LETTER

REPLY TO PARTRIDGE ET AL.:

Complementary bioinformatics and experimental approaches to investigate the transfer of AMR genes

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We would like to thank Sally R. Partridge et al. for the letter (1) and appreciate the opportunity to clarify our methods and thinking in relation to the concerns raised. We developed Plascad (2) for automated plasmid classification based on previously suggested schemes and standards (3–6) and extended the plasmid classification results in the existing database. We agree that the number of conjugative plasmids could be underestimated when the classification is relying on the presence of limited key genes. In fact, most of the misclassified plasmids in those Gram-positive bacteria mentioned by Partridge et al. are classified as mobilizable plasmids and also carry some genes related to conjugation based on our pipeline (Table 1), but do not meet the conserved criteria of the number of key indicators for conjugative plasmids previously proposed (5). Although mobilizable plasmids could exploit *trans*-acting conjugation apparatus and relaxases from coresident mobile genetic elements (MGEs) to increase the potential for transfer in some species, it's difficult to estimate the compatibility of these elements and how common this phenomenon is, based on plasmid sequence analysis alone. Despite the limitations of our methods, they will lay the foundation of opportunities for achieving a more balanced specificity and sensitivity in the future when more experimentally verified plasmids, as well as diverse underappreciated mechanisms that may be related to plasmid transfer, are integrated for analysis.

Regarding the insertion sequence (IS)-associated antimicrobial resistance (AMR) gene transfer, the identity-based criterion was introduced to infer the transfer under a molecular clock–based assumption following a criterion commonly used in the literature (7). We worked under the assumption that AMR genes having the highest percentage of identity (100% in our paper), plus the same IS–AMR gene distance in sequences from different species (< 97% 16S ribosomal RNA similarity), were likely to be more "recently" transferred than those with less identity, since the vertically inherited DNA in these distantly related genomes are nearly saturated with mutations at synonymous sites (8, 9). We chose 5 kb around each AMR gene to identify the most closely associated ISs that are more likely involved in the transfer of the AMR genes from a bioinformatic perspective (10), because most ISs (> 99%) are less than 5 kb in length. Although some relevant associations in complex multiresistance regions could be missed, we found that more than 77% (in terms of number) of the plasmid-borne AMR genes were closely associated with flanking ISs (figure 4 in ref. 2). We agree that the proximity of an AMR gene to an IS may not necessarily directly contribute to its movement, but the links between plasmids, ISs, and AMR genes, as well as the phylogenetic reach of these links, were systematically investigated in our study, which can help to explore vast amounts of potential IS-associated AMR gene transfer patterns over the limitation of the existing biological knowledge based on limited wet laboratory experiments and provide the candidate list for further validation. In our paper, we highlight the importance of integrating bioinformatics and experimental approaches to investigate the interactions of MGEs in mediating the transfer of AMR genes in future.

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Table 1. Plasmid typing results using Plascad

Plasmid	Accession ID	Size (bp)	Relaxase location (bp)	ATPase location (bp)	T4CP location (bp)	MPF systems location (bp)
pAMbeta1	GU128949.1	27,815	13,061–15,025	16,440–18,401	21,891–23,546	na
pCF10	AY855841.2	67,673	54,251–55,456	21,206–23,596	28,341–30,170	MPFT_virB6 (20,097–20,897)
pCP13	NC_003042.1	54,310	27,983–29,116	33,111–35,009	40,347–43,088	MPFI_traP (30,127–30,972)
pRE25	NC_008445.1	50,237	9,712–10,650	23,016–24,977	28,468–30,123	na
pSK41	AF051917.1	46,445	19,471–20,733	26,590–28,602	34,078–35,718	MPFG_44 (30,998–31,483)
pWBG4	KX149096.1	40,312	24,365–25,225	27,196–29,784	na	na
pMG1	AB206333.1	65,029	na	35,966–37,921	28,949–31,807	MPFI_traP (39,758–40,654)
pWBG749	NC_013327.1	38,087	na	11,077–13,074	4,877–7,648	MPFI_traK (27,923–28,168); MPFI_traP (14,828–15,673)
pCW3	DQ366035.1	47,263	na	na	na	na
pIP501	AJ505823.1	8,629	1,415–3,379	4,794–6,755	2,145–3,800	na
	L39769.1	8,136				

Complete sequence of plasmid pIP501 is not available in the National Center for Biotechnology Information plasmid database. Two fragments (AJ505823.1 and L39769.1) were used for analysis. MPF, mating pair formation. na, not detected.

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