11. McClure JA, Conly JM, Lau V *et al.* Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton–Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from -resistant staphylococci. *J Clin Microbiol* 2006; **44**: 1141–1144.
12. Oliveira DC, de Lencastre H. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2002; **46**: 2155–2161.
13. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000; **38**: 1008–1015.
14. Budimir A, Deurenberg RH, Plecko V, Vink C, Kalenic S, Stobberingh EE. Molecular characterization of methicillin-resistant *Staphylococcus aureus* bloodstream isolates from Croatia. *J Antimicrob Chemother* 2006; **57**: 331–334.
15. Kaplan SL, Hulten KG, Gonzalez BE *et al.* Three-year surveillance of community-acquired *Staphylococcus aureus* infections in children. *Clin Infect Dis* 2005; **40**: 1785–1791.
16. Lina G, Piemont Y, Godail-Gamot F *et al.* Involvement of Panton–Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis* 1999; **29**: 1128–1132.
17. Cirlan M, Saad M, Coman G *et al.* International spread of major clones of methicillin resistant *Staphylococcus aureus*: nosocomial endemicity of multi locus sequence type 239 in Saudi Arabia and Romania. *Infect Genet Evol* 2005; **5**: 335–339.
18. Hsu LY, Koh YL, Chlebicka NL *et al.* Establishment of ST30 as the predominant clonal type among community-associated methicillin-resistant *Staphylococcus aureus* isolates in Singapore. *J Clin Microbiol* 2006; **44**: 1090–1093.
19. Denis O, Deplano A, De Beenhouwer H *et al.* Polyclonal emergence and importation of community-acquired methicillin-resistant *Staphylococcus aureus* strains harbouring Panton–Valentine leucocidin genes in Belgium. *J Antimicrob Chemother* 2005; **56**: 1103–1106.
20. Gonzalez BE, Rueda AM, Shelburne SA, Musher DM, Hamill RJ, Hulten KG. Community-associated strains of methicillin-resistant *Staphylococccus aureus* as the cause of healthcare-associated infection. *Infect Control Hosp Epidemiol* 2006; **27**: 1051–1056.

RESEARCH NOTE

Effect of antibiotics, alone and in combination, on Panton–Valentine leukocidin production by a *Staphylococcus aureus* reference strain

O. Dumitrescu, C. Badiou, M. Bes, M.-E. Reverdy, F. Vandenesch, J. Etienne and G. Lina

INSERM U851 and Université Lyon 1, Centre National de Référence des Staphylocoques, Faculté Laennec, Lyon, France

ABSTRACT

The capacity of *Staphylococcus aureus* strain LUG855 to release Panton–Valentine leukocidin (PVL) in the presence of sub-inhibitory concentrations of anti-staphylococcal drugs was examined. Oxacillin enhanced PVL release 2.5-fold, while clindamycin, linezolid, fusidic acid and rifampicin were inhibitory, and vancomycin, pristinamycin, tetracycline, ofloxacin and cotrimoxazole had no effect. In combination with oxacillin, sub-inhibitory concentrations of clindamycin or rifampicin inhibited PVL induction significantly, linezolid was less inhibitory, and fusidic acid did not inhibit PVL induction by oxacillin. These data support the use of oxacillin in combination with clindamycin, rifampicin or linezolid for the treatment of PVL-positive *S. aureus* infections.

Keywords Clindamycin, fusidic acid, linezolid, oxacillin, Panton–Valentine leukocidin, rifampicin, *Staphylococcus aureus*

Original Submission: 17 August 2007; **Revised Submission:** 15 October 2007; **Accepted:** 14 November 2007

Clin Microbiol Infect 2008; **14:** 384–388
10.1111/j.1469-0691.2007.01947.x

Corresponding author and reprint requests: G. Lina, Centre National de Référence des Staphylocoques, 7 rue Guillaume Paradin, 69372 Lyon cedex 08, France
E-mail: gerard.lina@chu-lyon.fr

Staphylococcus aureus is a major human pathogen. Depending on the setting, 5–50% of *S. aureus* isolates produce Panton–Valentine leukocidin (PVL), a cytotoxin that causes tissue damage [1,2]. PVL production has been linked to severe infections such as necrotising pneumonia, necrotising fasciitis and osteomyelitis [3–6]. PVL-associated necrotising pneumonia has a mortality rate of 75%, and complications are more frequent in osteomyelitis caused by PVL-expressing strains.

It has been shown previously that sub-inhibitory concentrations of β-lactams augment PVL production, while agents such as clindamycin and linezolid reduce the release of PVL by *S. aureus* [7,8], suggesting that the choice of antibacterial agents for the treatment of PVL-positive staphylococcal infections should take into account their possible effect on toxin release. The present study extends previous work [7] by examining the effect of vancomycin, ofloxacin, co-trimoxazole, pristinamycin, clindamycin, fusidic acid, linezolid, tetracycline and rifampicin, alone or in combination with oxacillin, on PVL release *in vitro* by the methicillin-sensitive reference PVL-producing *S. aureus* strain LUG855 [7].

Experimental procedures were as close as possible to CSLI recommendations for MIC determinations [9]. PVL levels in culture supernatants were determined using a specific ELISA [7]. However, when Mueller–Hinton (MH) medium and CSLI procedures were used, PVL levels were close to the detection limit of the ELISA in the absence of antibiotics (data not shown). MH medium was thus replaced by casein hydrolysate and yeast extract (CCY) medium, which increased PVL levels 50-fold and MICs by one or two dilution steps, except for oxacillin and pristinamycin (data not shown). As MICs of rifampicin were extremely low (<0.006 mg/L), the effect of rifampicin on PVL production by LUG855 could not be investigated. Therefore, a *S. aureus* mutant with intermediate susceptibility to rifampicin (LUG855-R5) was obtained by culturing strain LUG855 on MH agar supplemented with rifampicin; this was assessed for the stability of its rifampicin resistance (MIC, 2 mg/L) as described previously [10]. As the PVL levels produced by LUG855-R5 and LUG855 were identical (results not shown), LUG855-R5 was then used to examine the effect of rifampicin on PVL production.

To examine the effect of antibiotics on PVL release, PVL was quantified in the culture supernatant of LUG855 incubated for 24 h in the presence of sub-inhibitory concentrations (0.5, 0.25 and 0.125 × MIC) of oxacillin, vancomycin, clindamycin, linezolid, pristinamycin, fusidic acid, tetracycline, ofloxacin and co-trimoxazole, and also in the culture supernatant of LUG855-R5 incubated for 24 h in the presence of sub-inhibitory concentrations of rifampicin. Bacterial counts were determined by the dilution and plating method, with PVL production expressed as μg of PVL/$\log_{10}$ CFU/mL.

PVL production was increased significantly (up to 2.5-fold) by oxacillin at 0.125 and 0.25 × MIC (Fig. 1). In contrast, clindamycin, linezolid, fusidic acid and rifampicin had a concentration-dependent inhibitory effect on PVL production at 0.125–0.5 × MIC. PVL production started to decrease significantly at 0.125 × MIC of clindamycin and rifampicin, and at 0.25 × MIC of linezolid and fusidic acid. Pristinamycin, tetracycline and ofloxacin inhibited PVL production at 0.5 × MIC, but not at lower concentrations. Co-trimoxazole and vancomycin had no effect on PVL production.

The effect of the strongest inhibitory drugs (i.e., clindamycin, linezolid, fusidic acid and rifampicin) on the enhancement of PVL production by oxacillin was then examined. A modified chequerboard method with CCY medium was used to determine the inhibitory effect of antibiotics in combination as recommended by the CLSI [9]. After incubation, bacterial counts and PVL levels were determined, and growth inhibition by antibiotic combinations was assessed using the fractional inhibitory concentration index, with antibiotic combinations defined as antagonistic, indifferent or synergic [11]. Combinations were indifferent, with the exception of oxacillin plus linezolid, which was synergic. PVL release was inhibited significantly by sub-inhibitory concentrations of oxacillin with either clindamycin or rifampicin in all the combinations tested (Fig. 2). When combined with 0.25 × MIC of oxacillin, linezolid inhibited PVL release at 0.5 and 0.25 × MIC, but not at 0.125 × MIC. When combined with 0.125 × MIC of oxacillin, linezolid inhibited PVL release at 0.5 × MIC but not at lower concentrations. In combination with oxacillin, fusidic acid inhibited PVL release only at 0.5 × MIC. With other concentrations of fusidic acid, PVL release was still increased in the presence of oxacillin.