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Short Communication
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Activity of Bacteriophages to Control *Listeria monocytogenes* and *Campylobacter jejuni* Antibiotic Resistant Strains

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Abstract

Phage pray and kill bacteria, are natural occurring agents, ubiquitous in nature, and could represent a global novel approach to therapy in both animals and humans. Nowadays, the problem of antimicrobial resistance, rapidly increasing in recent years, represents a major public health threat that particularly put interest in assessing the potential use of alternative antibacterial agents, including bacteriophages. Phage therapy has many advantages over traditional antibiotics such as specificity for the target organism, self-replicating activity, safety and the relative ease with which naturally occurring phages can be isolated from the environment and propagated in large numbers. The aim of this study was to evaluate the susceptibility of 12 *Listeria monocytogenes* (*L. monocytogenes*) and 3 *Campylobacter jejuni* (*C. jejuni*) strains used for initial phage isolation to bacteriophage activity and to some antibiotics frequently used in veterinary and human medicine. Moreover, we report an interesting finding related to *C. jejuni* 12662 strains and its apparent reversion to sensitivity to antimicrobials (ciprofloxacin, nalidixic acid and tetracycline) after been exposed to phage activity.

Introduction

The discovery of bacteriophages (phages) in the early 1920s was one of the most momentous events in microbiology [1]. Even not being a new issue, the dramatic rise of multidrug resistant bacteria has prompted scientists to re-evaluate bacteriophage therapy as an alternative to treat bacterial infectious diseases [2]. In particular, bacteriophages express very effective mechanisms to kill prokaryotes, being highly specific against some strains while are unable to affect commensal bacteria. As they are already present in the environment, their use does not constitute any further addition of new biologically active entities, thus reducing the possibility of side effects or the development of allergic responses [3]. Research has shown the potential for phages to control pathogens in live animals [4], humans but also in food for decontamination after production [5,6]. In our researches, we came across interesting findings in relation to phages and antibiotic resistant strains of *L. monocytogenes* and *C. jejuni*. In particular, in our activities on isolation of bacteriophages, we were able to demonstrate lytic activity towards a panel of *L. monocytogenes* and *C. jejuni* strains that were particularly resistant to some antibiotics. Moreover, by analysing *C. jejuni* strains before and after phage treatment, we demonstrated an interesting change in the antibiotic resistance profile. In particular, *C. jejuni* 12,662 strains that exhibited resistance to ciprofloxacin, nalidixic acid and tetracycline before being phage treated resulted in apparent reversion to sensitivity to the respective antibiotics after 24 hours phage exposure.

Materials and Methods

Antibiotic susceptibility testing

The assays were performed on *L. monocytogenes* strains of Table 1 and *C. jejuni* strains of Table 2 by using the microdilution method and the Sensititre automated system, according to manufacturer's guidelines (TREK Diagnostic Systems, USA). The strains were placed on Columbia agar (Oxoid, Germany) for *C. jejuni* and Blood agar (Oxoid, Germany) for *L. monocytogenes*, incubated for 22 hours ± 2 hours in growth conditions (37°C ± 1°C for *L. monocytogenes* and 42°C ± 1°C, in microaerophilic conditions- 85% nitrogen, 5% oxygen, and 10% carbon dioxide- for *C. jejuni*). Then bacterial colonies were seeded in Mueller Hinton broth supplemented with blood (Thermo scientific, Amsterdam) and dispensed into microtiter plates, containing known

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Table 1: *L. monocytogenes* strains used for phage isolation; they all showed sensitivity to ϕIZSAM-1.

ID	Serovar	Origin
13M	1/2C	ATCC 7644
2	1/2b	Fresh pork sausage
3	1/2a	Chicken meat
4	1/2b	Bovine meat
5	4b	Fresh pork sausage
6	1/2c	Pork minced meat
7	4b	*Pangasius* fillet
8	1/2c	Bovine meat
9	1/2a	Bovine meat
10	4b	Smoked salmon
11	4b	Smoked salmon
12	4b	Human cepahlorachidian fluid

scalar antimicrobial concentrations. The plates were incubated for 22 hours ± 2 hours at the same growth conditions specified before. The resistance profiles, for *C. jejuni* strains in particular, were evaluated according to the Commission Decision 2013 [7]. The antimicrobial sensitivity tests were performed 2 times for each bacterial strain.

Bacteriophage isolation and spot assays

L. monocytogenes phages were isolated from drains of Italian blue cheese plants, using double-layer agar techniques [8]. The activity of the newly isolated ϕ IZSAM-1 was assessed by spot assay technique against the *L. monocytogenes* strains listed in Table 1 [8].

C. jejuni phages were isolated from 51 samples of fresh chicken stool samples. Three *C. jejuni* strains were used for phage isolation chosen on the bases of their phenotypic and genotypic differences (Table 2) [9]. Moreover, 10 mL of *C. jejuni* strain 12,662 broth culture were seeded with a suspension of 100 µL of ϕ 7 and ϕ 16 (50 µL each phage) at MOI 0.1 and incubated at 42°C ± 1°C, in microaerophilic conditions, for 22 hours ± 2 hours [9]. *C. jejuni* strains recovered before and after phage treatment were assayed for antibiotic susceptibility test as described before.

Results and Discussion

The antibiotic resistance profiles (for *C. jejuni* strains) and MIC values (for *L. monocytogenes* and *C. jejuni* strains) are presented in Table 3 and 4, respectively. Repeatability of MIC values was obtained for all the strains. For *L. monocytogenes*, according to EUCAST tables (published in EUCAST Clinical Breakpoint Tables v.8.1, 2018), all the strains were sensitive to benzyl penicillin and erythromycin (data not shown). For the other antibiotics tested and shown in Table 3, even though there is no availability of indicative breakpoints for evaluation, the strains resulted still resistant at the maximum antimicrobial concentrations used in the plates for the assay. Some updated results in relation to antibiotic susceptibility of *L. monocytogenes* strains isolated from food and human samples have been recently reported by Noll et al. [10].

Table 3: MIC values of *L. monocytogenes* strains used in the study.

Strains	Antibiotics	Range (µg/mL)	MIC (µg/mL)
13M	Chloramphenicol	2-32	>32
	Lincomycin	1-8	>8
	Linezolid	0.5-8	>8
	Nitrofurantoin	2-64	>64
2	Lincomycin	1-8	>8
	Nitrofurantoin	2-64	>64
3	Nitrofurantoin	2-64	>64
4	Lincomycin	1-8	>8
	Nitrofurantoin	2-64	>64
5	Chloramphenicol	2-32	16
	Nitrofurantoin	2-64	>64
6	Lincomycin	1- 8	>8
	Nitrofurantoin	2-64	>64
7	Lincomycin	1-8	>8
	Linezolid	0.5-8	>8
	Nitrofurantoin	2-64	>64
8	Lincomycin	1-8	>8
	Nitrofurantoin	2-64	>64
9	Lincomycin	1-8	>8
	Nitrofurantoin	2-64	>64
10	Chloramphenicol	2-32	>32
	Lincomycin	1-8	>8
	Nitrofurantoin	2-64	>64
11	Lincomycin	1-8	>8
	Nitrofurantoin	2-64	>64
	Linezolid	0.5-8	>8
12	Chloramphenicol	2-32	>32
	Lincomycin	1-8	>8
	Nitrofurantoin	2-64	>64
	Linezolid	0.5-8	>8

Moreover, one phage against *L. monocytogenes* (ϕ IZSAM-1) was isolated [11], and it showed a broad lytic spectrum, resulting active against all antibiotic resistant Listeria strains of Table 3.

The results of the antibiotic profiles for the *C. jejuni* strains showed resistance to ciprofloxacin and nalidixic acid for all the 3 strains tested. Resistance to tetracycline was confirmed in 2 strains (218M and IZS-Hum). Our results are in accordance with other works, highlighting the emergent multi-drug resistance of Campylobacter, in particular to ciprofloxacin, tetracycline and erythromycin [12].

Two phages against *C. jejuni* (ϕ7 and ϕ16) were isolated in our research and they were active against the 3 antibiotic resistant strains of Table 2 [9].

Table 2: *C. jejuni* strains used for phage isolation; they all showed sensitivity to ϕ7 and ϕ16.

ID	Penner serotype	PFGE type (SmaI)	PFGE type (Kpn)	FlaA-SVR	Origin
218M	HS:5j	-	-	-	NCTC 12662
252gM/12A	HS:55	7	VII	265	Poultry
IZS-Hum	-	2	II	-	Human

Table 4: Antibiotic susceptibility and MIC values of *C. jejuni* strains used in the study, resistance/sensitivity profiles are reported according to EUCAST ECOFF. BFi: before phage exposure; AFi: after phage exposure.

Strains	Antibiotics	Range (µg/mL)	MIC (µg/mL)	Results
218M	Ciprofloxacin	0.12-16	BFi: 16	R
			AFi: <-0.12	S
	Nalidixic acid	1-64	BFi: 64	R
			AFi: <-1	S
	Tetracycline	0.5-64	BFi: 64	R
			AFi: 32	R
252gM	Ciprofloxacin	0.12-16	16	R
	Nalidixic acid	1-64	64	R
IZS-Hum	Ciprofloxacin	0.12-16	16	R
	Nalidixic acid	1-64	64	R
	Tetracycline	0.5-64	64	R

Moreover, *C. jejuni* 12662 strains analyzed before and after phage exposure showed a very interesting finding: the strain after phage treatment lost the resistance to ciprofloxacin, nalidixic acid and tetracycline (Table 4). The only reference about the ability of phage resistant mutant bacteria to revert into strains sensitive to antibiotics is reported in an EFSA Opinion, 2016 [13]. In this document, two *L. monocytogenes* phage resistant strains are described to acquire sensitivity to ciprofloxacin and another one to erythromycin, after infection with ϕP100. The scientific reasons at the bases of this interesting and useful phenomenon are still unclear and needs more elucidation. In the future, the authors will investigate on the possibility that the *Listeria* and *Campylobacter* strains assayed in this study could change their antibiotic resistance profiles and MIC values after phage infection.

Conclusion

Phages are the most prevalent replicating forms on Earth and their variety supports the preparation of an almost unlimited number of combinations of them in order to kill bacteria. Phages can be a useful weapon to use above all in case of prokaryotes that show antibiotic resistance patterns. The *L. monocytogenes* and *C. jejuni* phages that we isolated in this work showed the ability to kill several bacterial strains, also characterized by resistance to some of the most common antibiotics used in human and veterinary medicine. Interesting was the finding that 1 *C. jejuni* strain lost its resistance to antimicrobials after phage exposure. This results needs to be further investigated. The strategy of administering phages in case of diseases caused by antibiotic resistant bacteria and then the application of specific antibiotics after bacteria have re-acquired their sensitivity to the drugs could be exploited as an intelligent tool to apply in the future.

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