Title: Phage sensitivity and prophage carriage in *Staphylococcus aureus* isolated from foods in Spain and New Zealand

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Abstract

Bacteriophages (phages) are a promising tool for the biocontrol of pathogenic bacteria, including those contaminating food products and causing infectious diseases. However, the success of phage preparations is limited by the host ranges of their constituent phages. The phage resistance/sensitivity profile of eighty seven *Staphylococcus aureus* strains isolated in Spain and New Zealand from dairy, meat and seafood sources was determined for six phages (Φ11, K, ΦH5, ΦA72, CAPSa1 and CAPSa3). Most of the *S. aureus* strains were sensitive to phage K (*Myoviridae*) and CAPSa1 (*Siphoviridae*) regardless of their origin. There was a higher sensitivity of New Zealand *S. aureus* strains to phages isolated from both Spain (ΦH5 and ΦA72) and New Zealand (CAPSa1 and CAPSa3). Spanish phages had a higher infectivity on *S. aureus* strains of Spanish dairy origin, while Spanish strains isolated from other environments were more sensitive to New Zealand phages. Lysogeny was more prevalent in Spanish *S. aureus* compared to New Zealand strains. A multiplex PCR reaction, which detected ΦH5 and ΦA72 sequences, indicated a high prevalence of these prophages in Spanish *S. aureus* strains, but were infrequently detected in New Zealand strains. Overall, the correlation between phage resistance and lysogeny in *S. aureus* strains was found to be weak.

Keywords: Bacteriophage, *Staphylococcus aureus*, lysogeny, phage resistance, biocontrol.

1. Introduction

Staphylococcus aureus is a serious threat to human health, due to its ability to cause a multitude of skin and respiratory infections and foodborne illnesses. It is part of the normal microbiota on human skin and in mucous, and is the main cause of *Staphylococcus* infections in hospitals (Figueiredo and Ferreira, 2014) and food contamination during handling (Wattinger et al., 2012). Its ability to form biofilms can lead to persistent contamination of food processing (Gutiérrez et al., 2012; Herrera et al., 2007; Spanu et al., 2013) and hospital environments (Otto, 2013). Recently, the exponential increase in livestock-associated methicillin-resistant *S. aureus* strains (LA-MRSA), such as clone CC398, have become a concern due to their emergence along the whole farm to fork chain (farm animals, meat product and humans) (Fluit, 2012). Moreover, the emergence of vancomycin resistant *S. aureus* strains (VRSA) narrows the antibiotic arsenal available to treat staphylococcal infections (Weigel et al., 2003).

Phages and phage lytic proteins have been proposed as alternative treatments to reduce food contamination and combat infections caused by pathogenic bacteria (García et al., 2010). Some phage-based products are already available in the market to be used in the food industry. These include Listex™ P100 (www.micreosfoodsafety.com) and ListShield™ (www.intralytix.com) that have been recognized as safe by the US Food and Drug Administration (FDA) and approved by the US Department of Agriculture (USDA) as antimicrobial processing aids to combat *Listeria monocytogenes* in foods, and on food processing surfaces.

One of the key factors for the success of phage-based products is likely to be a sufficiently wide host range to ensure efficacy against the majority, if not all, strains of the pathogen. Due to their specificity for certain receptors on the cell wall, phages typically have relatively a narrow host range, and so to overcome this, the use of phage

69 mixtures is usually preferred (Chan et al., 2013; Hagens and Loessner, 2010). Another
70 factor to be considered in the use of phage biocontrol in foods is lysogeny in the target
71 bacterium. Prophages typically impart immunity to super-infection of related phages to
72 the host cell (Berngruber et al., 2010), and so this could be a potential barrier to the
73 successful use of phage biocontrol. Prophages are very often present in the chromosome
74 of pathogenic bacteria, and the majority of *S. aureus* isolates harbor at least one
75 prophage (Goerke et al., 2009).
76 Previously, we have characterized the temperate phages ΦA72 and ΦH5 isolated from
77 the dairy environment in Spain, and their lytic derivatives, vB_SauS-phiIPLA35 and
78 vB_SauS-phiIPLA88, both belonging to the *Siphoviridae* family (García et al., 2007,
79 2009a). These phages were able to inhibit *S. aureus* growth in milk, curd and cheese
80 manufacturing processes (Bueno et al., 2012; García et al., 2007, 2009b). More recently,
81 biocontrol candidate phages CAPSa1 and CAPSa3 were isolated from milk samples in
82 New Zealand. They are virulent phages that belong to the *Siphoviridae* family
83 (unpublished).
84 The present work aims to address the efficacy of biocontrol using phages and hosts
85 from distant geographical areas such as Spain and New Zealand. To do this, we have
86 determined phage sensitivity/resistance profiles in a representative *S. aureus* collection
87 containing strains from both countries. In addition, we have characterized the incidence
88 of lysogeny and the carriage of prophages ΦA72 and ΦH5 and its relationship with
89 bacterial resistance.
90

91 **2. Material and Methods**

92 **2.1 Bacterial strains, phages, media and growth conditions.**

Sixty four *S. aureus* strains from three food environments (dairy, meat and seafood) were isolated in Spain, and 23 strains from dairy and one strain from meat were obtained from New Zealand (Table 1). Staphylococcal cells were isolated on Baird Parker Agar (BP) supplemented with egg yolk, and routinely cultured in TSB broth (Triptone Soy Broth, Scharlau) at 37ºC with shaking or in TSB plates containing 2% (w/v) bacteriological agar (TSA).

Phages were routinely propagated as previously described (García et al., 2007). Phage K (O'Flaherty et al., 2005), Φ11 (Iandolo et al., 2002), ΦA72 and ΦH5 were propagated in *S. aureus* Sa9, while *S. aureus* NZRM2016 was used as host strain for phages CAPSa1 and CAPSa3. Phage enumeration was performed by the double-layer technique (Gutiérrez et al., 2010) using soft TSA medium (0.7% agar plus 10 mM $CaCl_2$ and 10 mM $MgSO_4$) in the upper layer.

2.2 Lysogeny determination.

The presence of resident prophages in the *S. aureus* strain collection was determined by mitomycin C induction as previously described (Gutiérrez et al., 2010). Briefly, mid-exponential-phase cultures were treated with 0.5 µg/ml of mitomycin C (Sigma-Aldrich, St. Louis, MO) for three hours at 37ºC and shaking. Supernatants were filtered and spotted into agar overlayed lawns of all the *S. aureus* strains.

2.3 Phage host range.

The host range of each phage was obtained against a collection of *S. aureus* strains by spotting 5 µl (10^9 pfu/ml) of the phage suspension into the lawn of each strain using the double-layer technique. Efficiency of plating (EOP) was calculated using *S. aureus* Sa9 as the reference strain (Gutiérrez et al., 2010).

118

119 **2.4 Multiplex PCR.**

120 The genomic nucleotide sequences of ΦA72 and ΦH5 (García et al., 2009a) were
121 subjected to progressive MAUVE alignment, using the default settings
122 (http://gel.ahabs.wisc.edu/mauve/). Regions with no homology were analyzed to design
123 specific primers for each phage, and these primers were submitted to *in silico* PCR
124 amplification (http://insilico.ehu.es/PCR/) to verify their specificity. For ΦA72, one pair
125 of primers was designed surrounding the *orf*2 (522 bp, from nucleotide 1350 to 1872)
126 and another pair in the region corresponding to a methyl transferase, *orf22* (324 bp,
127 from 11331 to 11655). For ΦH5, pairs of oligonucleotides were designed in *orf*29 (704
128 bp, from 151-855) and in the integrase region, *orf1* (225 bp, from 13013-13238). Total
129 DNA was extracted by GenElute^{TM} Bacterial Genomic DNA Kit (Sigma-Aldrich,
130 Madrid, Spain), according to the manufacturer´s instructions. PCR reactions were
131 performed with PureTaq Ready-To-Go^{TM} PCR Beads (GE Healthcare, Munich,
132 Germany), 10 ng of DNA and 1 μM of each primer. PCR reactions were based on phage
133 ΦA72 (GenBank NC_011612) using four primers (gp2F:
134 5'GATAATTACAACTGGGATACC3'; gp2R:
135 5'GTATTCAGACAATGTTTTGAAG3'; metrF 5'ATAGAATGCAACATTCACC3';
136 metrR 5'GATAACAACCATTCTGGTAC3') and the other based on ΦH5 (GenBank
137 NC_011614) (int88F:5' ATCATTGTGTAATAGATAAGAGC3'; int88R:
138 5'GTTATTACAGATAAAGCTTATGC3'; gp29F:
139 5'CATGATTGAAGAGACCATC3'; gp29R: 5'CTACTGCGTCATTTAAATTTC3').
140 As positive control, pure phage DNA from ΦA72 and ΦH5 was used. PCR was
141 performed in a thermocycler (Bio-Rad, Hercules, USA) under the following thermal

142 cycling conditions: one cycle at 95 ºC for 5 min; 35 cycles at 95 ºC for 1 min, 54 ºC for
143 1 min and 72 ºC for 1 min; and a final step of 10 min at 75 ºC.
144
145 **2.5 Statistical analyses**
146 Statistical analyses were performed using R (R Core Team, 2013).The unpaired t-test
147 and Chi-square test was conducted to compare the sensitivity/resistance of
148 staphylococcal strains isolated in Spain and New Zealand to six phages. The Chi-square
149 test was used to compare the sensitivity/resistance of Spanish staphylococcal strains
150 isolated from different food environments (dairy, meat, seafood) to the same phages. A
151 significance level of 0.05 was chosen for these purposes. Fisher's exact two tailed test
152 was run using R (R Core Team, 2013), where the null hypothesis was that phage
153 sensitivity and lysogeny are independent. McNemar's Chi-squared test with continuity
154 correction was also run on R (R Core Team, 2013), with the null hypothesis being the
155 proportion of hosts sensitive is the same as the proportion of hosts lysogenic, i.e.
156 concordance between the two.
157
158 **3. Results and Discussion**
159 The recent interest in the use of phages as biocontrol agents of pathogenic bacteria in
160 food (García et al., 2010; Sillankorva et al., 2012) raises the question of their efficacy in
161 a globalized market. The use of next generation sequencing technology has profoundly
162 improved our understanding of the dynamic evolutionary processes, transmission and
163 prevalence of staphylococcal clonal lineages in different geographical locations
164 (Castillo-Ramírez et al., 2012; Parkhill and Wren, 2011). However, data on phage
165 susceptibility of this species, and others, is scarce (Argudín et al., 2012).

166 A collection of *S. aureus* strains composed by isolates from distant locations, Spain (SP)
167 and New Zealand (NZ), was gathered. The Spanish collection encompassed sixty three
168 strains isolated from food-contact surfaces from dairy, meat and seafood factories
169 (Gutiérrez et al., 2012) and also from milk and dairy products (García et al., 2007)
170 (Table 1). These strains had been previously characterized as described in the above
171 references and were chosen on the basis of their distinct phenotypic properties
172 (antibiotic resistance, biofilm as well as genetic diversity based on their RAPD-PCR
173 profiles and presence/absence of endotoxin and biofilm genes. The New Zealand
174 collection was composed by twenty three *S. aureus* strains from dairy samples and one
175 from meat. The genetic relatedness within these strains is not known but they have been
176 gathered from different sources and locations. These collections were used to test the
177 susceptibility to the well characterized lytic phage K, temperate phage Φ11, phages
178 ΦA72 and ΦH5 isolated in Spain, and phages CAPSa1 and CAPSa3 isolated in New
179 Zealand. The spot-on-the-lawn technique was chosen as a quick and easy screening tool
180 to test all the phages on the same host plates together. Furthermore, to avoid
181 overestimating of the host range due to lysis-from-without, appropriate dilutions were
182 also spotted to confirm the presence of lytic plaques when deemed necessary and the
183 efficiency of plating was calculated using as reference strain *S. aureus* Sa9, susceptible
184 to all tested phages (Supplementary table 1).
185
186 **3.1 Infectivity of *S. aureus* with respect to geographic origin of the phage and host**
187 As expected from previous reports, phage K was the phage with the broadest host range
188 showing lysis on all but 4 SP and 1 NZ strains. Phage K is a lytic phage representative
189 of the *Myoviridae* family, infecting staphylococcal strains from human and bovine

190 origin (O'Flaherty et al., 2005), and our results further confirm the polyvalent nature of
191 this phage.
192 Φ11 was chosen as a representative of temperate phages belonging to *Siphoviridae*
193 family (Iandolo et al., 2002). This phage displayed a narrow host range and lysed 29%
194 of NZ strains compared to 48% of the SP *S. aureus,* respectively (Fig. 1). Nevertheless,
195 this difference was not statistically significant ($P>0.05$).
196 On the contrary, the geographical origin of the *S. aureus* host significantly ($P<0.01$)
197 determined the infectivity of the Spanish phages ΦA72 and ΦH5. Less than one third of
198 the SP strains were infected, whereas these phages infected more than 90 % of *S. aureus*
199 strains isolated in New Zealand (Fig. 1).
200 For the phages isolated in New Zealand, CAPSa1 had a broad host range, infecting 62%
201 of SP strains and 71% of NZ strains. By contrast, CAPSa3 preferentially infected NZ
202 over SP strains ($P<0.01$) (Fig. 1). Overall, NZ *S. aureus* strains were more susceptible
203 to all the phages than SP strains (Fig. 1).
204 Taken together these results indicate no clear association of infectivity of phages against
205 *S. aureus* strains based on the geographic region of host or phage isolation. These data
206 contrast with those obtained from studies in ecosystems like soil, where phages appear
207 to adapt to become more infective against bacteria living closely (Vos et al., 2009). A
208 possible explanation for the observed differences may be the globalization of the food
209 industry and the increased frequency of international travel for animals and humans,
210 which are key vectors of *S. aureus*. Therefore, the future design of phage preparations
211 for international markets should consider the potential for both local micro- and global
212 macro-scale interactions of phages and hosts.
213

3.2 Infectivity of *S. aureus* with respect to environmental origin of the phage and host

Phages ΦA72 and ΦH5 are derivatives of phages isolated from milk, and were previously determined to preferentially infect *S. aureus* strains from dairy compared to clinical origin (García et al., 2007). This observation suggested that the environmental source of hosts, and potentially of phages, may be relevant in the preparation of a phage biocontrol for foods. So we wished to test this further by expanding the panel of strains and phages, including the NZ phages CAPSa1 and CAPSa3 which were also isolated from milk, Φ11, a temperate phage from a clinical *S. aureus* isolate (Novick, 1963), and phage K, the origin of which is unclear (Burnet and Lush, 1935).

Only SP hosts were tested in this series of experiments as there were only two non-dairy host isolates available from NZ (Table 1). For phage K, Φ11 and ΦH5, the source of the host was not a significant factor for phage infectivity (Fig. 2). Whereas, for the other three phages ΦA72 (P<0.05), CAPSa1 (P<0.01) and CAPSa3 (P <0.05) there were significant differences in infectivity with hosts from dairy meat and seafood (Fig. 2). ΦA72 infected mostly dairy strains (40%), with no infection of seafood strains. CAPSa1 infected all meat and seafood strains, but fewer (31%) dairy strains. CAPSa3 was most active on strains from meat (60%), than either dairy (17%) or seafood (11%) (Fig. 2). These results highlight the need of developing specific phage preparations tailored to the food, and food-specific strains, in which they are to be applied.

3.3 Inducible prophages in *S. aureus* strains

The presence of prophages integrated in the bacterial chromosome confers immunity to superinfection and thus resistance to phage attack by closely related phages (Berngruber et al., 2010). Microarray studies have shown that prophages integrated in the bacterial

239 chromosomes are the most widespread mobile genetic elements in *S. aureus* strains,
240 with most of them carrying between one and four prophages (Goerke et al., 2009;
241 Pantucek et al., 2004). However, there is scarce data about the prevalence and diversity
242 of phage populations in specific geographical areas (Rahimi et al., 2012). Some
243 prophages have been determined to be specific for MRSA lineages and are linked to
244 geographical variants, such as the φSPβ-like prophage which is characteristic of the
245 ST239 'Asian clade' (Wang et al., 2014).
246 Mitomycin C induction of our *S. aureus* collection was performed and the presence of
247 phages tested in the culture supernatants. Results showed that only four (16.7%) of the
248 NZ strains were lysogenic for at least one phage, as the supernatants from the induced
249 cultures produced plaques on several strains (Table 2). By contrast, the presence of
250 prophages was detected in 78% of Spanish strains, (note data for some SP strains
251 previously described by Gutiérrez et al. (2012) and García et al. (2007). It is
252 noteworthy, that lysogeny seems to be more widespread in dairy environment, with
253 97% of dairy strains positive for lysogeny, compared to the meat (30% strains) and
254 seafood (67% strains) environments.
255 Fisher's Test's suggested the association between *S. aureus* lysogeny and phage
256 sensitivity was not significant, with the exception of CAPSa1 with SP hosts ($p<0.001$).
257 Similarly, McNemar's Test for dependence between phage sensitivity and lysogeny was
258 rejected for all the phages ($\alpha=0.05$), with the exception of Φ11 in both SP and NZ
259 strains, and ΦCAPSa1 in SP strains. Overall, the evidence for an association between
260 inducible prophages in the host and phage resistance is weak.
261

262 **3.4 Prevalence of prophages ΦH5 and ΦA72 in geographically distant *S. aureus***
263 **strains**

The prevalence of prophages ΦH5 and ΦA72 in SP and NZ *S. aureus* strains was determined by multiplex PCR. Two primer pairs were designed for each phage as described in the methods and the PCR results for each strain are compiled in supplementary table 1. Within all strains, at least one ΦA72-like sequence was detected in 41% of strains, and 40% of the strains harbored a ΦH5-like sequence (Table 2). Both ΦA72-like sequences were detected in 23% of the strains; while only eight strains had both ΦH5-like sequences (Table 2). These results likely reflect the mosaic nature of *S. aureus* phage genomes (Kahankova et al., 2010).

Interestingly, phages ΦA72 and ΦH5 seem to be widely spread among SP strains (32% had both ΦA72-like sequences, and 11% both ΦH5-like sequences), but they were rare in NZ strains (Table 2). No ΦH5-like sequences were detected in the NZ strains, and only three strains were positive for ΦA72 sequences. The absence of ΦA72 and ΦH5 related prophages on NZ strains could explain their higher sensitivity to these phages. Similarly, the prevalence of ΦA72-like prophages among the SP seafood strains might also contribute to the high frequency of strains resistant to this phage (100%).

4. Conclusion

The sensitivity of wild *S. aureus* strains to phage infection was not determined by the geographical origin of the strains. However, there appeared to be an association between phage infectivity and the environment in which the strains were isolated for some phages, but not all. The implications for biocontrol are that it may be more important to isolate and use phages with a naturally broad host range, than to isolate phages from the same region or environment as the target microorganism. Whilst the prevalence of lysogeny appeared to differ between two distant geographic locations, there was no

strong correlation between resistance of these strains to phage infection and the presence of prophages.

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Table 1. Strains used in this study, geographical isolation and origin.

Country	Food Industry	Origin	*S. aureus* strain	Reference
Spain	Dairy 1	Milk	Sa1, Sa2, Sa3, Sa4, Sa5, Sa6, Sa8, Sa9, Sa10, Sa11, Sa12, Sa13, Sa14, Sa15, Sa16	Garcia et al., 2009b
	Dairy 2	Milk	AAAC9, AAAC10, AAAC11, AFG1, AFG2, GDC3, GDC6, GDC9, GRA16, GRA17, GRA20, JFL2, JFL4, JFL6, JFL8	
	Dairy 3	Milk	IPLA19, IPLA20, IPLA24	Unpublished
	Dairy 4	Food-contact surfaces	IPLA1, IPLA3	Gutiérrez et al., 2012
	Meat 1	Food-contact surfaces	IPLA5, IPLA6, IPLA7, IPLA8, IPLA13, IPLA14, IPLA15, IPLA16, IPLA17, IPLA18	
	Sea food	Food-contact surfaces	IIM201, IIM208, IIM214, IIM222, IIM228, IIM229, IIM233, IIM234, IIM235, IIM237, IIM238, IIM239, IIM240, IIM241, IIM242, IIM245, IIM246 IIM249	
New Zealand	Dairy 5	Raw milk	PHCFAP1, PHCFAP2, PHCFAP3, S34, S36, S38, S39, S41, S43, S45, S46, S47	Unpublished
	Dairy 6	Cheese	S52, S51, FM31, FM34	
	Dairy 7	Milk powder	S100, S12	
		Skimmed milk powder	S27, S28	
		Dairy product	S14	
		Cream pie	NZRM3372	
	Meat 2	Ham	NZRM3374A	
	Bovine	-	NZRM2016	

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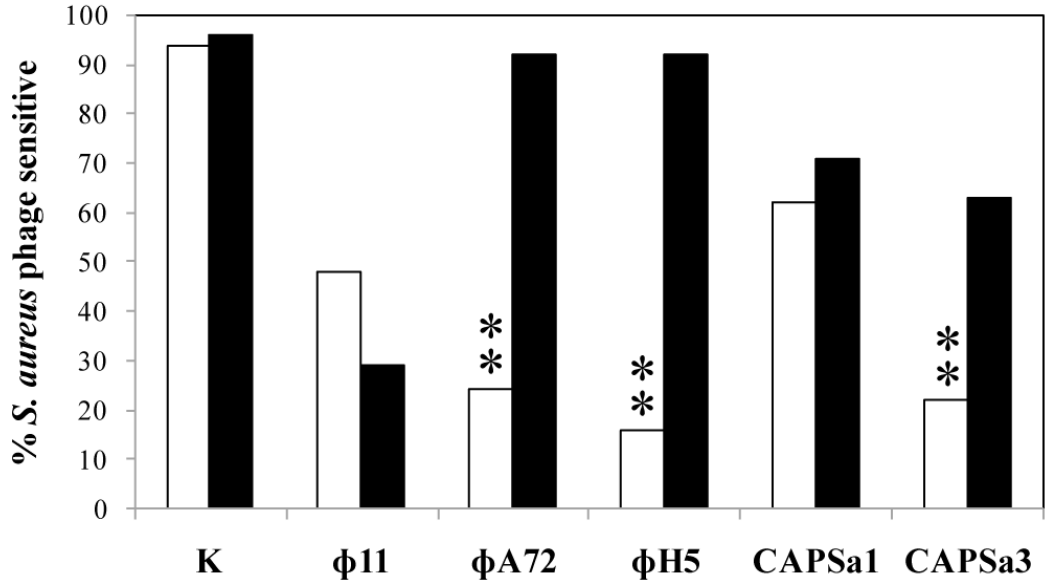
Table 2. Lysogeny and detection of ΦH5- and ΦA72-like sequences by multiplex PCR in *S. aureus* isolated from distantly located regions and environments.

S. aureus (no. strains tested)	Lysogeny[a]	No. strains positive					
		Prophage carriage					
		ΦA72			ΦH5		
		*orf*2	*orf*22	both	*orf*1	*orf*29	both
Spain							
Dairy 1 (15)	15	0	6	0	1	8	0
Dairy 2 (15)	15	1	6	1	11	0	1
Dairy 3 (3)	3	0	0	2	0	1	1
Dairy 4 (2)	2	0	1	0	0	0	1
Meat 1 (10)	3	0	1	4	0	7	2
Seafood (18)	12	1	1	13	0	11	3
Presence (%)		**3**	**24**	**32**	**19**	**43**	**11**
New Zealand							
Dairy 5 (12)	3	0	1	0	0	0	0
Dairy 6 (4)	1	1	0	0	0	0	0
Dairy 7 (6)	0	1	0	0	0	0	0
Meat 2 (1)	0	0	0	0	0	0	0
Bovine (1)	0	0	0	0	0	0	0
Presence (%)		**8**	**4**	**0**	**0**	**0**	**0**
TOTAL (%)		**5**	**18**	**23**	**14**	**31**	**9**

[a] Lysogeny determined by induction with Mitomycin C

401 **Figure 1.** Phage sensitivity of *S. aureus* isolated from Spain (white bars, n=63) and
402 New Zealand (black bars, n=23) and. **, $P<0.01$.



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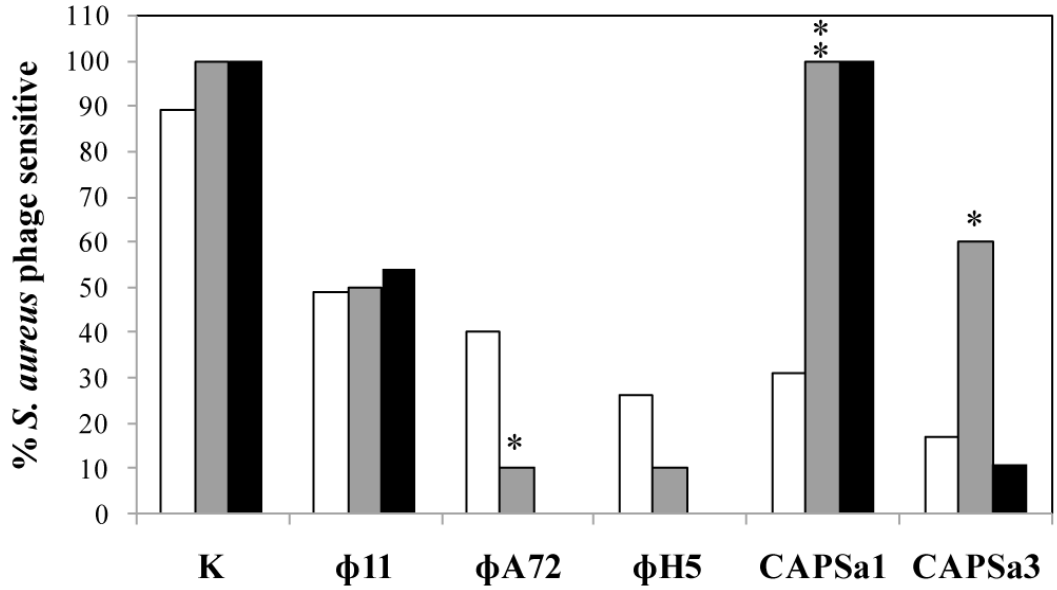
405 **Figure 2.** Phage sensitivity of *S. aureus* isolated from different food environments.
406 Dairy (white columns, n=35), meat (grey columns, n=10) and seafood (black columns,
407 n=18). *, $P<0.05$; ** $P<0.01$.



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Suplementary table 1. (A) Efficiency of plating (EOP) of phage K, Φ11, ΦA72, ΦH5, CAPSa1 and CAPSa3. *Staphylococcus aureus* Sa9 was used as a reference host. Empty cells show strains resistant to phage infection that gave neither lysis halo by spot-on-the-lawn nor single lysis plaques. With the exception of EOPs on strains isolated in New Zealand (see Table 1), data provided are means ± standard deviation of two independent EOP determination. *: positive by spot-on-the-lawn but EOP not determined. Shaded cells are results taken from García et al. (2009b). (B) Detection of ΦA72- and ΦH5-like sequences by PCR shown by +. Empty cells show absence of the PCR product.

A

S. aureus	Bacteriophage					
	K	Φ11	ΦA72	ΦH5	CAPSa1	CAPSa3
Sa1	0.58 ± 0.18		1.01 ± 0.07	0.72 ± 0.03		
Sa2	0.81 ± 0.05		0.39 ± 0.02		0.74 ± 0.03	
Sa3	0.26 ± 0.04		1.02 ± 0.18			
Sa4	0.38 ± 0.03		1.32 ± 0.06			
Sa5	1.04 ± 0.31	0.0018 ± 0.0003				
Sa6	0.22 ± 0.03					
Sa8	0.45 ± 0.11				0.62 ± 0.05	
Sa9	1.00 ± 0.21	1.00 ± 0.03	1.00 ± 0.07	1.00 ± 0.03	1.00 ± 0.12	1.00 ± 0.07
Sa10	0.42 ± 0.05	0.26 ± 0.01			0.77 ± 0.01	
Sa11	0.32 ± 0.02					
Sa12	0.16 ± 0.01	0.39 ± 0.02	0.26 ± 0.05			0.73 ± 0.03
Sa13	0.11 ± 0.01					0.35 ± 0.08
Sa14	0.41 ± 0.05	0.0017 ± 0.0002				
Sa15	0.34 ± 0.01					
Sa16	0.76 ± 0.03	0.0189 ± 0.0041				
AAAC9	0.33 ± 0.01		0.82 ± 0.02	0.60 ± 0.10		
AAAC10	0.29 ± 0.01					
AAAC11	0.25 ± 0.02		0.75 ± 0.01	0.82 ± 0.08	0.56 ± 0.03	
AFG1	0.25 ± 0.29	0.0276 ± 0.0007	4.59 ± 5.19	0.12 ± 0.00	0.53 ± 0.01	
AFG2	0.41 ± 0.00	0.0300 ± 0.0007				
GDC3	0.41 ± 0.05		0.50 ± 0.05			
GDC6	0.35 ± 0.00		0.51 ± 0.01	0.76 ± 0.01		
GDC9	0.23 ± 0.02					
GRA16	0.43 ± 0.03		0.94 ± 0.02	0.75 ± 0.03		0.74 ± 0.07
GRA17	0.39 ± 0.02					
GRA20	0.47 ± 0.03					
JFL2		0.18 ± 0.04	0.60 ± 0.02	0.85 ± 0.01		
JFL4		0.0027 ± 0.0001				
JFL6		0.189 ± 0.007	0.54 ± 0.01			
JFL8		0.42 ± 0.09				
IPLA1	0.47 ± 0.09	1.07 ± 0.0685			0.47 ± 0.53	1.19 ± 0.08
IPLA3	0.39 ± 0.04	0.1646 ± 0.01			0.57 ± 0.08	1.12 ± 0.10
IPLA5	0.81 ± 0.05	0.037 ± 0.004	0.66 ± 0.05	0.79 ± 0.02	0.70 ± 0.03	0.98 ± 0.05
IPLA6	0.33 ± 0.02				0.85 ± 0.01	
IPLA7	0.50 ± 0.06				0.53 ± 0.07	0.83 ± 0.02
IPLA8	0.76 ± 0.04	).00024 ± 0.00001			0.37 ± 0.12	
IPLA13	0.31 ± 0.01	0.026 ± 0.001			0.59 ± 0.03	
IPLA14	0.58 ± 0.06	0.027 ± 0.001			0.34 ± 0.01	0.59 ± 0.08
IPLA15	0.38 ± 0.03				0.51 ± 0.03	1.10 ± 0.06
IPLA16	0.49 ± 0.06				0.93 ± 0.02	
IPLA17	0.51 ± 0.06	).00013 ± 0.00002			0.57 ± 0.01	0.83 ± 0.02
IPLA18	0.47 ± 0.03				0.69 ± 0.03	0.84 ± 0.06
IPLA19	0.34 ± 0.01	0.62 ± 0.03			0.61 ± 0.05	
IPLA20	0.43 ± 0.08	0.484 ± 0.007		0.93 ± 0.03	0.76 ± 0.03	
IPLA24	0.31 ± 0.01	0.35 ± 0.03			0.32 ± 0.02	
IIM201	0.47 ± 0.02	0.0047 ± 0.0003			0.82 ± 0.03	
IIM208	0.82 ± 0.06	0.007 ± 0.001			0.52 ± 0.01	
IIM214	0.40 ± 0.03	0.055 ± 0.007			0.09 ± 0.00	
IIM222	0.25 ± 0.02				0.46 ± 0.01	0.46 ± 0.05
IIM228	0.98 ± 0.13				0.57 ± 0.02	
IIM229	0.33 ± 0.01	0.0006 ± 0.0001			0.78 ± 0.05	
IIM233	0.31 ± 0.00				0.88 ± 0.04	
IIM234	0.41 ± 0.06				0.81 ± 0.01	
IIM235	0.26 ± 0.01				0.80 ± 0.01	
IIM237	0.41 ± 0.01				0.50 ± 0.03	
IIM238	0.48 ± 0.03	.00002 ± 0.000004			0.60 ± 0.03	0.35 ± 0.00
IIM239	0.74 ± 0.01	0.008 ± 0.003			0.65 ± 0.03	
IIM240	0.50 ± 0.01	0.063 ± 0.004			0.79 ± 0.03	
IIM241	0.83 ± 0.08				0.52 ± 0.02	
IIM242	0.64 ± 0.09				0.57 ± 0.01	
IIM245	0.34 ± 0.03				0.67 ± 0.01	
IIM246	0.52 ± 0.09				0.57 ± 0.01	
IIM249	0.29 ± 0.02	0.038 ± 0.003			0.75 ± 0.01	
PHCFAP1	1.08				*	*
PHCFAP2	0.78		0.32	*	*	*
PHCFAP3	0.04	*	*	*	*	*
S34	*		*	*	*	*
S36	*		*	*	*	*
S38	0.05		*	2.51	*	
S39	*		*	*	*	
S41	*		*	*	*	*
S43	0.02	*	*	*	*	*
S45	0.06	*	*	*	*	*
S46	*		*	*	*	*
S47	0.03	*	0.53	0.01	*	*
S52	*		*	*		*
FM31	*		*	*		
S100	*	*	2.16	*	*	*
S12						
S27	*		*	*	*	*
S28	*		*	*		
S14	*		*	*		
S51	*		*	*		
FM34	*		*	*		
NZRM3372	*		*	*	*	
NZRM3374A	*	*	*	*	*	*
NZRM2016	*	*	*	*	*	*

B

S. aureus	Presence of prophage			
	ΦA72		ΦH5	
	orf2	*orf22*	*orf1*	*orf29*
Sa1				+
Sa2				
Sa3				+
Sa4				+
Sa5				+
Sa6				+
Sa8				+
Sa9				+
Sa10				+
Sa11		+		
Sa12		+		
Sa13		+		
Sa14		+		
Sa15		+	+	
Sa16		+		
AAAC9		+	+	
AAAC10		+	+	
AAAC11		+		
AFG1		+	+	
AFG2		+	+	
GDC3		+		
GDC6			+	
GDC9			+	+
GRA16	+		+	
GRA17	+	+	+	
GRA20				
JFL2			+	
JFL4			+	
JFL6			+	
JFL8			+	
IPLA1		+	+	+
IPLA3				
IPLA5		+		+
IPLA6	+	+	+	+
IPLA7				
IPLA8	+	+		+
IPLA13	+	+	+	+
IPLA14				+
IPLA15				+
IPLA16				+
IPLA17	+	+		+
IPLA18				+
IPLA19	+	+	+	+
IPLA20	+	+		
IPLA24				+
IIM201			+	+
IIM208	+	+		
IIM214			+	+
IIM222			+	+
IIM228	+	+		
IIM229	+	+		+
IIM233	+	+		+
IIM234	+	+		+
IIM235		+		+
IIM237	+	+		+
IIM238	+	+		
IIM239	+	+		+
IIM240	+	+		+
IIM241	+	+		+
IIM242	+			
IIM245	+	+		+
IIM246	+	+		+
IIM249	+	+		+
PHCFAP1				
PHCFAP2				
PHCFAP3				
S34				
S36				
S38				
S39				
S41				
S43		+		
S45				
S46				
S47				
S52				
FM31				
S100				
S12				
S27				
S28	+			
S14				
S51	+			
FM34				
NZRM3372				
NZRM3374A				
NZRM2016				

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