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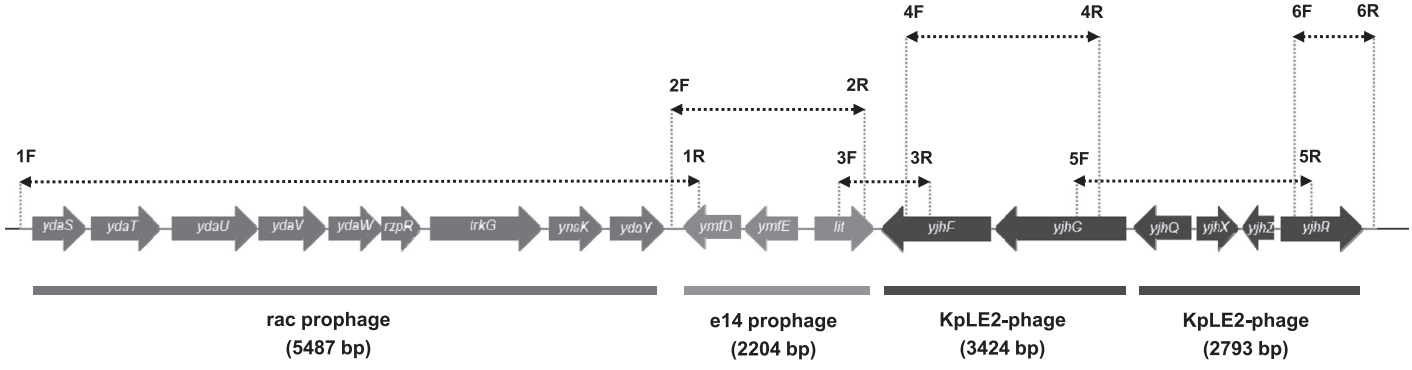
Letter to the Editor

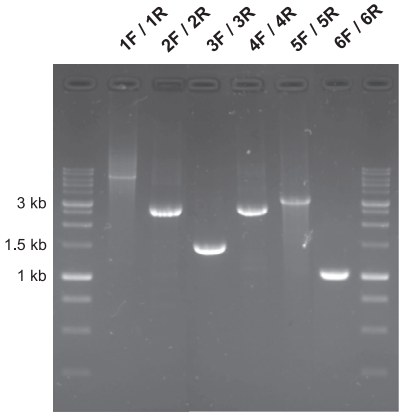
Prophages enhance resistance to antibiotic stress in a *bla*$_{NDM-1}$-carrying bacterial host: authors' reply

Sir,

We thank Dr Jobling for his valuable comment on our recent article regarding the cryptic prophages in an antibiotic resistance plasmid [1]. In our paper, we reported that the cryptic prophages in the plasmid may contribute to adaptation of the bacterial host to antibiotic stress [2]. The prophage features may allow the antibiotic-resistant strain to persist against antibiotic pressure and disseminate worldwide. We agree partially with Dr Jobling's opinion that our results may not be sufficient to support the conclusion stated in the paper. We acknowledge that we need to pay more attention to our experiments to justify the stated conclusions.

One of limitations of our study is that we investigated the effect of the prophages in the *Escherichia coli* laboratory strain DH5α that intrinsically contains the prophages in its chromosome. As the





Primers	Sequence (5'→3')	Amplicon size (bp)
1F	ACG TCT CTG ACG CCA TTT GCT TAA TAT TCT	7580
1R	GCA ATT TAA CAA TAT TTG AGC GGG TGT AAG	
2F	CGT TAC AGC GAT ACC CGC TGG CAT GAA GAT	2750
2R	TCG CAA CCT GTT GTT TGC AAG TTT TTC CTT	
3F	GTA TAT ACT ACC TAG CCC AAC AAT GTA GAG	1562
3R	CTA TCG CGG GTA TTG CCG GTC TGC TGC TGA	
4F	GGA CAT CAG CGT CCA GGA TAA CAG CGT TTC	2750
4R	ACG CTT CGC CAA TGG CGA ATT ATC TCT ACA	
5F	GCA GGT TTG TTG AAC CAC CGA ACG CGG CAT	3150
5R	CTT TAG CGC GTG AGA CAG CAA CAT TGA GGA	
6F	CGG CGC AGG TTA ATG CTA TCA AAA TGT CAT	1063
6R	CTG TCG GCG AAG GTA AGT TGA TGA CTC ATG	

Fig. 1. Detection of prophages rac and e14 in the chromosome and plasmid of the transconjugant (DH5α/pNDM-A1) and deleted mutant (DH5α/pNDM-A1-rac::Km). The primers were constructed to overlap the four prophage regions, as shown in the figure. The amplified products were confirmed by gelelectrophoresis, and primer sequences used in the amplification reaction are also shown.

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plasmid is from a *Klebsiella pneumoniae* isolate, the prophages in our study might be acclimatised to *K. pneumoniae* and may have different effects on *E. coli*. To investigate the role of prophages in a bla_{NDM-1}-harbouring plasmid, we attempted to conjugate our plasmids repeatedly into several *K. pneumoniae* reference strains not harbouring prophage genes in their chromosome, although in vain. Therefore, we used the *E. coli* reference strain DH5α as a bacterial host instead of *K. pneumoniae*. However, plasmids with both the carbapenemase gene (e.g. bla_{NDM-1} in our study) and prophages have also been identified in *E. coli* isolates. Thus, we reasoned that it would be worthwhile to use *E. coli* as a host.

However, we did not consider that prophage genes in the chromosome of the bacterial host might also be removed when we tried to delete prophage genes in the plasmid. PCR using internal primers of prophages revealed that prophages were deleted both from the chromosome and the plasmid of the transconjugant. The supplemental data in our previous study and the results of quantitative real-time PCR using the prophage sequences of each region (Supplementary Fig. S2 in the original paper) support these results [2]. In addition, PCRs overlapping the four prophage regions showed that the prophages are indeed contiguous and correctly assembled in the *K. pneumoniae* plasmid (Fig. 1). The susceptibility of the bacterial host to antibiotics may be due to deletion of prophage genes both from the chromosome as well as the plasmid. Thus, rather than deleting the prophage genes after conjugation of the plasmid to the host, the plasmid with deleted prophage genes should be conjugated into a bacterial host such as *E. coli* DH5α that intrinsically conserves the prophage.

Although the PCR results did not support plasmid-specific gene deletion, they revealed that the prophages were located in the plasmid harbouring the carbapenemase gene. The observation that the prophages were located adjacent to each other may indicate that they have been transferred *en bloc*, and not from *E. coli* DH5α in the process of transconjugation. Therefore, we cannot accept Dr Jobling's misassembly hypothesis.

Several reports show that prophages in bacterial chromosomes confer benefits to the bacterial host [3]. For example, they assist the bacterial host in tolerating adverse environments [4]. However, to the best of our knowledge, the effect of the prophage against antibiotic stress has not been investigated. Although our experiment is limited by the fact that plasmid-specific prophage deletion was not accomplished, our observation that prophage genes may contribute to a bacterial host's adaptation to antibiotic pressure is notable. In addition, the prophage of the plasmid may partially play such a role. We are grateful to Dr Jobling once again for the valuable comments on our research and we hope to identify more diverse and accurate roles of prophages in bacteria in the future.

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Competing interests

None declared.

Ethical approval

Not required.

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