value, and NCBI identification number of best match.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Isolates** |  | **MALDI-TOF MS**  **Score Value** | | |
|  |  | **Matched Pattern** | **Score Value** | **NCBI Identifier** |
| ***MSSA*** |  |  |  |  |
| ATCC-29213 |  | Staphylococcus aureus ssp aureus DSM 3463 DSM | 2.20 | 46170 |
| 280/20 |  | Staphylococcus aureus ssp aureus DSM 3463 DSM | 2.16 | 46170 |
| 249/20 |  | Staphylococcus aureus ssp aureus DSM 3463 DSM | 2.12 | 46170 |
| 204/20 |  | Staphylococcus aureus ATCC 33862 THL | 2.15 | 1280 |
| 231/20 |  | Staphylococcus aureus ATCC 33591 THL | 2.12 | 1280 |
|  |  |  |  |  |
| ***MRSA*** |  |  |  |  |
| ATCC-33592 |  | Staphylococcus aureus ssp aureus DSM 3463 DSM | 2.12 | 46170 |
| DSMZ-23622 |  | Staphylococcus aureus ssp aureus DSM 3463 DSM | 2.19 | 46170 |
| 874/19 |  | Staphylococcus aureus ATCC 33591 THL | 2.04 | 1280 |
| 845/19 |  | Staphylococcus aureus ATCC 33591 THL | 2.12 | 1280 |
| 563/18 |  | Staphylococcus aureus ssp aureus DSM 799 DSM | 2.28 | 46170 |

\* MSSA, methicillin-susceptible Staphylococcus aureus; MRSA methicillin-resistant Staphylococcus aureus; ATCC, American type culture collection; DSMZ, german collection of microorganisms and cell cultures; MALDI-TOF MS, Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry; NCBI, National Center for Biotechnology Information

**Supplementary Table S2.** Oligonucleotides used for PCR analysis

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **Forward oligonucleotide (5’ – 3’)** | **Reverse oligonucleotide (5’ – 3’)** | **References** |
| **PBP1** | 5‘-ACCAATAACAAAAGATACTGCTGAA-3‘ | 5‘-CGTCACCCATAAAACTTACAAAATA-3‘ | [1] |
| **PBP2** | 5‘-TAACAGAAACCAAGCAACAGAT-3‘ | 5‘-TTTGTCGTAAAGCATCATAAATAGA-3‘ | [1] |
| **PBP2a** | 5‘-CAACTAATGAAACAGAAAGTCGTAA-3‘ | 5‘-TAATGTATGTGCGATTGTATTGCTA-3‘ | [1] |
| **PBP3** | 5‘-ACAAAATCCTAAAAATGGAGACA-3‘ | 5‘-ACCTTGGAAATGTAATGGTTCAT-3‘ | [1] |
| **PBP4** | 5‘-ACGATGTTTTACCAAGTGATTTTAG-3‘ | 5‘-ACCAATGATAGTGAATAATGGATGT-3‘ | [1] |
| **gap** | 5‘-CAAAATACACAAGACGCACC-3‘ | 5‘-CCGATAGCTTTAGCAGCAC-3‘ | [\*] |
| **mecA** | 5′-GTTGTAGTTGTCGGGTTTGG-3’ | 5′-CTTCCACATACCATCTTCTTTAAC-3’ | [2] |

PBP1-4, penicillin binding protein 1-4; gap, the Staphylococcus aureus gene encoding the glyceraldehyde-3-phosphate dehydrogenase

[1] Navratna V, Nadig S, Sood V, Prasad K, Arakere G, Gopal B. Molecular basis for the role of Staphylococcus aureus penicillin binding protein 4 in antimicrobial resistance. *J Bacteriol* 2010; **192**: 134–44.

[2] Terpstra S, Noordhoek GT, Voesten HG, Hendriks B, Degener J. Rapid emergence of resistant coagulase-negative staphylococci on the skin after antibiotic prophylaxis. The Journal of hospital infection. 1999

[\*] These primers were designed for the present study

**Supplementary Table S3.** Relative gene expression of penicillin binding proteins (PBP1, PBP2, PBP2`, PBP3 and PBP4) determined for one fosfomycin-susceptible (ATCC-33592) and one fosfomycin-resistant (DSMZ-23622) MRSA isolate using RT-PCR with gap as housekeeping gene and a no treatment control as reference. Bacteria were exposed to either cefazolin or fosfomycin at a concentration corresponding to 0.25 times of their respective minimum inhibitory concentrations (MIC) for a time period of 4 hours. Data is stated as mean (±SD) relative quantification values and individual Ct values

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | **MRSA 33592** | | **MRSA 23622** | |
|  |  | **Mean RQ (±SD)** | **Ct values** | **Mean RQ (±SD)** | **Ct values** |
| **NTC** | **pbp1** | 1.000 (±0.120) | |  | | --- | | 25,419 | | 25,465 | | 25,417 | | 25,238 | | 1.000 (±0.080) | |  | | --- | | 27,619 | | 27,753 | | 27,723 | | 27,720 | |
|  |  |  |  |  |  |
| **CEFA 0.25xMIC** | **pbp1** | 0.639 (±0.119) | |  | | --- | | 26,827 | | 26,738 | | 27,304 | | 26,971 | | 0.405 (±0.034) | |  | | --- | | 28,021 | | 28,212 | | 28,116 | |
|  |  |  |  |  |  |
| **FOF 0.25xMIC** | **pbp1** | 0.420 (±0.061) | |  | | --- | | 26,149 | | 26,284 | | 26,618 | | 26,147 | | 2.807 (±0.079) | |  | | --- | | 24,939 | | 24,966 | | 24,960 | | 25,001 | |
|  |  |  |  |  |  |
| **NTC** | **pbp2** | 1.000 (±0.129) | |  | | --- | | 25,328 | | 25,179 | | 25,476 | | 25,265 | | 1.000 (±0.145) | |  | | --- | | 28,332 | | 27,937 | | 28,079 | | 27,887 | |
|  |  |  |  |  |  |
| **CEFA 0.25xMIC** | **pbp2** | 0.784 (±0.089) | |  | | --- | | 26,615 | | 26,580 | | 26,654 | | 26,519 | | 0.476 (±0.085) | |  | | --- | | 28,484 | | 28,452 | | 28,080 | | 27,937 | |
|  |  |  |  |  |  |
| **FOF 0.25 xMIC** | **pbp2** | 0.376 (±0.027) | |  | | --- | | 26,483 | | 26,430 | | 26,259 | | 26,385 | | 3.718 (±0.219) | |  | | --- | | 24,967 | | 24,908 | | 24,985 | | 24,804 | |
|  |  |  |  |  |  |
| **NTC** | **pbp2a** | 1.000 (±0.110) | |  | | --- | | 24,203 | | 24,258 | | 24,117 | | 24,147 | | 1.000 (±0.102) | |  | | --- | | 27,127 | | 27,263 | | 27,034 | |
|  |  |  |  |  |  |
| **CEFA 0.25xMIC** | **pbp2a** | 0.966 (±0.112) | |  | | --- | | 25,204 | | 25,134 | | 25,077 | | 25,227 | | 2.109 (±0.144) | |  | | --- | | 25,205 | | 25,141 | | 25,234 | | 25,113 | |
|  |  |  |  |  |  |
| **FOF 0.25xMIC** | **pbp2a** | 0.151 (±0.009) | |  | | --- | | 26,536 | | 26,518 | | 26,681 | | 26,541 | | 2.773 (±0.151) | |  | | --- | | 24,359 | | 24,496 | | 24,475 | | 24,356 | |
|  |  |  |  |  |  |
| **NTC** | **pbp3** | 1.000 (±0.128) | |  | | --- | | 24,964 | | 24,844 | | 24,974 | | 24,716 | | 1.000 (±0.131) | |  | | --- | | 27,718 | | 27,571 | | 27,509 | | 27,299 | |
|  |  |  |  |  |  |
| **CEFA 0.25xMIC** | **pbp3** | 0.437 (±0.061) | |  | | --- | | 26,964 | | 27,103 | | 26,813 | | 27,112 | | 0.409 (±0.048) | |  | | --- | | 27,775 | | 28,086 | | 27,803 | | 28,032 | |
|  |  |  |  |  |  |
| **FOF 0.25xMIC** | **pbp3** | 0.159 (±0.009) | |  | | --- | | 27,171 | | 27,278 | | 27,138 | | 2.711 (±0.262) | |  | | --- | | 24,964 | | 24,953 | | 24,751 | | 24,680 | |
|  |  |  |  |  |  |
| **NTC** | **pbp4** | 1.000 (±0.191) | |  | | --- | | 25,720 | | 25,471 | | 25,195 | | 1.000 (±0.077) | |  | | --- | | 27,894 | | 27,904 | | 27,993 | | 27,976 | |
|  |  |  |  |  |  |
| **CEFA 0.25xMIC** | **pbp4** | 0.389 (±0.043) | |  | | --- | | 27,725 | | 27,786 | | 27,747 | | 0.507 (±0.113) | |  | | --- | | 27,810 | | 27,935 | | 28,556 | | 27,821 | |
|  |  |  |  |  |  |
| **FOF 0.25xMIC** | **pbp4** | 0.119 (±0.010) | |  | | --- | | 28,295 | | 28,142 | | 28,278 | | 28,058 | | 3.160 (±0.123) | |  | | --- | | 25,000 | | 25,083 | | 25,064 | | 24,986 | |
|  |  |  |  |  |  |
| **NTC** | **gap** |  | |  | | --- | | 21,708 | | 21,767 | | 21,415 | | 21,588 | |  | |  | | --- | | 26,132 | | 26,174 | | 25,970 | | 25,977 | |
|  |  |  |  |  |  |
| **CEFA 0.25xMIC** | **gap** |  | |  | | --- | | 22,560 | | 22,632 | | 22,688 | | 22,315 | |  | |  | | --- | | 25,268 | | 25,139 | | 25,107 | |
|  |  |  |  |  |  |
| **FOF 0.25xMIC** | **gap** |  | |  | | --- | | 21,342 | | 21,250 | | 21,309 | | 21,237 | |  | |  | | --- | | 24,831 | | 24,813 | | 24,771 | | 24,844 | |

\*MRSA, methicillin-resistant Staphylococcus aureus; mean RQ, mean relative quantification values (±standard deviation) calculated by ΔΔCt using a no treatment control as reference; NTC, no treatment control; pbp, penicillin binding protein; CEF, cefazolin; FOF, fosfomycin; MIC, minimum inhibitory concentration: gap, the Staphylococcus aureus gene encoding the glyceraldehyde-3-phosphate dehydrogenase

**Supplementary Figure S1.** Survival curves of *G.mellonella* larvae infected with methicillin-resistant *Staphylococcus aureus* (ATCC-33592) followed by treatment with fosfomycin at doses of 200mg/kg (20 larvae), 50mg/kg (19 larvae), 25mg/kg (19 larvae), 12.5mg/kg (20 larvae), 6.25mg/kg (20 larvae) and 0.1mg/kg (12 larvae). The control curve represents the pooled data of three experiments performed on separate days (32 larvae, 11 larvae, 20 larvae). Data sets of fosfomycin 200mg/kg, 50mg/kg, 25mg/kg and 12.5mg/kg were nudged to prevent an overlap.

**Supplementary Figure S2.** Agarose gel electrophoresis for the detection of the mecA resistance gene. 1: molecular weight marker (100bp Plus Opti DNA Marker, ABMGood, Canada); 2. 280/20; 3. 249/20; 4. 204/20; 5. 231/20; 6. 874/19; 7. 845/19; 8. 563/18; 9. ATCC-29213 (internationally standardized methicillin-susceptible Staphylococcus aureus strain); 10. ATCC-33592 (internationally standardized methicillin-resistant Staphylococcus aureus strain); 11. DSMZ-23612; 12. molecular weight marker (100bp Plus Opti DNA Marker, ABMGood, Canada); 13. negative controlC:\Users\Labor 6\Matthias ownCloud\3P\Studie_FOF-CEFA_Lena\mecA-pcr-matthias-16-04-2021.Tif