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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), aminoglycosides (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 SaPIn1 to SaPIn3 (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 β-lactam-induced lysogenic staphylococcal strains[a]

Donor strain	Phage	Inducer[b]	Phage titer[c]
RN27	ϕ80α	MC	3.4×10^{10}
		A	2.4×10^{8}
		P	7.3×10^{8}
		CL	2.4×10^{8}
		CE	3.2×10^{8}
		NI	8.0×10^{5}
RN451	ϕ11	MC	3.6×10^{9}
		A	3.6×10^{6}
		P	2.8×10^{6}
		CL	2.3×10^{6}
		CE	3.0×10^{6}
		NI	1.4×10^{5}
RN1030 (*recA* mutant)	ϕ11	MC	<10
		A	<10
		P	<10
		NI	<10
JP83 [RN27 LexA (G94E)]	ϕ80α	A	4.5×10^{6}
		NI	4.0×10^{5}
JP84 [RN451 LexA (G94E)]	ϕ11	A	1.3×10^{6}
		NI	3.0×10^{4}

[a] Results from a representative experiment are shown.
[b] MC, mitomycin C (2 μg/ml); A, ampicillin (10 μg/ml); P, penicillin G (10 μg/ml); CL, cloxacillin (10 μg/ml); CE, ceftriaxone (10 μg/ml); NI, not induced.
[c] 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-β-lactam antibiotics tested induced phage replication (data not shown). Since the β-lactam antibiotics (ampicillin and penicillin) utilized have low clinical relevance in the treatment of staphylococcal infections, we repeated the phage inductions with the β-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 ϕ11 (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 transciption-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 β-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′-CGCGGATCCGGCTGTTTGCTCCTTTGCT TCTTC-3′), lexA-2c (5′-CTCAGCCATTAATGAATTCTAT TGGTC-3′), lexA-3m (5′-GGTAAAGTCACAGCTGAGGT TCCTATTACCGC-3′), and lexA-4c (5′-GCGGTAATAGGA ACCTCAGCTGTGACTTTACC-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 ϕ11, 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 ϕ11.

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 β-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 μg/ml); as shown in Table 2, all the β-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

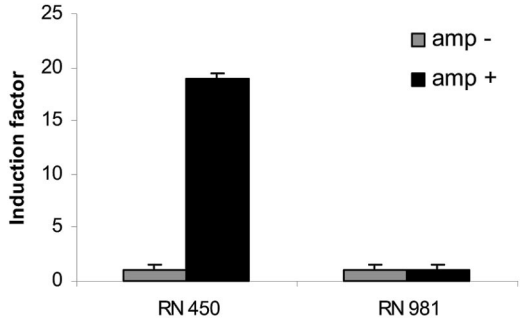

FIG. 1. Ampicillin-mediated induction factor of the *lexA* gene in *S. aureus* RN450 or its *recA*-mutant strain RN981. The induction factor was measured by quantitative reverse transciption-PCR, and in all cases, it is the ratio of the relative *lexA* mRNA concentration in ampicillin-treated cells to 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 μg/ml; Amp−, no ampicillin added.

TABLE 2. Transduction frequencies of β-lactam-induced SaPI-containing lysogenic strains[a]

Donor strain	Phage	SaPI	Inducer[b]	Transduction titer[c]
JP44	ϕ80α	SaPIbov1	MC	7.2×10^9
			A	2.7×10^7
			P	3.0×10^7
			CL	1.6×10^7
			CE	3.2×10^7
			NI	8.1×10^4
JP47	ϕ11	SaPIbov1	MC	2.1×10^6
			A	6.0×10^4
			P	5.8×10^4
			CL	4.4×10^4
			CE	5.6×10^4
			NI	1.7×10^3
JP50 (*recA* mutant)	ϕ11	SaPIbov1	MC	<10
			A	<10
			NI	<10
JP85 [JP83 LexA (G94E)]	ϕ80α	SaPIbov1	MC	5×10^5
			A	4×10^4
			NI	2.3×10^3
JP52	ϕ11 cIG131E	SaPIbov1	MC	<10
			A	<10
			NI	<10
RN8667	ϕ80α	SaPI1	A	2.1×10^5
			P	1.6×10^5
			CL	2.2×10^5
			CE	2.9×10^5
			NI	6.0×10^3

[a] Results from a representative experiment are shown.
[b] MC, mitomycin C (2 μg/ml); A, ampicillin (10 μg/ml); P, penicillin G (10 μg/ml); CL, cloxacillin (10 μg/ml); CE, ceftriaxone (10 μg/ml); NI, not induced.
[c] 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 ϕ11, 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 ϕ11, that carries a mutation eliminating the ϕ11 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 SaPI1, the prototypical *S. aureus* pathogenicity island, could also be excised, replicated, and transferred at a high frequency after antibiotic treatment. For that purpose, RN8667 (SaPI1 positive, lysogenic for ϕ80α [6]) was grown with ampicillin, penicillin, ceftriaxone, or cloxacillin, as described above. As expected, the β-lactam-activated SOS response increased SaPI1 replication and transfer (Table 2). Thus, activation of the SOS response by β-lactam antibiotics greatly stimulates the transfer of *S. aureus* pathogenicity islands.

Recently, it has been reported that β-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 β-lactam antibiotics (12). Whether corresponding genes are involved in SOS induction by β-lactams in *S. aureus* remains to be determined. Nevertheless, our findings indicate that β-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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REFERENCES

1. **Beaber, J. W., B. Hochhut, and M. K. Waldor.** 2004. SOS response promotes horizontal dissemination of antibiotic resistance genes. Nature **427:**72–74.
2. **Courcelle, J., A. Khodursky, B. Peter, P. O. Brown, and P. C. Hanawalt.** 2001. Comparative gene expression profiles following UV exposure in wild-type and SOS-deficient *Escherichia coli*. Genetics **158:**41–64.
3. **Dinamarca, M. A., I. Aranda-Olmedo, A. Puyet, and F. Rojo.** 2003. Expression of the *Pseudomonas putida* OCT plasmid alkane degradation pathway is modulated by two different global control signals: evidence from continuous cultures. J. Bacteriol. **185:**4772–4778.
4. **Fernandez De Henestrosa, A. R., T. Ogi, S. Aoyagi, D. Chafin, J. J. Hayes, H. Ohmori, and R. Woodgate.** 2000. Identification of additional genes belonging to the LexA regulon in *Escherichia coli*. Mol. Microbiol. **35:** 1560–1572.
5. **Kuroda, M., H. Kuroda, T. Oshima, F. Takeuchi, H. Mori, and K. Hiramatsu.** 2003. Two-component system VraSR positively modulates the regulation of cell-wall biosynthesis pathway in *Staphylococcus aureus*. Mol. Microbiol. **49:** 807–821.
6. **Lindsay, J. A., A. Ruzin, H. F. Ross, N. Kurepina, and R. P. Novick.** 1998. The gene for toxic shock toxin is carried by a family of mobile pathogenicity islands in *Staphylococcus aureus*. Mol. Microbiol. **29:**527–543.
7. **Little, J. W.** 1993. LexA cleavage and other self-processing reactions. J. Bacteriol. **175:**4943–4950.
8. **Miller, C., L. E. Thomsen, C. Gaggero, R. Mosseri, H. Ingmer, and S. N. Cohen.** 2004. SOS response induction by beta-lactams and bacterial defense against antibiotic lethality. Science **305:**1629–1631.
9. **Novick, R.** 1967. Properties of a cryptic high-frequency transducing phage in *Staphylococcus aureus*. Virology **33:**155–166.
10. **Novick, R. P.** 2003. Mobile genetic elements and bacterial toxinoses: the superantigen-encoding pathogenicity islands of *Staphylococcus aureus*. Plasmid **49:**93–105.
11. **Novick, R. P., P. Schlievert, and A. Ruzin.** 2001. Pathogenicity and resistance islands of staphylococci. Microbes Infect. **3:**585–594.
12. **Perez-Capilla, T., M. R. Baquero, J. M. Gomez-Gomez, A. Ionel, S. Martin, and J. Blazquez.** 2005. SOS-independent induction of *dinB* transcription by β-lactam-mediated inhibition of cell wall synthesis in *Escherichia coli*. J. Bacteriol. **187:**1515–1518.

13. **Phillips, I., E. Culebras, F. Moreno, and F. Baquero.** 1987. Induction of the SOS response by new 4-quinolones. J. Antimicrob. Chemother. **20:**631–638.
14. **Ruzin, A., J. Lindsay, and R. P. Novick.** 2001. Molecular genetics of SaPI1—a mobile pathogenicity island in *Staphylococcus aureus*. Mol. Microbiol. **41:**365–377.
15. **Singh, V. K., R. K. Jayaswal, and B. J. Wilkinson.** 2001. Cell wall-active antibiotic induced proteins of *Staphylococcus aureus* identified using a proteomic approach. FEMS Microbiol. Lett. **199:**79–84.
16. **Ubeda, C., E. Maiques, E. Knecht, I. Lasa, R. P. Novick, and J. R. Penades.** 2005. Antibiotic-induced SOS response promotes horizontal dissemination of pathogenicity island-encoded virulence factors in staphylococci. Mol. Microbiol. **56:**836–844.
17. **Ubeda, C., M. A. Tormo, C. Cucarella, P. Trotonda, T. J. Foster, I. Lasa, and J. R. Penades.** 2003. Sip, an integrase protein with excision, circularization and integration activities, defines a new family of mobile *Staphylococcus aureus* pathogenicity islands. Mol. Microbiol. **49:**193–210.
18. **Utaida, S., P. M. Dunman, D. Macapagal, E. Murphy, S. J. Projan, V. K. Singh, R. K. Jayaswal, and B. J. Wilkinson.** 2003. Genome-wide transcriptional profiling of the response of *Staphylococcus aureus* to cell-wall-active antibiotics reveals a cell-wall-stress stimulon. Microbiology **149:**2719–2732.
19. **Zhang, X., A. D. McDaniel, L. E. Wolf, G. T. Keusch, M. K. Waldor, and D. W. Acheson.** 2000. Quinolone antibiotics induce Shiga toxin-encoding bacteriophages, toxin production, and death in mice. J. Infect. Dis. **181:**664–670.