

Twelve years of genomic surveillance of vancomycin-resistant *Enterococcus faecium*: emergence of linear *vanA* and bacteriocin-carrying plasmids challenging infection control

Ana C. Almeida-Santos^{a,b}, Ana P. Tedim^c, Bárbara Duarte^{a,b}, Ana P. Pereira^{a,b}, Luís M. Silva^d, Júlio Teixeira^d, Ana P. Castro^d, Louise Roer^e, Anette M. Hammerum^e, Henrik Hasman^e, Teresa M. Coque^{f,g}, Carla Novais^{a,b}, Ana R. Freitas^{a,b,h*}, Luísa Peixe^{a,b*}; ESCMID Study Group for Epidemiological Markers (ESGEM), and ESCMID Study Group on Mobile Elements and Plasmids (ESGMAP)

^aUCIBIO, Unidade de Ciências Biomoleculares Aplicadas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal

^bLaboratório Associado i4HB, Instituto para a Saúde e a Bioeconomia, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal

^cDepartamento de Anatomía Patológica, Microbiología, Medicina Preventiva y Salud Pública, Medicina Legal y Forense, Facultad de Medicina de la Universidad de Valladolid, Valladolid, Spain.

^dServiço de Microbiologia, Centro Hospitalar do Porto, Porto, Portugal

^eBacteria, Parasites and Fungi, Statens Serum Institut, Copenhagen, Denmark

^fHospital Universitario Ramón y Cajal -IRYCIS, Madrid, Spain;

^gCIBER in Infectious Diseases (CIBERINFEC), Madrid, Spain

^hUCIBIO, Unidade de Ciências Biomoleculares Aplicadas, Instituto Universitário de Ciências da Saúde (1H-TOXRUN, IUCS-CESPU), Gandra, Portugal

***Corresponding authors: Luísa Peixe:** UCIBIO, Laboratory of Microbiology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira 228, 4050-313, Porto, Portugal. Tel. +351 220428580. **Ana R. Freitas:** Laboratory of Microbiology, Department of Sciences, University Institute of Health Sciences (IUCS), CESPU, CRL, 4585-116 Gandra, Portugal. Phone: +351 224157213; E-mail: ana.freitas@iucs.cespu.pt.

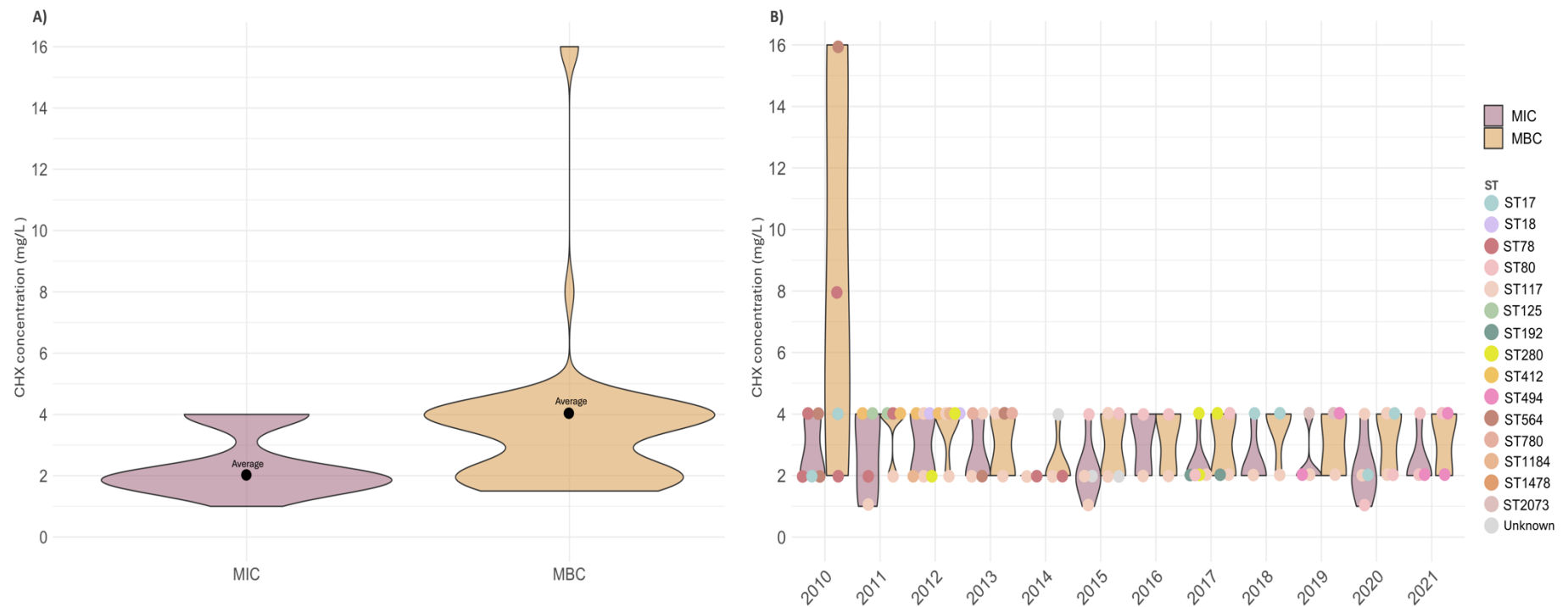


Fig. S1. A) Average minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of chlorhexidine in a representative subset of isolates (n=63). B) Distribution of MICs and MBCs by year and sequence type.

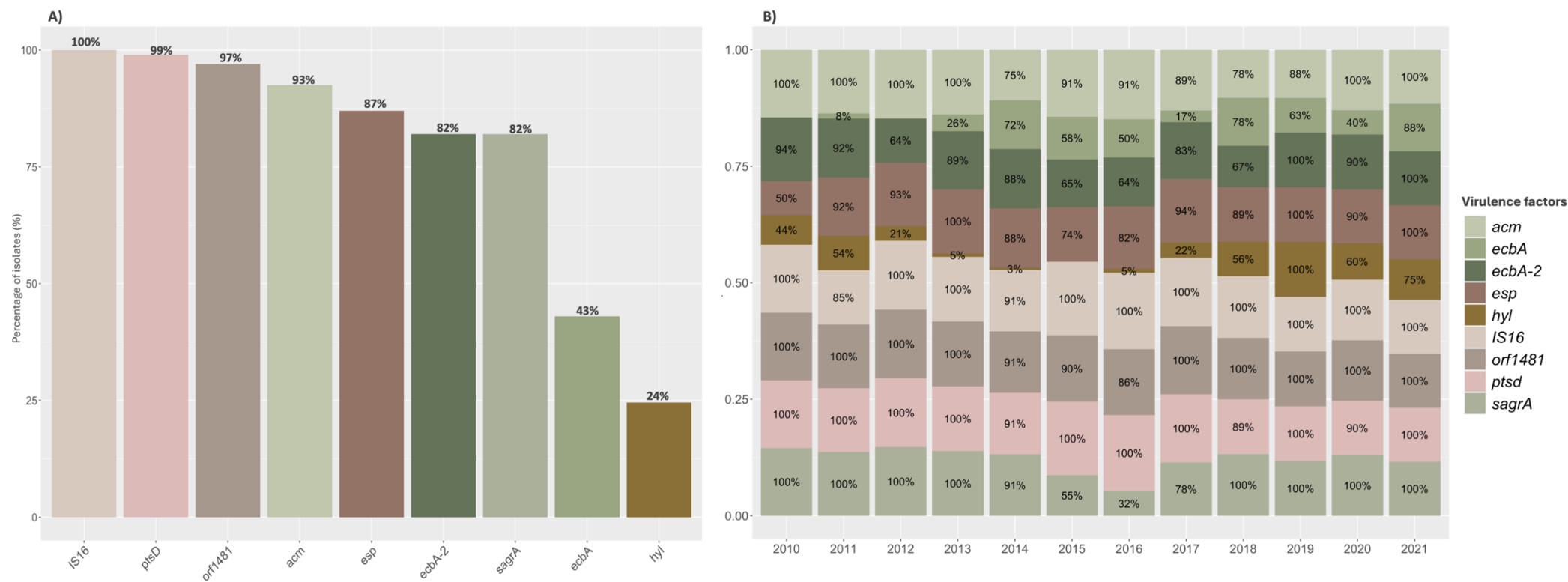


Fig. S2. A) Overall percentage of virulence markers detected by PCR. B) Annual distribution of virulence markers detected by PCR.

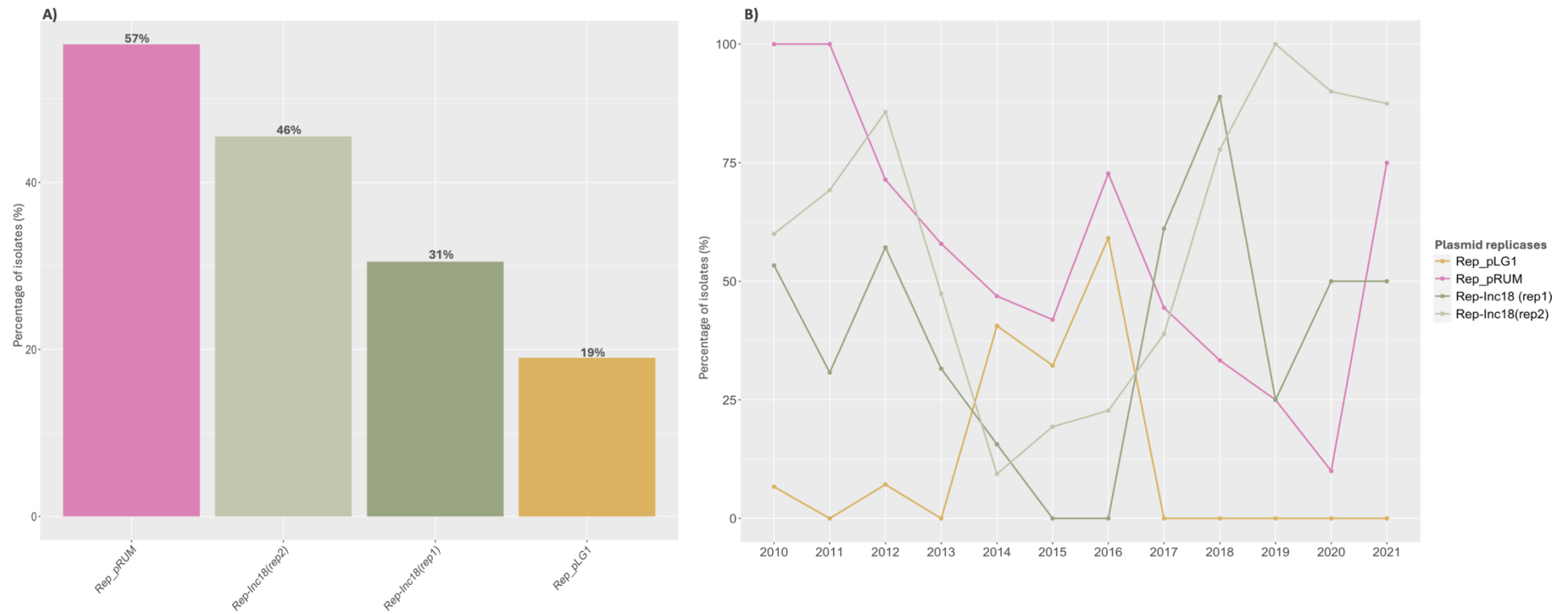


Fig. S3. A) Overall percentage of plasmid replicases [rep₁₇_pRUM (GenBank accession no.: AF507977) , rep₂₀_pLG1 (GenBank accession no.:CP004064), and rep₁_pSM19035 (GenBank accession no.:AY357120) and rep₂_pRE25 (GenBank accession no.:X92945), by PCR. B) Temporal dynamics of the same plasmid replicases detected by PCR.

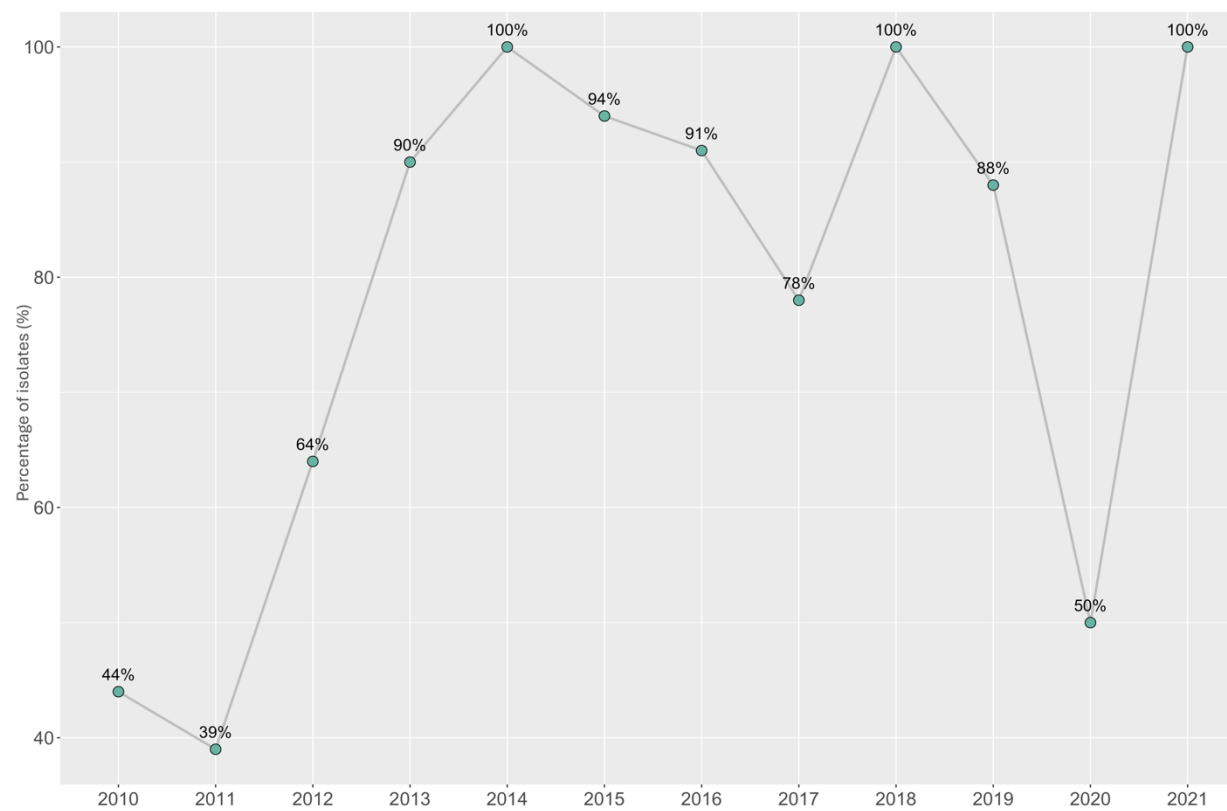


Fig. S4. Distribution of the bacteriocin 43 gene over a 12-year period.