

## β-Lactam Antibiotics Induce the SOS Response and Horizontal Transfer of Virulence Factors in *Staphylococcus aureus*

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**Antibiotics that interfere with DNA replication and cell viability activate the SOS response. In *Staphylococcus aureus*, the antibiotic-induced SOS response promotes replication and high-frequency horizontal transfer of pathogenicity island-encoded virulence factors. Here we report that β-lactams induce a bona fide SOS response in *S. aureus*, characterized by the activation of the RecA and LexA proteins, the two master regulators of the SOS response. Moreover, we show that β-lactams are capable of triggering staphylococcal prophage induction in *S. aureus* lysogens. Consequently, and as previously described for SOS induction by commonly used fluoroquinolone antibiotics, β-lactam-mediated phage induction also resulted in replication and high-frequency transfer of the staphylococcal pathogenicity islands, showing that such antibiotics may have the unintended consequence of promoting the spread of bacterial virulence factors.**

Previous studies have revealed that certain genes of *Staphylococcus aureus*, including those encoding proteins involved in cell wall metabolism and stress responses, are upregulated after treatment with β-lactam antibiotics (5, 15, 18), suggesting the existence of a cell wall stimulon induced in response to cell wall-active agents. In addition, it is well documented that in *Escherichia coli*, the SOS response is induced by antibiotics that interfere with cell wall synthesis (8, 12) as well as DNA replication (13). The SOS system represents a global response to DNA damage that upregulates genes involved in DNA repair and cell survival (2, 4). The SOS response is governed by the LexA and RecA proteins. The LexA protein binds to operator sites of SOS-regulated genes, effectively repressing their expression. Conversely, the presence of DNA lesions activates RecA, which promotes the autocatalytic cleavage of LexA at a specific Ala-Gly bond (7). Cleaved LexA is unable to bind DNA, leading to the derepression of SOS genes. Once DNA damage has been addressed, newly synthesized RecA and LexA restore repression to the system. Furthermore, the SOS response has been shown to induce the lateral transfer of antibiotic resistance encoded by the *Vibrio cholerae* integrating conjugative element SXT (1), of pathogenicity island-encoded virulence factors in staphylococci (16), and of prophage-encoded Shiga toxin in *E. coli* (19).

In this study we investigated the influence of subinhibitory concentrations of different antibiotics, including β-lactams (ampicillin, penicillin, ceftriaxone, and cloxacillin), macrolide-lincosamide-streptogramin B antibiotics (erythromycin), ami-

noglycosides (kanamycin), chloramphenicol, and tetracycline, on the replication and transfer of superantigen-carrying staphylococcal pathogenicity islands (SaPIs). In *S. aureus*, several related pathogenicity islands have been described, including SaPI1 to SaPI4, SaPbov1, SaPIbov2, and SaPIin1 to SaPIin3 (for reviews see references 10 and 11). These elements most commonly encode TSST-1 plus two or more other superantigen toxins, with the exception of SaPIbov2, which encodes the biofilm-associated protein Bap (17). Phage-assisted replication, transduction, and site-specific integration in a *recA* mutant strain demonstrated the mobility of SaPI1 (6, 14) and SaPIbov1 (16). Additionally, we demonstrated that the fluoroquinolone-induced SOS response is fully effective for the mobilization of SaPIbov1 and SaPI1 and, by implication, for that of all other SOS-induced SaPIs (16). In view of these results and of the results of Cohen and coworkers (8), we analyzed the possibility that other antibiotics used in clinical practice could also induce the SOS response, resulting in the dissemination of virulence factors in staphylococci. The results of our study show that β-lactams induce SaPI replication and transfer in an SOS-dependent manner and imply that they, as well as other SOS-inducing antibiotics, could thus increase microbial strain diversification and promote the spread of temperate phages and phage-inducible pathogenicity islands, resulting in the dissemination of superantigens and other virulence factors.

In the experiments presented here, we employed *S. aureus* strains RN27 and RN451, carrying SOS-inducible prophages 80α and φ11, respectively (9). Induction of the SOS response by any of the antibiotics analyzed would be expected to induce phage replication in these strains. Bacteria grown in Trypticase soy broth to an optical density at 540 nm of 0.4 were tested for prophage induction by the addition of subinhibitory concentrations (ranging from 0.05 μg/ml to 10 μg/ml) of ampicillin,

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TABLE 1. Phage titers of  $\beta$ -lactam-induced lysogenic staphylococcal strains<sup>a</sup>

Donor strain	Phage	Inducer <sup>b</sup>	Phage titer <sup>c</sup>
RN27	$\phi$ 80 $\alpha$	MC	$3.4 \times 10^{10}$
		A	$2.4 \times 10^8$
		P	$7.3 \times 10^8$
		CL	$2.4 \times 10^8$
		CE	$3.2 \times 10^8$
		NI	$8.0 \times 10^5$
RN451	$\phi$ 11	MC	$3.6 \times 10^9$
		A	$3.6 \times 10^6$
		P	$2.8 \times 10^6$
		CL	$2.3 \times 10^6$
		CE	$3.0 \times 10^6$
		NI	$1.4 \times 10^5$
RN1030 ( <i>recA</i> mutant)	$\phi$ 11	MC	<10
		A	<10
		P	<10
		NI	<10
JP83 [RN27 LexA (G94E)]	$\phi$ 80 $\alpha$	A	$4.5 \times 10^6$
		NI	$4.0 \times 10^5$
JP84 [RN451 LexA (G94E)]	$\phi$ 11	A	$1.3 \times 10^6$
		NI	$3.0 \times 10^4$

<sup>a</sup> Results from a representative experiment are shown.

<sup>b</sup> MC, mitomycin C (2  $\mu$ g/ml); A, ampicillin (10  $\mu$ g/ml); P, penicillin G (10  $\mu$ g/ml); CL, cloxacillin (10  $\mu$ g/ml); CE, ceftriaxone (10  $\mu$ g/ml); NI, not induced.

<sup>c</sup> Number of plaque-forming phages per milliliter of induced culture, using RN4220 as the indicator strain.

penicillin, erythromycin, chloramphenicol, tetracycline, or kanamycin. Cultures were grown at 32°C with slow shaking (80 rpm). After 16 h, phage titers were determined by plating suitable dilutions on RN4220. Phage replication was stimulated by exposure of bacteria to ampicillin and penicillin (Table 1), although the phage titers were lower than those with mitomycin C induction (Table 1). In contrast, none of the non- $\beta$ -lactam antibiotics tested induced phage replication (data not shown). Since the  $\beta$ -lactam antibiotics (ampicillin and penicillin) utilized have low clinical relevance in the treatment of staphylococcal infections, we repeated the phage inductions with the  $\beta$ -lactam antibiotics ceftriaxone and cloxacillin, which are extensively used in the treatment of staphylococcal infections. As shown in Table 1, exposure of bacteria to ceftriaxone and cloxacillin also increased the phage titers.

To determine whether ampicillin-mediated phage induction was SOS dependent, we measured the phage titers after antibiotic treatment of strain RN1030, a *recA*-defective strain lysogenic for  $\phi$ 11 (9). As is evident in Table 1, no induction was observed in the presence of the antibiotic.

To resolve the basis of ampicillin-mediated SOS induction further, we investigated the effect of ampicillin on expression of *lexA*. Thus, reverse transcription-PCR analysis using internal oligonucleotides specific to the *S. aureus* *lexA* gene were carried out as previously reported (3). As shown in Fig. 1, our experiments showed that the presence of ampicillin produces an increase of approximately 19-fold in *S. aureus* *lexA* expression from strain RN450 (9), while expression of the gene in its derivative *recA* mutant strain RN981 (9) was not affected, indicating that this  $\beta$ -lactam induces the SOS response.

This conclusion was additionally confirmed by mutational inactivation of the LexA protein and testing for phage induction. The substitution of glutamate for glycine at the Ala-Gly RecA cleavage site in the LexA protein results in a noncleavable repressor that was predicted to be less sensitive to SOS induction (7). The noncleavability mutation was introduced into *lexA* in strains RN27 and RN451, using oligonucleotides *lexA*-1mB (5'-CGCGGATCCGGCTGTTTGCTCCTTTGCTTCTTC-3'), *lexA*-2c (5'-CTCAGCCATTAATGAATTCTATTGGTC-3'), *lexA*-3m (5'-GGTAAAGTCACAGCTGAGGTTCCTATTACCGC-3'), and *lexA*-4c (5'-GCGGTAATAGGAACCTCAGCTGTGACTTTACC-3'), as previously described (16). The resulting strains, JP83 and JP84, respectively, each encoded noncleavable LexA [LexA (G94E)]. Strain JP83 [RN27 LexA (G94E)] showed diminished phage titers upon ampicillin treatment in comparison to those induced by ampicillin in strain RN27, confirming the role of LexA in the ampicillin-mediated SOS response. Surprisingly, although ampicillin induced  $\phi$ 11, similar phage titers were obtained upon ampicillin treatment of strains JP84 [RN451 LexA (G94E)] and RN451 (Table 1), suggesting the existence of a LexA-independent pathway in ampicillin-mediated SOS induction of  $\phi$ 11.

We have previously shown that mitomycin C induction of the SOS response in lysogenic SaPI1- and SaPIbov1-containing *S. aureus* induced replication and high-frequency transduction of the island (6, 14, 16). Similarly, growth of lysogenic SaPI1 or SaPIbov1 donor cells in ciprofloxacin, a widely used fluoroquinolone antibiotic that activates the SOS response (13), also increased SaPI1 and SaPIbov1 replication and transfer (16). To determine whether the  $\beta$ -lactam-mediated SOS response could induce SaPIbov1, strains JP44 (RN27 SaPIbov1 *tst::tetM* [16]) and JP47 (RN451 SaPIbov1 *tst::tetM* [16]) were grown with ampicillin, penicillin, ceftriaxone, or cloxacillin (10  $\mu$ g/ml); as shown in Table 2, all the  $\beta$ -lactam antibiotics analyzed also increased SaPIbov1 replication and transfer.

To confirm that the stimulation of SaPIbov1 transfer by ampicillin was a consequence of SOS induction, we induced

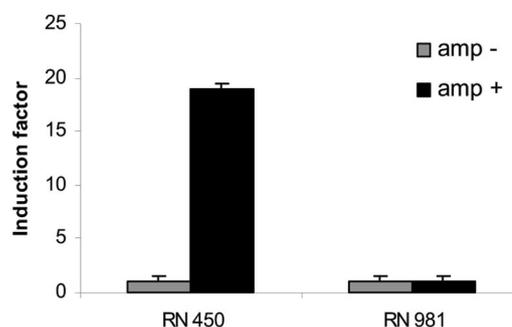


FIG. 1. Ampicillin-mediated induction factor of the *lexA* gene in *S. aureus* RN450 or its *recA*-mutant strain RN981. The induction factor was measured by quantitative reverse transcription-PCR, and in all cases, it is the ratio of the relative *lexA* mRNA concentration in ampicillin-treated cells to that in untreated cells. The relative *lexA* mRNA concentration was calculated as described previously (3). Values were calculated 5 h after ampicillin addition. In each case, the mean value from three independent experiments (each in triplicate) is shown. Amp+, addition of ampicillin at 0.2  $\mu$ g/ml; Amp-, no ampicillin added.

TABLE 2. Transduction frequencies of  $\beta$ -lactam-induced SaPI-containing lysogenic strains<sup>a</sup>

Donor strain	Phage	SaPI	Inducer <sup>b</sup>	Transduction titer <sup>c</sup>
JP44	$\phi$ 80 $\alpha$	SaPIbov1	MC	$7.2 \times 10^9$
			A	$2.7 \times 10^7$
			P	$3.0 \times 10^7$
			CL	$1.6 \times 10^7$
			CE	$3.2 \times 10^7$
			NI	$8.1 \times 10^4$
JP47	$\phi$ 11	SaPIbov1	MC	$2.1 \times 10^6$
			A	$6.0 \times 10^4$
			P	$5.8 \times 10^4$
			CL	$4.4 \times 10^4$
			CE	$5.6 \times 10^4$
			NI	$1.7 \times 10^3$
JP50 ( <i>recA</i> mutant)	$\phi$ 11	SaPIbov1	MC	<10
			A	<10
			NI	<10
JP85 [JP83 LexA (G94E)]	$\phi$ 80 $\alpha$	SaPIbov1	MC	$5 \times 10^5$
			A	$4 \times 10^4$
			NI	$2.3 \times 10^3$
JP52	$\phi$ 11 cIG131E	SaPIbov1	MC	<10
			A	<10
			NI	<10
RN8667	$\phi$ 80 $\alpha$	SaPII	A	$2.1 \times 10^5$
			P	$1.6 \times 10^5$
			CL	$2.2 \times 10^5$
			CE	$2.9 \times 10^5$
			NI	$6.0 \times 10^3$

<sup>a</sup> Results from a representative experiment are shown.

<sup>b</sup> MC, mitomycin C (2  $\mu$ g/ml); A, ampicillin (10  $\mu$ g/ml); P, penicillin G (10  $\mu$ g/ml); CL, cloxacillin (10  $\mu$ g/ml); CE, ceftriaxone (10  $\mu$ g/ml); NI, not induced.

<sup>c</sup> Number of transductants per milliliter of induced culture, using RN4220 as the recipient strain.

JP50 (a *recA* mutant *S. aureus* strain, a derivative of RN1030, lysogenic for  $\phi$ 11, that carries SaPIbov1 *tst::tetM* [16]) and JP85 (a derivative of JP83 that carries SaPIbov1 *tst::tetM*). As expected, neither replication nor significant transfer was observed upon ampicillin induction of strain JP50 (Table 2), while a significant reduction in SaPIbov1 transfer was observed after ampicillin induction of strain JP85. Additionally, the role of ampicillin in the SOS-mediated transfer of SaPIbov1 was confirmed by analysis of strain JP52, a SaPIbov1-positive strain, lysogenic for  $\phi$ 11, that carries a mutation eliminating the  $\phi$ 11 phage repressor (*cI*) cleavage site (16). Ampicillin did not increase SaPIbov1 transfer in this strain (Table 2), suggesting that *cI* cleavage mediates the SOS enhancement of SaPIbov1 transfer.

Finally, we addressed the question of whether SaPII, the prototypical *S. aureus* pathogenicity island, could also be excised, replicated, and transferred at a high frequency after antibiotic treatment. For that purpose, RN8667 (SaPII positive, lysogenic for  $\phi$ 80 $\alpha$  [6]) was grown with ampicillin, penicillin, ceftriaxone, or cloxacillin, as described above. As expected, the  $\beta$ -lactam-activated SOS response increased SaPII replication and transfer (Table 2). Thus, activation of the SOS response by  $\beta$ -lactam antibiotics greatly stimulates the transfer of *S. aureus* pathogenicity islands.

Recently, it has been reported that  $\beta$ -lactam antibiotics induce the SOS response in *E. coli* through the DpiBA two-component signal transduction system (8). This event, which requires the SOS-promoted cleavage of RecA and LexA as well as *dpiA*, transiently halts bacterial cell division, enabling the organisms to survive otherwise lethal antibiotic exposure. In addition, it has been reported that transcription of the SOS-regulated *dinB* gene, encoding DNA polymerase IV, is induced by inhibition of cell wall synthesis by  $\beta$ -lactam antibiotics (12). Whether corresponding genes are involved in SOS induction by  $\beta$ -lactams in *S. aureus* remains to be determined. Nevertheless, our findings indicate that  $\beta$ -lactam antibiotics are extracellular stimuli of the SOS response in *S. aureus* as well as in *E. coli* and demonstrate a novel mechanism for horizontal dissemination of staphylococcal virulence factors. Thus, our results, even though anticipated, reinforce the need for great caution in the use of SOS response-inducing antibiotics. Such antibiotics not only promote the dissemination of antibiotic resistance genes and the production of toxins regulated by repressors sensitive to RecA cleavage but also promote the induction of prophages and SaPIs, staphylococcal elements that frequently encode virulence factors.

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