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RESEARCH NOTE

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

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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*

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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.

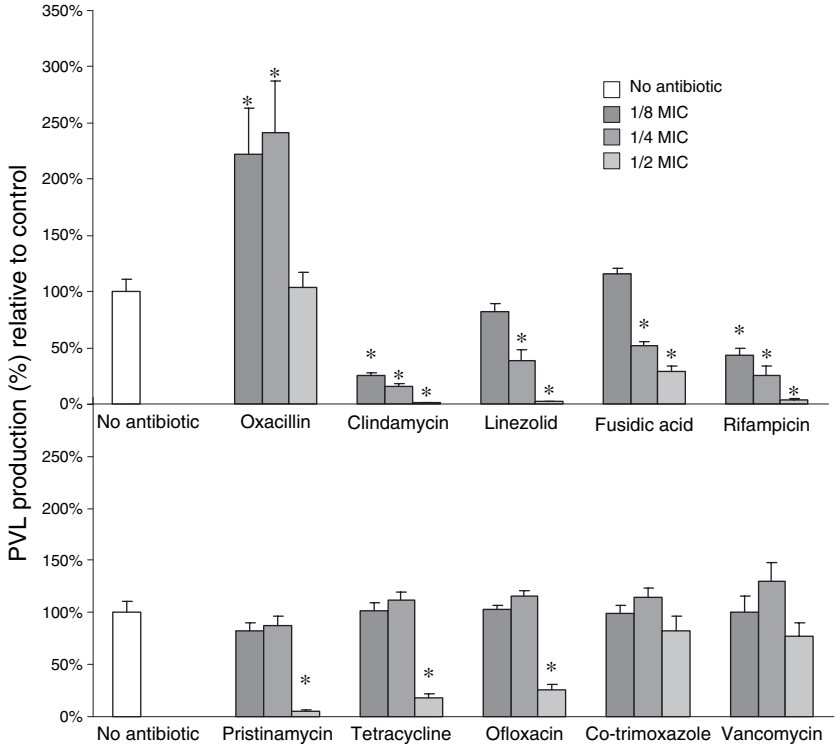

Fig. 1. Effect of antibiotics on production of Panton–Valentine leukocidin (PVL). Results are given as the ratio (expressed as a percentage) of PVL (μg)/$\log_{10}$ CFU of bacteria cultured in the presence of the indicated concentration of antibiotic to the mean PVL μg/$\log_{10}$ CFU of bacteria cultured without antibiotics. Values are means ± SD of three different experiments. *denotes a statistically significant difference ($p < 0.05$) to the control (the corresponding isolate grown without antibiotic), according to one-way ANOVA followed by *a posteriori* Dunnett's test.

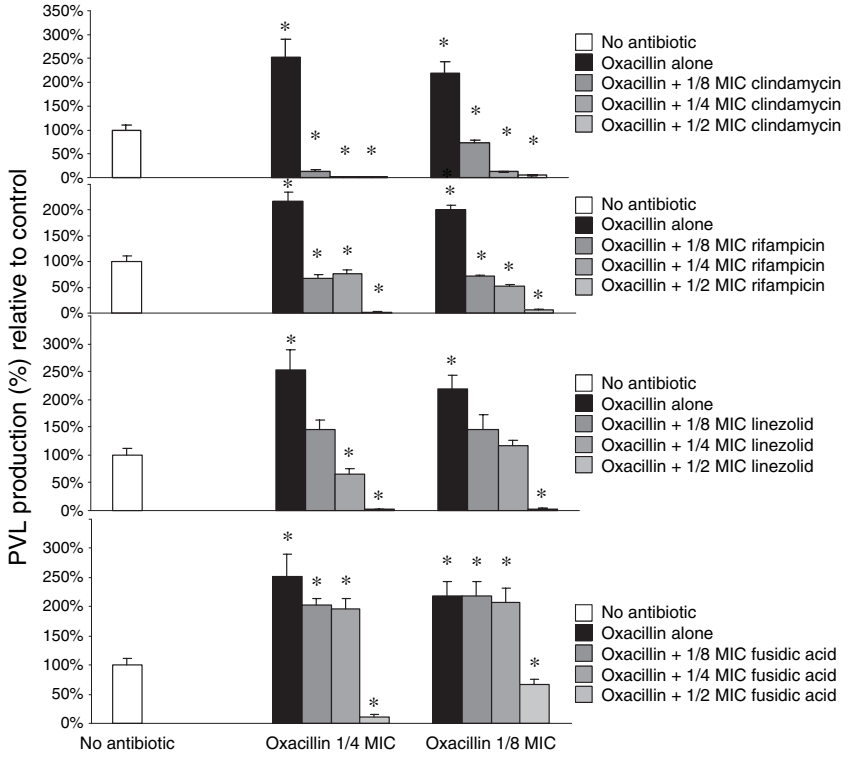

Fig. 2. Effects of clindamycin, linezolid, fusidic acid and rifampicin in combination with oxacillin on the production of Panton–Valentine leukocidin (PVL). Results are given as the ratio (expressed as a percentage) of PVL (μg)/$\log_{10}$ CFU of bacteria cultured in the presence of the indicated concentration of antibiotic to the mean PVL (μg)/$\log_{10}$ CFU of bacteria cultured without antibiotics. Values are means ± SD of three different experiments. *denotes a statistically significant difference ($p < 0.05$) to the control (the corresponding isolate grown without antibiotic) according to one-way ANOVA followed by *a posteriori* Dunnett's test.

Thus, in summary, sub-inhibitory antibiotic concentrations could either up-regulate (oxacillin) or down-regulate (clindamycin, rifampicin, linezolid and fusidic acid) PVL release by *S. aureus*. Increased release of toxins in the presence of β-lactams has also been observed

with other *S. aureus* toxins [8,12], and seems to be related to transcriptional activation. Clindamycin and linezolid have been shown previously to reduce the production of other toxins [13–15], possibly through their impact on protein synthesis and transcription [8,12]. There are no published data concerning the inhibitory effect of rifampicin on *S. aureus* toxin production, but rifampicin inhibits the transcription of other bacterial genes [16,17]. Pristinamycin, tetracycline and ofloxacin inhibited PVL production only when used at concentrations close to the MIC. This was unexpected, as pristinamycin and tetracycline both inhibit the synthesis of bacterial proteins, including toxins [18,19]. Finally, sub-inhibitory concentrations of co-trimoxazole and vancomycin had no effect on PVL release. Thus, sub-inhibitory oxacillin concentrations enhanced PVL production, while clindamycin, linezolid, fusidic acid and rifampicin inhibited PVL production, and the other antibiotics tested had little or no effect.

As semi-synthetic penicillins are still the most widely prescribed anti-staphylococcal agents, subsequent studies then investigated whether the drugs with the strongest inhibitory effect could abolish the increase in PVL production mediated by oxacillin. Clindamycin and rifampicin both significantly reduced PVL production in the presence of oxacillin, while linezolid and fusidic acid had an inconsistent effect. However, as reported previously [20], linezolid was the only antibiotic that inhibited bacterial growth synergically with oxacillin.

Taken together, these data confirm that β-lactam agents up-regulate PVL release, and that PVL induction is suppressed in combination with clindamycin, rifampicin or linezolid. This provides a logical basis for future in-vivo studies designed to examine whether antibiotic combinations that inhibit both bacterial replication and PVL release could improve the outcome of severe infections caused by PVL-producing *S. aureus* strains.

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RESEARCH NOTE

Seroprevalence of IgG antibodies against *Bordetella pertussis* in healthy individuals aged 4–24 years in Turkey

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ABSTRACT

The distribution of IgG antibodies to *Bordetella pertussis* was investigated in serum samples from 550 subjects, aged 4–24 years, to determine the optimal age for booster immunisation. Levels of antibody to *B. pertussis* antigens were determined using an ELISA that measures a mixture of pertussis toxin, filamentous haemagglutinin and lipopolysaccharide. Geometric mean titres of anti-pertussis antibodies in subjects aged 4–6 years were significantly lower than those in other age groups, which reflects waning immunity following vaccination. High positive titres in older children and adolescents suggested acquired *B. pertussis* infection, and booster doses at the ages of 7 and 15 years are therefore suggested.

Keywords Antibodies, *Bordetella pertussis*, ELISA, immunity, seroprevalence, vaccination recommendations

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Even if the cellular and humoral immune responses are both involved in conferring protection against *Bordetella pertussis* [1], determination of the seroepidemiology of pertussis makes possible the evaluation of patterns of pertussis immunity in a given population, and helps define the target population for pertussis booster vaccination [2,3]. The aims of the present study were to determine the distribution of IgG antibodies to *B. pertussis* among different age groups in Turkey, to evaluate the rate of decrease in vaccine-acquired immunity, and to determine the optimal age and frequency for booster immunisations.

Antibody levels to *B. pertussis* antigens were measured in serum samples obtained from 550 (305 male, 245 female) healthy subjects, aged 4–24 years, who visited the Gazi University Medical School well-child clinic, or the paediatric and adolescent health examination clinics, for check-up between April and June 2006, and who did not have a prolonged history of coughing in the preceding month. All study subjects had received whole-cell pertussis vaccine three times in the first year of life, followed by a booster at the age of 18 months. The whole-cell pertussis vaccines used in Turkey for the last 20 years have been obtained from several different foreign companies, and most recently from the Serum Institute (Pune, India). Each single 0.5-mL dose contains diphtheria toxoid ≤25 Lf, tetanus toxoid ≥5 Lf and *B. pertussis* ≥4 IU, adsorbed on aluminium phosphate ≥1.5 mg, with thiomersal 0.01% w/v as preservative (http://www.seruminsitute.com/content/products/product_list.htm). Informed