Journal of Global Antimicrobial Resistance 36 (2024) 135–138

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Genome Note

Genomic data of global clones of CTX-M-65-producing *Escherichia coli* ST10 from South American llamas inhabiting the Andean Highlands of Peru

Adriana R. Cardenas-Arias[a], Elder Sano[a], Brenda Cardoso[a], Bruna Fuga[a,b], Fábio P. Sellera[c,d], Fernanda Esposito[b], Valentina Aravena-Ramírez[e], Dennis Carhuaricra Huaman[f], Carla Duran Gonzales[f], Luis Luna Espinoza[f], Lenin Maturrano Hernández[f], Nilton Lincopan[a,b,*]

[a] *Department of Microbiology, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil*
[b] *Department of Clinical Analysis, School of Pharmacy, University of São Paulo, São Paulo, Brazil*
[c] *Department of Internal Medicine, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil*
[d] *School of Veterinary Medicine, Metropolitan University of Santos, Santos, Brazil*
[e] *Departamento de Patología y Medicina Preventiva, Facultad de Ciencias Veterinarias, Universidad de Concepción, Chillán, Chile*
[f] *Research Group in Biotechnology Applied to Animal Health, Production and Conservation (SANIGEN), Laboratory of Biology and Molecular Genetics, Faculty of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Lima, Peru*

ARTICLE INFO

Article history:
Received 20 April 2023
Revised 20 November 2023
Accepted 28 November 2023
Available online 10 December 2023

Editor: Stefania Stefani

Keywords:
WHO critical-priority pathogens
Enterobacterales
ESBL
Camelids
One Health
Genomic surveillance

ABSTRACT

Background: The global spread of extended-spectrum β-lactamase (ESβL)-producing *Escherichia coli* has been considered a One Health issue that demands continuous genomic epidemiology surveillance in humans and non-human hosts.
Objectives: To report the occurrence and genomic data of ESβL-producing *E. coli* strains isolated from South American llamas inhabiting a protected area with public access in the Andean Highlands of Peru.
Methods: Two ESβL-producing *E. coli* strains (*E. coli* L1LB and L2BHI) were identified by MALDI-TOF. Genomic DNAs were extracted and sequenced using the Illumina NextSeq platform. De novo assembly was performed by CLC Genomic Workbench and in silico prediction was accomplished by curated bioinformatics tools. SNP-based phylogenomic analysis was performed using publicly available genomes of global *E. coli* ST10.
Results: *Escherichia coli* L1LB generated a total of 4 000 11 and L2BHI a total of 4 002 54 paired-end reads of ca.164 × and ca. 157 ×, respectively. Both *E. coli* strains were assigned to serotype O8:H4, *fim*H41, and ST10. The $bla_{CTX\text{-}M\text{-}65}$ ESβL gene, along with other medically important antimicrobial resistance genes, was predicted. Broad virulomes, including the presence of the *astA* gene, were confirmed. The phylogenomic analysis revealed that *E. coli* L1LB and L2BHI strains are closely related to isolates from companion animals and human hosts, as well as environmental strains, previously reported in North America, South America, Africa, and Asia.
Conclusion: Presence of ESβL-producing *E. coli* ST10 in South American camelids with historical and cultural importance supports successful expansion of international clones of priority pathogens in natural areas with public access.

1. Introduction

The global spread of extended-spectrum-β-lactamase (ESβL)-producing *Escherichia coli* has been considered a One Health threat that urgently demands mitigation strategies and continuous genomic epidemiology surveillance for mapping its incidence in humans and non-human hosts [1]. Because of its rapid

* Corresponding author at: Department of Microbiology, Instituto de Ciências Biomédicas, Universidade de São Paulo, Av. Prof. Lineu Prestes 1374 - 05508-000, São Paulo, Brazil.
E-mail address: lincopan@usp.br (N. Lincopan).

https://doi.org/10.1016/j.jgar.2023.11.011

dissemination and clinical implications on human patients, ESβL-producing *E. coli* was recently classified as a critical priority pathogen by the World Health Organization (WHO) [2]. *Escherichia coli* belonging to sequence type 10 (ST10) are host generalist, being frequently recovered form humans and other mammals and avian species [3]. Additionally, it has been increasingly recovered from environmental samples, making this a potential pandemic One Health clone [1].

South American camelids are ruminant animals that belong to the Camelidae family. This group is constituted by two wild species: guanaco (*Lama guanicoe*) and vicuña (*Vicugna vicugna*); and two other that are domesticated: llama (*Lama glama*) and alpaca (*Vicugna pacos*) [4]. These animals are important historical components of the Andean biocultural heritage, being used by Andean human groups since ancient times [4]. Specifically, llamas have been kept as multipurpose animals, being used for wool and meat production, to haul loads over the mountains, and as companion and therapy animals [4].

In this study, we present the draft genome sequences of two CTX-M-65-producing *E. coli* belonging to the international ST10 isolated from two South American llamas from Cuzco, Peruvian highlands. Additionally, a phylogenomic analysis of *E. coli* ST10 circulating between humans, animals, and the environment is addressed.

2. Materials and methods

In November 2021, a local surveillance study was conducted to monitor the presence of ESβL-producing bacteria in Llamas in Urubamba city, Cuzco, Peruvian highlands. Fresh faecal samples of Llamas were collected. Samples were immediately transported to a microbiology laboratory. Samples were streaked onto MacConkey agar plates supplemented with ceftriaxone (2 µg/mL) and incubated for 24 h at 37 °C. Two ceftriaxone-resistant *E. coli* strains (L1LB and L2BHI) were recovered, being identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). *Escherichia coli* L1LB and L2BHI strains displayed identical multidrug-resistant (MDR) profiles to aztreonam, ceftriaxone, cefotaxime (MIC> 32 µg/mL), cefepime (MIC> 32 µg/mL), ciprofloxacin, and nalidixic acid, remaining susceptible to carbapenems, as determined by the disk diffusion and/or E-test methods, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (2022).

The genomes of L1LB and L2BHI *E. coli* strains were sequenced on an Illumina NextSeq 550 platform with 150-bp paired-end reads (Illumina, San Diego, CA). The sequenced reads were both trimmediand de novo assembled using CLC genomics workbench V 12.0.3 (Qiagen, Hilden, Germany). Sequences were annotated using NCBI Prokaryotic Genome Annotation Pipeline version v4.10 (http://www.ncbi.nlm.nih.gov/genome/annotation_prok/).

Genomic analyses of sequenced strains were performed using ResFinder v.4.1, VirulenceFinder v2.0, PlasmidFinder v.2.0, MLST v2.0, SerotypeFinder v2.0, and FimTyper v1.0 tools from CGE (http://genomicepidemiology.org/). We also used ABRicate v0.9.8 (https://github.com/tseemann/abricate) to predict virulence genes profiling through the VFDB database (https://github.com/haruosuz/vfdb). On the other hand, an in-house built database, constructed through the BacMet2 (http://bacmet.biomedicine.gu.se) and GenBank (https://www.ncbi.nlm.nih.gov/genbank/) databases, was used for identifying heavy metal, disinfectants, and pesticide resistance genes. The *E. coli* phylogroup was performed using ClermonTyping v1.4.0 (http://clermontyping.iame-research.center/). Identity and coverage threshold were set to 95% and 80%, respectively. iTOL v6 (https://itol.embl.de) was used for midpoint rooting.

To assess phylogenomic relatedness, we performed a search of 5395 *E. coli* genome ST10 genomes on Enterobase, with data for country, source, and collection date. FastANI v1.32 (https://github.com/ParBLiSS/FastANI) was used to select the 148 assemblies with highest average nucleotide identity (ANI) to L1LB and L2BHI, for further phylogenetic comparison. Next, an approximately maximum-likelihood SNP-based phylogenetic tree was built using CSI Phylogeny v1.4 (https://cge.cbs.dtu.dk/services/CSIPhylogeny) with default settings. The chromosome sequence of *E. coli* ST10 LAU-OXA strain (RefSeq accession number: NZ_CP045277.1) was used as reference.

3. Results and discussion

The genome size of *E. coli* L1LB was calculated at 4,000,11 bp, with 164 × of coverage, comprising 4839 total genes, 75 tRNAs, 3 rRNAs, 11 ncRNAs, and 11 pseudogenes (accession number: JAMASU000000000). On the other hand, genome size of L2BHI was calculated at 4,002,54 bp, with 157 × of coverage, comprising 4901 total genes, 71 tRNAs, 3 rRNAs, 11 ncRNAs, and 199 pseudogenes (accession number: JAMASV000000000).

Both *E. coli* L1LB and L2BHI strains were classified as serotype O8:H4-*fim*H41 and phylogroup A. Multilocus sequence typing analysis revealed that both *E. coli* strains belonged to ST10, which has been recognized as a pandemic lineage, being broadly distributed at the human-animal-environment interface [1].

L1LB and L2BHI resistome analysis identified determinants encoding resistance to aminoglycosides, β-lactams, fosfomycin, sulphonamides, tetracyclines, and trimethoprim. Genes conferring resistance to heavy metals (arsenic and tellurite), biocides (bile salt, triclosan, chlorhexidine, benzalkonium chloride, quaternary ammonium compounds, and hydrogen peroxide), and pesticides (glyphosate) were also identified. Both strains also carried broad virulomes, which included important virulence factors, such as the *astA* gene. Moreover, plasmid incompatibility (Inc) types IncFIA, IncFIB, IncFII, and IncI were detected (Table 1). The $bla_{\text{CTX-M-65}}$ gene of both L1LB and L2BHI strains was flanked upstream by the *tonB* gene and an IS*903B* family transposase; while downstream, an IS*1380* family transposase was located.

The 148 *E. coli* strains of ST10 selected for phylogenomic analysis shared an ANI value of ≥99.6% with L1LB and L2BHI strains. The SNP-based phylogenetic tree revealed 0–3710 SNP differences between all *E. coli* analysed, with L1LB and L2BHI strains being clustered (SNP difference ranging from 1–83) with 3 *E. coli* strains isolated from companion animals (United States of America) and 2 human strains from Brazil and the USA, respectively, 1 environmental strain from Japan, and 4 strains from Ethiopia without isolation sources reported (Fig. 1; Supplementary Table S1).

In summary, we report the draft genome sequences of two MDR CTX-M-65-producing *E. coli* ST10 isolated from South American Llamas inhabiting the Andean Highlands of Peru. The widespread and broad host range and environmental adaptability of this *E. coli* clone make continuous genomic epidemiological surveillance imperative. Our data could add valuable genomic information about the dissemination of ESβL-producing *E. coli* in Peru,and may also be useful for comparative analysis of this One Health clone. In this regard, next-generation sequencing technologies and *in silico* analysis of bacterial genomes using online bacterial sequence typing and source tracking databases have allowed for the revelation of origins and successful expansion of clinically relevant high-risk clones and their resistance mechanisms. Moreover, detailed analyses of bacterial genomes could also provide valuable information that contributes to prevention and control strategies to tackle the global antimicrobial resistance crisis [5]. Finally, since South American camelids possesses historical and cultural importance, especially for Andean populations, monitoring the occurrence of ESβL-producing bacteria in these animals should be encouraged.

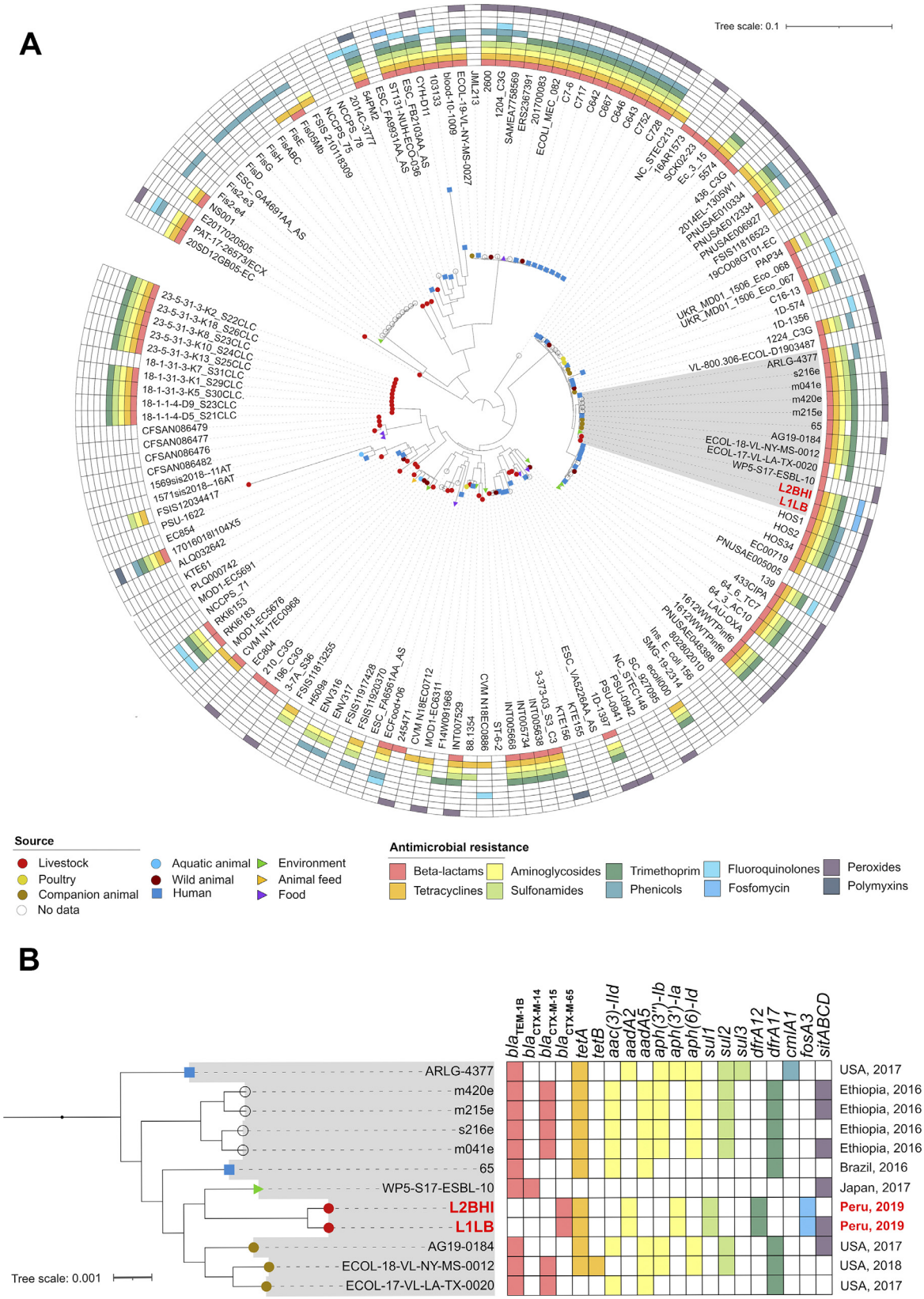

Fig. 1. (A) Phylogenomic analysis showing that L1LB and L2BHI are genetically close to each other, differing in only one of the analysed SNPs. In the tree, they formed a clade with other 10 isolates from companion animals ($n = 3$), humans ($n = 2$), and environmental sources ($n = 1$), plus 4 isolates with no data for source, isolated between 2016 and 2018 from the USA ($n = 4$), Ethiopia ($n = 4$), Brazil ($n = 1$), and Japan ($n = 1$). Among the 12 isolates in this clade, SNP counts ranged between 1 and 83 SNPs. (B) Subtree with the clade highlighted in grey, showing the resistome identified on ABRicate/Resfinder 4.1, source, country, and year of isolation.

Table 1
Genomic analysis of CTX-M-65-producing *Escherichia coli* L1LB and L2BHI strains isolated from South American Llamas inhabiting the Andean Highlands of Peru[a].

Characteristics	L1LB	L2BHI
Serotype	O8:H17	O8:H17
fimH-type	*fimH*41	*fimH*41
Phylogroup	A	A
MLST Sequence Type (ST)	ST10	ST10
Virulome		
EAEC heat-stable enterotoxin (EAST1)	*astA*	*astA*
Heat shock-induced protein	*gndA*	*gndA*
Colicin-M, colicin-V	-	*cma, cvaC*
Hemolysin	-	*hlyF*
Yersiniabactin siderophore	*fyuA, irp1, irp2, ybtAESQPUTX*	*fyuA, irp1, irp2, ybtAETUPSQX*
Salmochelin siderophore	-	*iroBCDEN*
Aerobactin siderophore	-	*iutA, iucBCD*
Enterobactin siderophore	*fes, fepABCD, entABCDEFS*	*fes, fepABCD, entABCDEFS*
Type II secretion system (T2SS)	*gspCDEFHIJKLM*	*gspCDEFHIJKLM*
Type III secretion system (T3SS)	*espL1, espL4, espX1, espX5*	*espL1, espL4, espX1, espX5*
Type VI secretion system (T6SS)	*tssA, tssD1, tssM*	*tssD1, tssAMH*
EAEC heat-resistant agglutinin	*hra*	*hra*
Hsp100/Clp protein	*clpV*	-
E. coli common pilus	*ecpAR, ecpBCE*	*ecpABCDER*
Fimbrial protein	*fimBCDEFGHI*	*fimBEICDFGH*
Curli production	*cgsBCDEFG*	*cgsCBDFFG*
Glutamate decarboxylase (tolerance to acid pH)	*gad*	*gad*
Increased serum survival	*iss*	*iss*
Invasion of brain microvascular endothelial cells	*ibeBC*	*ibeBC*
Omptin (cleaves proteamine P1)	-	*ompT*
Resistome		
Aminoglycosides	*aadA2, aph(3')-Ia*	*aadA2, aph(3')-Ia*
β-lactams	$bla_{CTX\text{-}M\text{-}65}$	$bla_{CTX\text{-}M\text{-}65}$
Fosfomycin	*fosA3*	*fosA3*
Sulphonamides	*sul1*	*sul1*
Tetracyclines	*tetA*	*tetA*
Trimethoprim	*dfrA12*	*dfrA12*
Quinolones (mutations)	*gyrA* (S83L, D87N), *parC* (S80I)	*gyrA* (S83L, D87N), *parC* (S80I)
Biocides (bile salt, triclosan, chlorhexidine, benzalkonium chloride, quaternary ammonium compounds, hydrogen peroxide)	*acrAB–tolC, acrD, acrE, acrF, cpxA, qacE*Δ*1, emrK, emrD, yjiO, mdtEF, mdtK, mdtN, sitABCD*	*acrAB–tolC, acrD, acrE, acrF, cpxA, qacE*Δ*1, emrK, emrD, yjiO, mdtEF, mdtK, mdtN, sitABCD*
Heavy metals (tellurite, arsenic)	*tehAB, arsBCR, arsH*	*tehAB, arsBCR*
Pesticides (glyphosate)	*phnPONMLKJIHGFEDC*	*phnOPNMLKJIHGFEDC*
Plasmid (Inc-type)	IncI, IncFIA, IncFII	IncI, IncFIA, IncFIB, IncFII
GenBank accession number	JAMASU000000000	JAMASV000000000

[a] Resistomes, virulomes, and functional information of proteins were analysed and obtained using ResFinder (https://cge.food.dtu.dk/services/ResFinder/), Comprehensive Antibiotic Resistance Database (https://card.mcmaster.ca/analyse), BacMet: antibacterial biocide and metal resistance genes database (http://bacmet.biomedicine.gu.se/index.html), BioCyc (https://biocyc.org/gene-search.shtml), and UniProt (https://www.uniprot.org/) tools.

The Whole Genome Shotgun projects of L1LB and L2BHI have been deposited at DDBJ/ENA/GenBank under the accessions numbers: JAMASU000000000 and JAMASV000000000, respectively.

Funding: This study was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (2020/08224-9, 2019/15778-4), Conselho Nacional de Desenvolvimento Científico e Tecnológico (422984/2021-3 and 314336/2021-4), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (88882.333054/2019-01). NL is a research fellow of CNPq (314336/2021-4). BC was researcher fellows of CAPES (88882.333054/2019-01). FE was a research fellow of FAPESP (2019/15578-4). BF and FPS were researcher fellows of PNPD/CAPES (88887.358057/2019-00 and 88887.463868/2019-00).

Competing interests: None declared

Ethical approval: Not required

Acknowledgments: FAPESP, CAPES, and CNPq research grants are gratefully acknowledged. NL is a research fellow of CNPq (314336/2021–4). We thank Cefar Diagnóstica Ltda. (São Paulo, Brazil) and CEFAP-GENIAL facility for kindly supplying antibiotic discs for susceptibility testing and Illumina sequencing, respectively.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2023.11.011.

References

[1] Fuga B, Sellera FP, Cerdeira L, Esposito F, Cardoso B, Fontana H, et al. WHO critical priority *Escherichia coli* as One Health challenge for a post-pandemic scenario: genomic surveillance and analysis of current trends in Brazil. Microbiol Spectr 2022;10:e0125621. doi:10.1128/spectrum.01256-21.
[2] Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 2018;18:318–27. doi:10.1016/S1473-3099(17)30753-3.
[3] Zong Z, Fenn S, Connor C, Feng Y, McNally A. Complete genomic characterization of two *Escherichia coli* lineages responsible for a cluster of carbapenem-resistant infections in a Chinese hospital. J Antimicrob Chemother 2018;73:2340–6. doi:10.1093/jac/dky210.
[4] Vilá B, Arzamendia Y. South American Camelids: their values and contributions to people. Sustain Sci 2022;17:707–24. doi:10.1007/s11625-020-00874-y.
[5] Ellington MJ, Ekelund O, Aarestrup FM, Canton R, Doumith M, Giske C, et al. The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee. Clin Microbiol Infect 2017;23:2–22. doi:10.1016/j.cmi.2016.11.012.