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Effect of Antibiotic Treatment on Bacteriophage Production by a Cystic Fibrosis Epidemic Strain of *Pseudomonas aeruginosa*▽

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Phage production in response to antibiotics varied among four isolates of a *Pseudomonas aeruginosa* cystic fibrosis (CF) epidemic strain. Whereas ciprofloxacin induced higher levels of phage production, other CF-relevant antibiotics led to reduced production. We detected free phages directly in CF patient sputum samples by both plaque (40% positive) and PCR (76% positive) assays. Our observations suggest that the choice of antibiotics could influence the number of free phages within the CF lung environment.

The Liverpool epidemic strain (LES) is the most common cystic fibrosis (CF) epidemic strain of *Pseudomonas aeruginosa* in the United Kingdom (12). The genome of the earliest LES isolate (LESB58) has been sequenced, revealing the presence of six prophages, five of which can produce active phage progeny (14). Specific mutations within LES prophages 2, 3, and 5 resulted in reduced competitiveness in a rat chronic lung infection model (14). The aim of this study was to determine the effects of different CF-relevant antibiotics on the level of phage production by LES isolates.

The *P. aeruginosa* LES isolates (LES400, LES431, LESB58, and LESB65) represent different phenotypic and pathogenic variants (6). MICs of antimicrobial agents were determined according to British Society for Antimicrobial Chemotherapy guidelines (1). Sputum samples were collected from adult CF patients chronically infected with the LES. Sputum was treated with an equal volume of Sputasol (Oxoid, Basingstoke, United Kingdom). PCR assays were performed as described previously (3, 5) for LES phages 2 and 3, respectively, on supernatants from culture and directly from CF patient sputum. All PCR assays were performed in duplicate. Cultures were grown to an optical density (A_{600}) of 0.5, and phage induction was performed by adding antibiotics at the MIC (norfloxacin, 50 μg/ml; tobramycin, 4 μg/ml; colistin, 1 μg/ml; ceftazidime, 64 μg/ml; meropenem, 2 μg/ml; ciprofloxacin, 4 μg/ml) and incubation for 1 h, followed by 1 h of recovery in fresh medium. Induced active phage particles were enumerated using plaque assays in which 100 μl of culture supernatant was added to 100 μl (A_{600} of 0.5) of *P. aeruginosa* PAO1 (the indicator strain) in 5 ml of molten 0.4% Luria agar and poured onto L agar in triplicate. PCR assays (3, 5) showed that the plaques obtained were caused by a combination of LES phages 2 and 3. Statistical analysis was performed using either a Kruskal-Wallis test or a Mann-Whitney U test for significant differences.

The background level of spontaneous phage production was low (50 to 1,000 PFU/ml), but there were significant differences among the four LES isolates tested (Fig. 1). Ciprofloxacin and norfloxacin caused a level of phage induction higher than that in uninduced cultures of each of the isolates. Cultures treated with the other four CF-relevant antibiotics resulted in reduced numbers of phages from all of the LES isolates tested (Fig. 1). For LESB58 and LES400, the presence of each different antibiotic significantly affected the number of phages produced (Table 1).

Ciprofloxacin and norfloxacin, which induced the highest phage numbers, affect DNA replication and repair, triggering the SOS response and induction of the phage lytic cycle. Upregulation of phage-like genes in the presence of ciprofloxacin has been shown previously for strain PAO1 (3). In the presence of other CF-relevant antibiotics, the level of phage production was less than the spontaneous induction level, suggesting that some antibiotics may have an inhibitory effect on phage production. Colistin can inhibit phage production in *Mycobacterium tuberculosis* (4), possibly due to interruption of the cell membrane, thereby disrupting binding sites required for phage assembly (7). Colistin and meropenem target the cell membrane and cell wall, respectively, and could have a similar effect on the production of LES phages. A recent study showed that the production of late gene phage products was affected differently by different classes of subinhibitory antibiotic concentrations (10). Antibiotics affecting DNA synthesis caused upregulation of the late gene responsible for Shiga toxin production (*stx*), whereas antibiotics affecting cell wall and protein synthesis had an inhibitory effect (10). This phenomenon was particularly pronounced in the presence of azithromycin. We found that the presence of azithromycin caused a significant decrease in phage production by three isolates (LESB58, LESB65, and LES400). Considerable variation in phage production was observed among the different LES isolates, adding further to the different characteristics exhibited by LES isolates (8).

PCR and plaque assays were performed on 58 sputum

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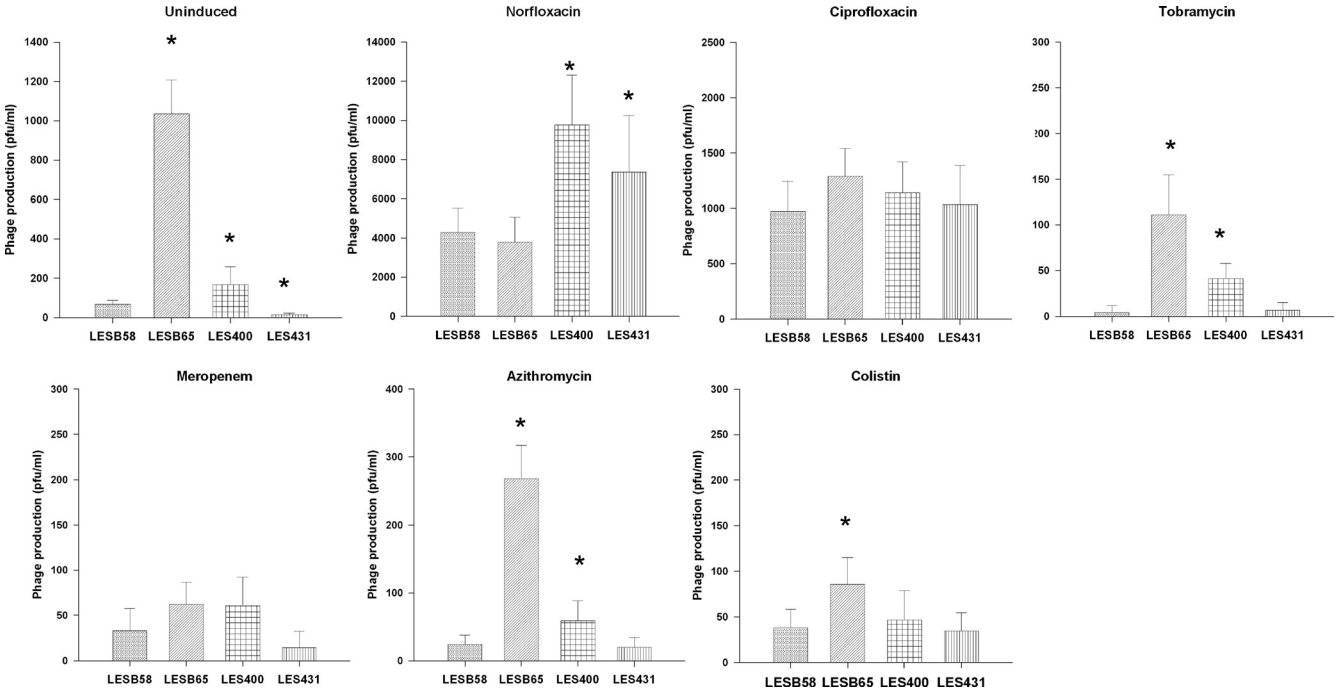

FIG. 1. Levels of phage production by four LES isolates in PFU per milliliter. Cultures ($n = 9$ for each isolate) were either uninduced (spontaneous phage induction) or treated with the appropriate antibiotic at the MIC. Asterisks signify isolates with phage production significantly different ($P < 0.05$) from that of LESB58 under each condition. Statistical analysis was performed using the Kruskal-Wallis test and Dunn's test for nonparametric data.

samples from CF patients chronically infected with the LES. Plaques caused by LES phage 2 or 3 were identified in 40% of the samples by plaque assays. Using PCR assays, LES phage 2 or 3 was identified in 74% of the Sputasol-treated, filter-sterilized, DNase I-treated sputum samples. Both phages were present together in 59% of the samples tested. The high rate of detection of these phages in CF sputum may be due to induction by the high levels of oxidative stress and the abundance of inflammatory cells in the CF lung (11). In addition, intensive antibiotic treatment regimens are often used particularly during periods of exacerbation.

Phages have been linked with horizontal gene transfer between closely related species and have been associated with virulence in many other bacterial hosts (5). *Pseudomonas* phages have also been associated with the formation of biofilms (13). The importance of lysogenic *Pseudomonas* phages remains poorly understood, but LES prophages 2 and 3 have been implicated in the establishment of chronic infection in an animal model (10). It is possible that the prophages contain genes that are only activated during the process of induction leading to phage production. As has been suggested for *Staphylococcus* (9), phage mobilization in *P. aeruginosa* populations may contribute to adaptation in the CF airway through the production of increased diversification (4).

This is the first demonstration of active LES phages (detected by plaque assay) present in CF sputum. Our data suggest that the choice of antibiotic used in CF lung infection management could dramatically affect the levels of free *Pseudomonas* phages in the CF lung. *P. aeruginosa* phage mobilization has been associated with increased diversification (4) and the transduction of antibiotic resistance (2). Given the roles of phages in promoting horizontal gene transfer (2), modulating biofilm formation (9), and increasing the competitiveness (10) of this important CF pathogen, our findings suggest that the choice of antibiotic therapy

TABLE 1. Phage production in the presence of antibiotics

Isolate	*P* value[a]					
	Norfloxacin	Ciprofloxacin	Tobramycin	Meropenem	Azithromycin	Colistin
LESB58	<0.001[b]	0.004[b]	<0.001[b]	0.006[b]	<0.001[b]	0.006[b]
LESB65	<0.001[b]	0.05	<0.001[b]	<0.001[b]	<0.001[b]	<0.001[b]
LES400	<0.001[b]	<0.001[b]	<0.001[b]	0.001[b]	<0.001[b]	<0.001[b]
LES431	<0.001[b]	<0.001[b]	0.063	0.222	0.796	0.031[b]

[a] Analysis was performed using a Mann-Whitney U test for statistically significant differences. For each LES isolate, the difference in phage production between uninduced cultures and antibiotic-treated cultures was tested ($n = 9$ for each isolate under each condition).
[b] Statistically significant difference associated with the presence of antibiotic.

may be important in the adaptation of *P. aeruginosa* populations to the CF lung through increased or decreased levels of free phage.

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