Induction of Prophages by Fluoroquinolones in *Streptococcus pneumoniae*: Implications for Emergence of Resistance in Genetically-Related Clones

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Abstract

Antibiotic resistance in *Streptococcus pneumoniae* has increased worldwide by the spread of a few clones. Fluoroquinolone resistance occurs mainly by alteration of their intracellular targets, the type II DNA topoisomerases, which is acquired either by point mutation or by recombination. Increase in fluoroquinolone-resistance may depend on the balance between antibiotic consumption and the cost that resistance imposes to bacterial fitness. In addition, pneumococcal prophages could play an important role. Prophage induction by fluoroquinolones was confirmed in 4 clinical isolates by using Southern blot hybridization. Clinical isolates (105 fluoroquinolone-resistant and 160 fluoroquinolone-susceptible) were tested for lysogeny by using a PCR assay and functional prophage carriage was studied by mitomycin C induction. Fluoroquinolone-resistant strains harbored fewer inducible prophages (17/43) than fluoroquinolone-susceptible strains (49/70) (**P** = 0.0018). In addition, isolates of clones associated with fluoroquinolone resistance [CC156 (3/25); CC63 (2/20), and CC81 (1/19)], had lower frequency of functional prophages than isolates of clones with low incidence of fluoroquinolone resistance [CC30 (4/21), CC230 (5/20), CC62 (9/21), and CC180 (21/30)]. Likewise, persistent strains from patients with chronic respiratory diseases subjected to fluoroquinolone treatment had a low frequency of inducible prophages (1/11). Development of ciprofloxacin resistance was tested with two isogenic strains, one lysogenic and the other non-lysogenic: emergence of resistance was only observed in the non-lysogenic strain. These results are compatible with the lysis of lysogenic isolates receiving fluoroquinolones before the development of resistance and explain the inverse relation between presence of inducible prophages and fluoroquinolone-resistance.

Citation: López E, Domènech A, Ferrándiz M-J, Frias MJ, Ardanuy C, et al. (2014) Induction of Prophages by Fluoroquinolones in *Streptococcus pneumoniae*: Implications for Emergence of Resistance in Genetically-Related Clones. PLoS ONE 9(4): e94358. doi:10.1371/journal.pone.0094358

Editor: Bernard Beall, Centers for Disease Control & Prevention, United States of America

Received January 17, 2014; Accepted March 13, 2014; Published April 9, 2014

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Funding: This study was supported by grants BIO2008-02154, SAF2009-10824 and BIO2011-25343 from Plan Nacional and PI 0901904 by FIS of Ministerio de Economía Competitividad. CIBER de Enfermedades Respiratorias (CIBERES) is an initiative of ISCIII. A.D. was supported by a grant from FPU of Ministerio de Educación, Spain. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

*Streptococcus pneumoniae* (the pneumococcus) is a major etiological agent of community-acquired pneumonia, meningitis and acute otitis media, as well as an important cause of acute exacerbations in patients with chronic respiratory diseases [1]. Antimicrobial resistance in the pneumococcus (including resistance to β-lactams, macrolides, tetracycline and co-trimoxazole) has expanded worldwide [2], influenced by patterns of antibiotic use and spread of a few international clones [3]. Therefore, fluoroquinolones (Fqs) are nowadays widely used for treating community-acquired pneumonia and other respiratory diseases in adults [4]. In Spain, the current prevalence of Fq resistance in pneumococci is lower than 3%, although it reaches 6.6% among strains isolated from acute exacerbations of chronic obstructive pulmonary disease [5,6]. We have found that CC156, CC63, and CC81 are the main Fq-resistant clones since 2002 in Spain [5,7].

Resistance to Fqs in pneumococci occurs mainly by alteration of their intracellular drug targets, i.e., DNA topoisomerase IV and DNA gyrase. Fqs inhibit these enzymes by forming a ternary complex of drug, enzyme, and DNA. Their killing effect has been related to the resolution of reaction intermediates of DNA-Fq-topoisomerase, which yield to the formation of irreparable double-stranded DNA breaks [8]. However, it has been also described that hydroxyl radical formation utilizing internal iron and the Fenton reaction are generated following gyrase poisoning and play an important role in cell killing by Fqs [9]. Fq resistance is acquired by point mutation as well as by intraspecific or interspecific recombination with streptococci of the mitis group [10–12]. A future increase in Fq resistance in *S. pneumoniae* would depend on the balance between antibiotic consumption and the cost that resistance imposes to bacterial fitness. A direct relationship between Fq consumption and increase in the
prevalence of resistance in *S. pneumoniae* was reported [13]. We and others have reported that specific Fq-resistant (Fq<sup>R</sup>) mutations confer a fitness cost to *S. pneumoniae* [14,15]. However, compensation of this fitness cost in isolates carrying recombinant topoisomerase genes has been observed [16].

In this context, if Fqs are able to induce pneumococcal prophages, they might have an important role in the emergence of Fq resistance in *S. pneumoniae* and would also modulate bacterial fitness in the presence of Fqs. Induction of prophages by Fqs has not been yet investigated in *S. pneumoniae*. This process has been described, among Gram-positive bacteria, only in *Staphylococcus aureus* [18,19], investigated in *S. pneumoniae* pneumoniae an important role in the emergence of Fq resistance in *S. pneumoniae* topoisomerase genes has been observed [16]. In this context, if *S. pneumoniae* isolates of presence of inducible prophages and Fq resistance in clinical samples, in which reach different values: a) 42% was deduced from induction of bacterial lysis with mitomycin C (MitC) [22], and b) 76% was proposed from hybridization with a *lytA* bacterial probe [23]. Recently, using a PCR protocol, pneumococcal prophages have been classified into three types [24]. A study performed in 240 isolates of the CC81 clone showed multiple recombination events at the prophage region [25], suggesting that the presence of phages genes does not always equate to the presence of a functional phage.

In this study we have performed experiments of phage induction in the presence of Fqs and investigated the relation between presence of inducible prophages and Fq resistance in clinical isolates of *S. pneumoniae*.

**Materials and Methods**

**Ethics statement**

This study and publication of the results were approved by the “Comité Étic d’Investigació Clínica del Hospital Universitari de Bellvitge” and the written or oral informed consent was considered not necessary, because the source of bacterial isolates was anonymized and the study was retrospective.

**Bacterial isolates**

Fq<sup>R</sup> [ciprofloxacin (CPX) MICs ≥ 4 mg/L] strains were isolated during the 2002–2009 period from 112 hospitals nationwide and previously published [5,7]. A randomized selection of Fq<sup>R</sup> isolates collected at Bellvitge Hospital during the same period was used as a control. Most isolates were from invasive sites (blood [90], cerebrospinal fluid [9], pleural fluid [18], synovial fluid [2]), and respiratory tract samples (sputum [125], bronchoalveolar lavage [12]). Clonal complex (CC) characterization was made on the basis of pulsed-field gel electrophoresis (PFGE) and assessed by multilocus sequence typing (MLST). Briefly, genomic DNA embedded in agarose plugs was restricted with SmaI or ApaI and fragments were separated by PFGE in a CHEF-DRIII apparatus (Bio-Rad). PFGE patterns were compared with representative clones of the Pneumococcal Molecular Epidemiology Network (PMEN), the world-wide epidemic clones [3]. Isolates with patterns varying by three or less bands were considered to represent the same PFGE type. In order to assess the identity with global pneumococcal clones, at least one isolate of each PFGE pattern/serotype combination was analyzed by MLST. Allele numbers and sequence types (ST) were assigned using the MLST web site (http://www.mlst.net).

**Detection of phage DNA**

PCR detection of the *hol1* gene, indicative of the presence of phage, was performed as described previously [24]. Strains with *hol1* positive PCR were tested for the presence of *int1*, *int2* and *mtp* with specific oligonucleotide pairs to identify phage types.

**Southern blot hybridizations**

Three additional isolates, one of CC156 (CipR-6.87, with two prophages of types 1 and 2), and two of CC63 with prophages of type 2 (CipS-6.3 and CipS-6.10). Phage induction was also followed by Southern blotting. First, phage induction was tested in CipR-6.55 (no prophages), which suggested prophage induction by the drug. In accordance, CipR-6.55 showed lysis after treatment with MitC (Figure 1). This lysis was also observed in three additional isolates, one of CC156 (CipR-6.37, with two phages of types 1 and 2) and two of CC63 with phages of type 2 (CipS-6.3 and CipS-6.10). Phage induction was also followed by Southern blotting. First, phage induction was tested in CipR-6.55 in the presence of CPX and LVX, MitC and the gyrase B inhibitor novobiocin. Phage DNA was purified, digested with *Bgl*II and hybridized with a *hol1* probe. As shown in Figure 2, discrete restriction bands were detected in the culture supernatants, which corresponded to phage DNA. Some basal spontaneous induction of phage was also observed in the untreated cultures. However, the amount of phage DNA, as detected by hybridization, was higher when Fqs or MitC were used. These results showed induction of
prophages by both Fqs and MitC, but not by novobiocin. Given that induction by LVX was more efficient than by CPX, the first Fq was chosen for further studies. In three additional lysogenic isolates studied, induction with LVX and MitC was also observed (Figure 2).

Detection of prophages by PCR in a collection of S. pneumoniae isolates

A total of 265 clinical isolates were tested for the presence of phages [105 FqR and 160 Fq-susceptible (FqS)]. Of them, 113 (42.6%) carried prophage DNA, and no significant difference was found among FqR (40.9%; 43/105) and FqS (43.5%; 70/160). Likewise, no difference was observed among prophage carriage rates of isolates collected from invasive samples (36.9%; 45/122) and respiratory tract samples (46.7%; 64/137). Types 1 and 2 prophages were more abundant than those of type 3. A majority (77/113) of isolates carried prophages of a single type, (51 isolates with type 1 phages, 25 isolates with type 2 phages and 1 isolate with a type 3 phage), 16 isolates carried prophages of two types (11 isolates with types 1 and 2, and 5 isolates with types 2 and 3), and 6 isolates carried prophages of the three types. Fourteen isolates showed amplification with hol1 oligonucleotides but not with those specific for int1, int2 or mtp suggesting the presence of prophage remnants or of temperate phages of an unknown type.

Presence of inducible prophages among isolates of prevalent clones

Since we showed a correlation between prophage induction by MitC and Fqs (Figures 1 and 2), MitC induction was chosen to test the functionality of the prophages detected by PCR in all lysogenic isolates. The kinetics of growth in the presence of MitC was analyzed as described in methods. Among 113 lysogenic isolates, only 66 (58.4%) exhibited detectable lysis after treatment with MitC. The frequencies of functional prophages were statistically lower ($P=0.0018$) in FqR (17/43) than in FqS (49/70) isolates (Table 1). No difference was observed in the distribution of functional phages among invasive (28/122) and respiratory tract samples (37/137).

As shown in Table 1, several clones had low presence of phage DNA: CC156; CC81; CC63; and CC306. No association between the type of phage carried and the genotype was observed. Isolates of the same clone carried different phage types or combinations, with the exception of CC180 in which 20 out of 22 isolates carried a type 1 prophage. Four clones: CCT156 (3/25); CC63 (2/20);
CCT81 (1/19) and CC306 (1/29) showed low rates of functional prophage carriage. The first three are the main Fq R Spanish clones since 2002 [5,7] whereas, CC306, is usually antimicrobial susceptible. On the other hand, isolates belonging to CC180 (21/30); CC30 (4/21); CC230 (5/20); and CC62 (9/21) showed higher frequencies of functional prophage carriage. These last four clones were low prevalent among FqR Spanish pneumococci [5,7].

Low frequency of inducible prophages among persistent strains isolated from patients that received Fq treatment

To test the role of Fq therapy in phage induction in vivo, 11 persistent pneumococci collected from 10 adult patients with chronic respiratory diseases were studied (Table 2). Details of these strains have been previously reported [26]. All isolates from each patient were clonally related (same MLST) and were repeatedly isolated throughout the period (27 to 165 weeks) in which these patients received multiple Fq therapy courses. Only isolates of two patients (7 and 11) showed a positive detection of the holI gene. Moreover, only prophages from the pneumococci of patient 7 were induced with MitC. Four strains were FqR since the first isolation and did not carry any inducible prophages, although one of them had a phage remnant (patient 11). Among the five FqR strains that did not carry prophages, two developed resistance after Fq course (patients 7 and 9). Patient 7 was sequentially colonized...
## Table 1. Relevant characteristics of S. pneumoniae isolates and their prophages analyzed in this study.

| Clonal Complex (no. of isolates) | Phenotype<sup>b</sup> | hol1<sup>c</sup> | MitC<sup>d</sup> | Type of phage |
|----------------------------------|------------------------|-----------------|----------------|----------------|
| CC180 (30)                       | 29 S                   | 1 R             |                | 12 3 1          |
|                                 | 1 R                    | 1 R             |                | 3 2 1           |
| CC306 (29)                       | 28 S                   | 1 R             |                | 19 8 3          |
|                                 | 1 R                    | 1 R             |                | 3 2 1           |
| CC156 (25)                       | 6 S                    | 1 R             |                | 4 4 3           |
|                                 | 1 R                    | 1 R             |                | 1 2 2           |
| CC30 (21)                        | 17 S                   | 4 R             |                | 16 4 2          |
|                                 | 4 R                    | 4 R             |                | 1 2 2           |
| CC62 (21)                        | 19 S                   | 8 R             |                | 11 1 1          |
|                                 | 2 R                    | 2 R             |                | 2 1 2           |
| CC63 (20)                        | 7 S                    | 2 R             |                | 2 1 2           |
|                                 | 13 R                   | 1 R             |                | 1 1 1           |
| CC230 (20)                       | 18 S                   | 6 R             |                | 17 2 4          |
|                                 | 2 R                    | 2 R             |                | 1 1 1           |
| CC81 (19)                        | 7 S                    | 2 R             |                | 11 1 3          |
|                                 | 12 R                   | 3 R             |                | 3 1 3           |
| CC97 (6)                         | 3 S                    | 3 R             |                | 2 1 3           |
|                                 | 3 R                    | 3 R             |                | 3 0 2           |
| CC433 (6)                        | 3 S                    | 3 R             |                | 2 1 2           |
|                                 | 4 S                    | 4 R             |                | 1 1 0           |
| CC42 (5)                         | 1 S                    | 2 R             |                | 2 1 2           |
|                                 | 1 R                    | 1 R             |                | 1 1 1           |
| CC717 (5)                        | 1 S                    | 1 S             |                | 1 1 1           |
|                                 | 2 R                    | 2 R             |                | 2 1 2           |
| CC17 (4)                         | 2 R                    | 2 R             |                | 2 1 2           |
| CC90 (4)                         | 2 S                    | 2 R             |                | 2 1 2           |
|                                 | 2 R                    | 2 R             |                | 2 1 2           |
| CC260 (4)                        | 2 S                    | 2 R             |                | 2 1 2           |
|                                 | 2 R                    | 2 R             |                | 2 1 2           |
| CC67 (3)                         | 2 S                    | 2 R             |                | 2 1 2           |
|                                 | 1 R                    | 1 R             |                | 1 1 1           |
| CC191 (3)                        | 2 S                    | 1 R             |                | 1 1 1           |
|                                 | 1 R                    | 1 R             |                | 1 1 1           |
| CC88 (2)                         | 2 S                    | 0 R             |                | 1 0 1           |
|                                 | 0 R                    | 0 R             |                | 0 0 0           |
| CC247 (2)                        | 2 S                    | 0 R             |                | 1 0 1           |
|                                 | 0 R                    | 0 R             |                | 0 0 0           |
| CC989 (2)                        | 1 S                    | 1 R             |                | 1 0 1           |
|                                 | 1 R                    | 1 R             |                | 1 0 1           |
| Others (34)                      | 1 S                    | 1 S             |                | 1 0 1           |
|                                 | 33 R                   |                 |                | 19 8 4 3 1 3 1 3 3 5 |

<sup>a</sup>Clones are named by their clonal complex number. Those showed in boldface and underlined are the main clones involved in Fq resistance in Spain since 2002.

<sup>b</sup>Isolates are separated on the basis of their Fq susceptibility: S, susceptible (CPX MICs ≤2 mg/L); R, resistant (MICs ≥4 mg/L).

<sup>c</sup>PCR detection for hol1 gene.

<sup>d</sup>Functional phages caused cell lysis in the presence of MitC.

doi:10.1371/journal.pone.0094358.t001
The presence of a prophage affects development of CPX resistance

The observation that Fq\(^R\) isolates have lower rates of inducible prophages than Fq\(^S\) pneumococci, together with the results of induction of phages by Fqs, suggest that under Fq selective pressure, lysogenic pneumococci will be prone to die due to phage-mediated lysis, while non-lysogenic isolates would be able to develop Fq resistance. Resistance would be hence more likely to arise in isolates that do not carry prophage. To test this hypothesis, two isogenic strains, R36A (wild type) and R36AP (an R36A derivative carrying an inducible prophage) [27] were cultured for 4 h in the presence of 1\(\mu\)g/ml of CPX and LVX. Cultures were grown for 4 h and bacteria were recovered by centrifugation at 5000 g for 30 minutes. Cells were suspended in 25% glycerol at concentrations of 3.3 \(\times\) 10\(^6\) CFUs/ml. Cultures were grown for 4 h and bacteria were recovered by centrifugation at 5000 g for 30 minutes. Cells were suspended in 25% glycerol at concentrations of 3.3 \(\times\) 10\(^6\) CFUs/ml. Cultures were grown for 4 h and bacteria were recovered by centrifugation at 5000 g for 30 minutes. Cells were suspended in 25% glycerol at concentrations of 3.3 \(\times\) 10\(^6\) CFUs/ml. Cultures were grown for 4 h and bacteria were recovered by centrifugation at 5000 g for 30 minutes. Cells were suspended in 25% glycerol at concentrations of 3.3 \(\times\) 10\(^6\) CFUs/ml. Cultures were grown for 4 h and bacteria were recovered by centrifugation at 5000 g for 30 minutes. Cells were suspended in 25% glycerol at concentrations of 3.3 \(\times\) 10\(^6\) CFUs/ml.

Discussion

In this study we first showed that Fqs targeting topoisomerase IV, such as CPX and LVX, are able to induce the lytic cycle of pneumococcal temperate phages. Comparison of growth kinetics in the presence of 1\(\times\)MIC of these fluoroquinolones showed lower OD increases in isolates carrying inducible prophages, than in the non-lysogenic CipR-6.55 isolate. All these results suggest a role of the inhibition of topoisomerase IV in the lysis response. This could be a consequence of the cellular processes acting on the ternary complex formed by topoisomerase IV-Fq and DNA [8]. However, it could also be due to transcriptional regulation of phage or bacterial genes by changes in DNA supercoiling caused by inhibition of topoisomerase IV, given that treatment of S. pneumoniae with LVX causes a complex transcriptomic response [28]. On this respect, it has been shown that the transcription of the pneumococcal mcl gene, a competence-induced gene, is necessary for temperate phage induction [29], and that competence in S. pneumoniae, a bacterium lacking an SOS-like system, is induced by Fqs and MitC but not by other antibiotics [30].

Since our results showed that Fqs caused bacterial lysis by phage induction, we determined the rates of inducible phages in isolates of the most frequent clones causing pneumococcal diseases in adult patients. Using a PCR approach, we determined that about half of the isolates analyzed were lysogenic, a figure compatible with the previously reported value (42%) based on MitC induced bacterial lysis [22], but lower than the 76% estimated by detection of the prophage lytI-like gene by hybridization with a host lytI probe [23]. However, both the PCR detection and the hybridization approaches overestimate the rate of inducible, functional prophage carriage, since these methods detect also defective prophages, and experiments of induction with MitC were necessary to determine the functional phage rate.

We showed that Fq\(^R\) isolates have lower rates of inducible prophage carriage than Fq\(^S\) pneumococci. These findings,
together with the induction of phages by Fqs, and the inability to select FqR isolates in the R36aP strain in conditions when these arise readily in the non-lysogenic parental strain R36a. Suggest that under Fq pressure lysogenic pneumococci will be prone to die due to phage-mediated lysis, while non-lysogenic isolates are able to develop Fq resistance. Consistent with this hypothesis, isolates belonging to the three main Fq\(^{b}\) Spanish clones (CC156, CC63, and CC81), have a frequency of inducible prophages lower than clones not related with Fq resistance, such as CC30, CC62 or CC180. In contrast, the vast majority of isolates of CC306, which were Fq\(^{c}\), were non-lysogenic. There are two possible explanations for this finding. The first is that isolates of this clone usually cause invasive pneumococcal disease in children, who are not treated with Fqs. The second is that, since this clone is rarely found as a colonizer (neither in children nor adult patients with chronic obstructive pulmonary disease), it may seldom exchange DNA with other streptococci or have the chance to be infected by temperate bacteriophages.

Another possibility could be that the clones within which Fq resistance is most common are less likely to be lysogenic for unrelated reasons. However, our results shown that there are differences in the prevalence of inducible phage between different clones not commonly resistant to Fq, ranging from 1/29 to 21/30 (Table 1) and experiments with isogenic strains differing only in the carriage of a prophage support a role of prophages in preventing the development of Fq resistance.

Finally, we found a low frequency of functional prophages (1/11) in strains persistently colonizing patients which received multiple courses of Fq therapy. These results also support the role of prophage in cell lysis and development of in vivo Fq resistance in S. pneumoniae. This ecological niche is optimal for the development of antibiotic resistance; given that the patients had multiple infections with different pathogens and that they received multiple courses of antibiotic treatment. In relation with fluoroquinolone treatments, the doses of CPX that are able to kill Gram-negative bacteria are subinhibitory for S. pneumoniae and this would allow, both the development of resistance in this kind of patients, and also the induction of prophages.

The evolution of bacteria cannot be understood without the contribution of their prophages [31]. These could change from inducible to cryptic prophages (unable to excise from the chromosome and cause cell lysis), which contribute significantly to resistance to sub-lethal concentrations of Fqs and b-lactam antibiotics primarily through plasmid-encoded proteins that inhibit cell division, as recently demonstrated for Escherichia coli prophages that do not excise on MinC treatment [32]. Lysogeny is also important for interspecies competition, as showed by the killing of S. aureus by prophage induction caused by H\(_2\)O\(_2\) production by S. pneumoniae in the nasopharynx [19]. Activation of key proteins involved in phage-induced cell lysis, encoded either by the prophages or by the bacterial host, may be a novel way to fight antimicrobial resistance.

**Author Contributions**

Conceived and designed the experiments: M. J. Ferrándiz CA MR EG AGC. Performed the experiments: EL AD M. J. Ferrándiz M. J. Frias. Analyzed the data: EL AD M. J. Ferrándiz M. J. Frias CA MR EG JLG AGC. Contributed reagents/materials/analysis tools: EL AD M. J. Ferrándiz M. J. Frias CA MR EG JLG AGC. Wrote the paper: AGC.

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**References**

1. Austen R (1981) Some observations on the pneumococcus and on the current status of pneumococcal disease and its prevention. Rev Infect Dis 3 Suppl S1–17.
2. Jacobs MR, Feltham D, Appelbaum PC, Gruneberg RN, the Alexander Project Group (2003) The Alexander project 1990-2000: susceptibility of pathogens isolated from community-acquired respiratory tract infection to commonly used antimicrobial agents. J Antimicrob Chemother 52: 229–246.
3. McGee L, McDougal L, Zhou J, Spratt BG, Tenover FC, et al. (2001) Nomenclature of major antimicrobial-resistant clones of *Streptococcus pneumoniae* defined by the pneumococcal molecular epidemiology network. J Clin Microbiol 39: 2565–2571.
4. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, et al. (2011) *Staphylococcus aureus* and *Enterococcus faecalis* as niche competitors by remote-control bacteriophage induction. Proc Natl Acad Sci USA 108: 1234–1239.
5. Romero P, García E, Mitchell TJ (2009) Development of a prophage typing scheme for *Streptococcus pneumoniae*. J Antimicrob Chemother 5: 103–107.
6. Croucher NJ, Harris SR, Fraser C, Quail MA, Burton J, et al. (2011) Rapid molecular evolution perspectives on intraspecific lateral DNA transfer of *Streptococcus pneumoniae*. J Antimicrob Chemother 53: 822–830.
7. Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ (2007) A common mechanism of cellular death induced by bactericidal antibiotics. Cell 130: 797–807.
8. Selva L, Viana D, Regev-Yochay G, Trzcinski K, Corpas JM, et al. (2009) Killing niche competitors by remote-control bacteriophage induction. Proc Natl Acad Sci USA 106: 1234–1239.
9. Meessen-Pinard M, Sekulovic O, Forrier LC (2012) Evidence of in vivo prophage induction during *Clostridium difficile* infection. Appl Environ Microbiol 78: 7662–7670.
10. Bernheimer HP (1979) Lysogenic pneumococci and their bacteriophages. J Bacteriol 138: 618–624.
26. Domenech A, Ardanuy C, Balsalobre L, Marti S, Calatayud L, et al. (2012) Pneumococci can persistently colonize adult patients with chronic respiratory disease. J Clin Microbiol 50: 4047–4053.

27. Frias MJ, Melo-Cristino J, Ramirez M (2009) The autolysin LytA contributes to efficient bacteriophage progeny release in *Streptococcus pneumoniae*. J Bacteriol 191: 5428–5440.

28. Ferrández MJ, de la Campa AG (2014) The fluoroquinolone levofloxacin triggers the transcriptional activation of iron transport genes that contribute to cell death in *Streptococcus pneumoniae*. Antimicrob Agents Chemother 58: 247–257.

29. Martin B, García P, Castañé MP, Claverys JP (1995) The recA gene of *Streptococcus pneumoniae* is part of a competence-induced operon and controls lysogenic induction. Mol Microbiol 13: 367–379.

30. Prudhomme M, Attaiéch L, Sanchez G, Martin B, Claverys JP (2006) Antibiotic stress induces genetic transformability in the human pathogen *Streptococcus pneumoniae*. Science 313: 89–92.

31. Rodríguez-Valera F, Martín-Cuadrado AB, Rodríguez-Brito B, Pasic L, Thingstad TF, et al. (2009) Explaining microbial population genomics through phage predation. Nat Rev Microbiol 7: 828–836.

32. Wang X, Kim Y, Ma Q, Hong SH, Pokusaeva K, et al. (2010) Cryptic prophages help bacteria cope with adverse environments. Nat Commun 1: 147.