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Short Communication

A new OCH β-lactamase from a *Brucella pseudintermedia* (*Ochrobactrum pseudintermedium*) strain isolated from *Zophobas morio* larvae

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ABSTRACT

Objectives: OCH class C β-lactamases have been reported in several species belonging to the *Brucella* genus that were formerly known as *Ochrobactrum*. Moreover, only one complete genome of *Brucella pseudintermedia* has been published. In this work, we describe the genome of a *B. pseudintermedia* strain possessing a new *bla*$_{OCH}$ gene that was isolated from *Zophobas morio* larvae.
Methods: Hybrid whole-genome sequencing analysis (Illumina and Nanopore) was used to identify and characterise the strain (Ops-OCH-23). Phylogenetic analyses based on the 16S rRNA gene sequence and a core-genome alignment were performed to study the relationships among Ops-OCH-23 and deposited genomes. Moreover, all deposited *bla*$_{OCH}$ genes were compared to the one found in Ops-OCH-23.
Results: Ops-OCH-23 showed a susceptibility profile consistent with the production of AmpC β-lactamase(s). Its genome consisted of two chromosomes, of which one carried the *bla*$_{OCH}$ gene. Such gene encoded a new class C OCH β-lactamase among the fifteen so far reported. Two plasmids (120-Kb and 59-Kb) without any associated antimicrobial resistance genes were also found. Analysis of 16S rRNA revealed that Ops-OCH-23 shared 100% homology with four deposited *B. pseudintermedia* strains. Moreover, the core-genome analysis indicated that the closest match (279 ΔSNVs) to Ops-OCH-23 was strain CTOTU49018 isolated from an urban environment in Germany in 2013.
Conclusion: We described the second complete genome of a *B. pseudintermedia* that also encoded a new OCH β-lactamase variant. Overall, this report expands our knowledge regarding this rarely isolated *Brucella* species that have been reported so far only a few times in human sources.

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1. Introduction

OCH enzymes are a group of chromosomal AmpC β-lactamases that have been so far described in *Brucella anthropi*, *Brucella intermedia* and *Brucella tritici* [1–3]. These bacteria - formerly grouped in the *Ochrobactrum* genus [4] consist of Gram-negative, non-fermenting, aerobic bacilli isolated from various sources belonging to humans, animals, environment and plants [5,6]. In particular, they have been identified as opportunistic and nosocomial pathogens [3,7,8].

As a result of the production of OCH enzymes, the above-mentioned species exhibit *in vitro* non-susceptibility to most β-lactams (including extended-spectrum cephalosporins, ESCs) and standard β-lactam/β-lactamase inhibitor combinations, but not carbapenems [1,3]. Moreover, these three species are also reported as resistant to fluoroquinolones [3]. Lastly, while *B. anthropi* and *B. tritici* are generally described as sensitive to polymyxins, *B. intermedia* is naturally resistant to them [8–12].

The OCH β-lactamases have been frequently reported in *B. anthropi* strains, but data regarding its presence, function, and genetic characterisation in the other *Brucella* species are lacking [3]. Currently, fifteen *bla*$_{OCH}$ gene sequences are deposited in the NCBI database (i.e., *B. anthropi*, $n = 9$; *B. intermedia*, $n = 2$; *B. tritici*, $n = 3$; *Brucella* spp., $n = 1$). Remarkably, only one complete genome sequence of *B. pseudintermedia* (formerly

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Ochrobactrum pseudintermedium) has been described (GenBank: GCA_025118245.1); the strain (ASAG-D25) was isolated in China in 2019 from wheat ear [13].

In this work, we describe the complete genome of an ESC-resistant (ESC-R) *B. pseudintermedia* strain (Ops-OCH-23) producing a new OCH enzyme variant and isolated from *Zophobas morio* larvae.

2. Materials and methods

2.1. Isolation, species identification and antimicrobial susceptibility tests

Strain Ops-OCH-23 was isolated as previously described from the homogenised tissues of four *Z. morio* larvae purchased from a pet store in Bern (Switzerland) in 2023 [14]. These larvae were part of an ongoing project for the establishment of a new *in vivo* model of gut colonisation with ESC-R Enterobacterales (https://data.snf.ch/grants/grant/206400).

Bacterial identification (ID) was achieved using the matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS; Brucker, FlexControl v3.4 [build 135.14]). Antimicrobial susceptibility tests (ASTs) were performed using the MIC broth microdilution Sensititre^{TM} GNX2F panels (Thermo Fisher Scientific). Since no criteria are available for *B. pseudintermedia*, AST results were interpreted in accordance with the 2023 European Committee on Antimicrobial Susceptibility Testing (EUCAST) v13.1 cutoffs set for Enterobacterales (www.eucast.org/clinical_breakpoints).

2.2. Whole-genome sequencing

Genomic DNA (gDNA) was extracted using the PureLink^{TM} microbiome DNA Purification Kit (Thermo Fisher Scientific) starting from a bacterial glycerol stock that had been sub-cultivated overnight on a MacConkey II agar plate (Becton-Dickinson). Quality and quantity of gDNA were assessed using NanoDrop^{TM} and Qubit^{TM} 3 (Thermo Fisher Scientific). As previously described [15–18], the short-read whole-genome sequencing (WGS) was carried out on a NovaSeq 6000 instrument (2 $\times$ 150-bp paired-end reads; Illumina), while the long-read WGS was performed on a MinION sequencer (Oxford Nanopore Technologies) with a 48-hour run time using the SQK-RBK004 rapid barcoding kit and a FLO-MIN106D R9 flow cell. Trimming of adaptor sequences from short read data was accomplished using Trimmomatic v0.39 (http://www.usadellab.org/cms/?page=trimmomatic), while long-reads were preprocessed and quality filtered with Porechop v0.2.3 (https://github.com/rrwick/Porechop) and Filtlong v0.2.1 (https://github.com/rrwick/Filtlong; parameters: minimum read length of 1-kb and one billion target bases). A complete genome assembly was generated using the hybrid pipeline provided by Unicycler v0.4.7 (https://github.com/rrwick/Unicycler) and its coverage was calculated using QualiMap v2.2.1 (http://qualimap.conesalab.org/).

2.3. Genome assembly and characterisation

Genome assembly was analyzed using the tools provided by the Center for Genomic Epidemiology database (https://genomicepidemiology.org/) to define antimicrobial resistance genes (ARGs), plasmid replicon sequences, and *Brucella* spp. sequence type (ResFinder v4.1 [parameters: 30% threshold ID and 20% minimum length], PlasmidFinder v2.1 [parameters: Enterobacterales, 50% minimum identity and 20% minimum coverage], and MLST v2.0 [parameter: *Brucella* spp.], respectively). ARGs were also defined using the comprehensive antibiotic resistance database (CARD) resistance gene identifier (RGI) for protein homology models (https://card.mcmaster.ca/analyze/rgi).

Table 1
Susceptibility profile for *B. pseudintermedia* strain Ops-OCH-23.

Antibiotics	MIC value (μg/mL)[a]
Piperacillin-tazobactam	≥64/4 (R)
Ticarcillin-clavulanate	≤16/2 (S)
Ceftazidime	≥16 (R)
Cefotaxime	16 (R)
Cefepime	8 (R)
Aztreonam	≥16 (R)
Imipenem	≤1 (S)
Meropenem	≤1 (S)
Doripenem	0.5 (S)
Ertapenem	≤0.25 (S)
Gentamicin	≤1 (S)
Tobramycin	≤1 (S)
Amikacin	8 (S)
Ciprofloxacin	≤0.25 (S)
Levofloxacin	≤1 (S)
Colistin	≥4 (R)
Polymyxin B	≥4 (R)
Doxycycline	8 (NA)
Minocycline	≤2 (NA)
Tigecycline	1 (R)
Trimethoprim-sulfamethoxazole	≤0.5/9.5 (S)

R, resistant; S, susceptible; NA, not available; MIC, minimum inhibitory concentration.
[a] MICs were interpreted according to the 2023 EUCAST criteria for Enterobacterales (i.e., *E. coli*).

Species ID was confirmed with WGS data using the: (1) Type Strain Genome Server (TYGS; https://tygs.dsmz.de/); (2) ribosomal multilocus sequence typing (rMLST) available on the public PubMLST (https://pubmlst.org/); and (3) average nucleotide identity (ANI) based on BLAST (ANIb) from JSpeciesWS (https://jspecies.ribohost.com/jspeciesws/) and OrthoANIu (https://www.ezbiocloud.net/tools/ani) [19]. The genome assembly was automatically annotated using the NCBI Prokaryotic Genome Annotation Pipeline (annotation method: GeneMarkS-2 + v6.6).

2.4. Database search, 16S rRNA and core-genome phylogeny analysis

The 16S rRNA genes of the genomes of *B. pseudintermedia* (n = 3), *B. intermedia* (n = 5), *B. anthropi* (n = 4), and *B. tritici* (n = 1) available in GenBank (downloaded on 10 October 2023) were analyzed using Barrnap v0.9 (https://github.com/tseemann/barrnap) to predict the location of 16S rRNA genes for each strain. Then, 16S rRNA sequences were aligned using Geneious Prime v2022.0.2 (Biomatters). After that, IQ-TREE v2.2.2.7 (http://www.iqtree.org/) was employed for carrying out phylogenetic analyses [ModelFinder parameter: "F81+F + I", 1000 ultrafast bootstraps (UFBoot; parameter: "-bb"), and the SH-aLRT (SH-like approximate likelihood ratio) test (parameter:"-alrt")].

All *B. pseudintermedia* genomes available in NCBI (n = 4; downloaded on 6 October 2023), together with Ops-OCH-23, also underwent a core-genome alignment to the reference genome *B. pseudintermedia* ASAG-D25 using Snippy v3.1 (https://github.com/tseemann/snippy). Then, as previously described [16–18], IQ-TREE was used to conduct phylogenetic analyses. The resulting trees were visualised and annotated using iTOL v6.8.1 (https://itol.embl.de/).

2.5. Multiple sequence alignment of OCH β-lactamases

The bla_{OCH} nucleotide sequences were obtained from the genomes of *B. pseudintermedia* (n = 4) and *B. intermedia* (n = 4) available in GenBank (downloaded on 6 and 10 October 2023). Subsequently, Geneious Prime was used to translate the nucleotide

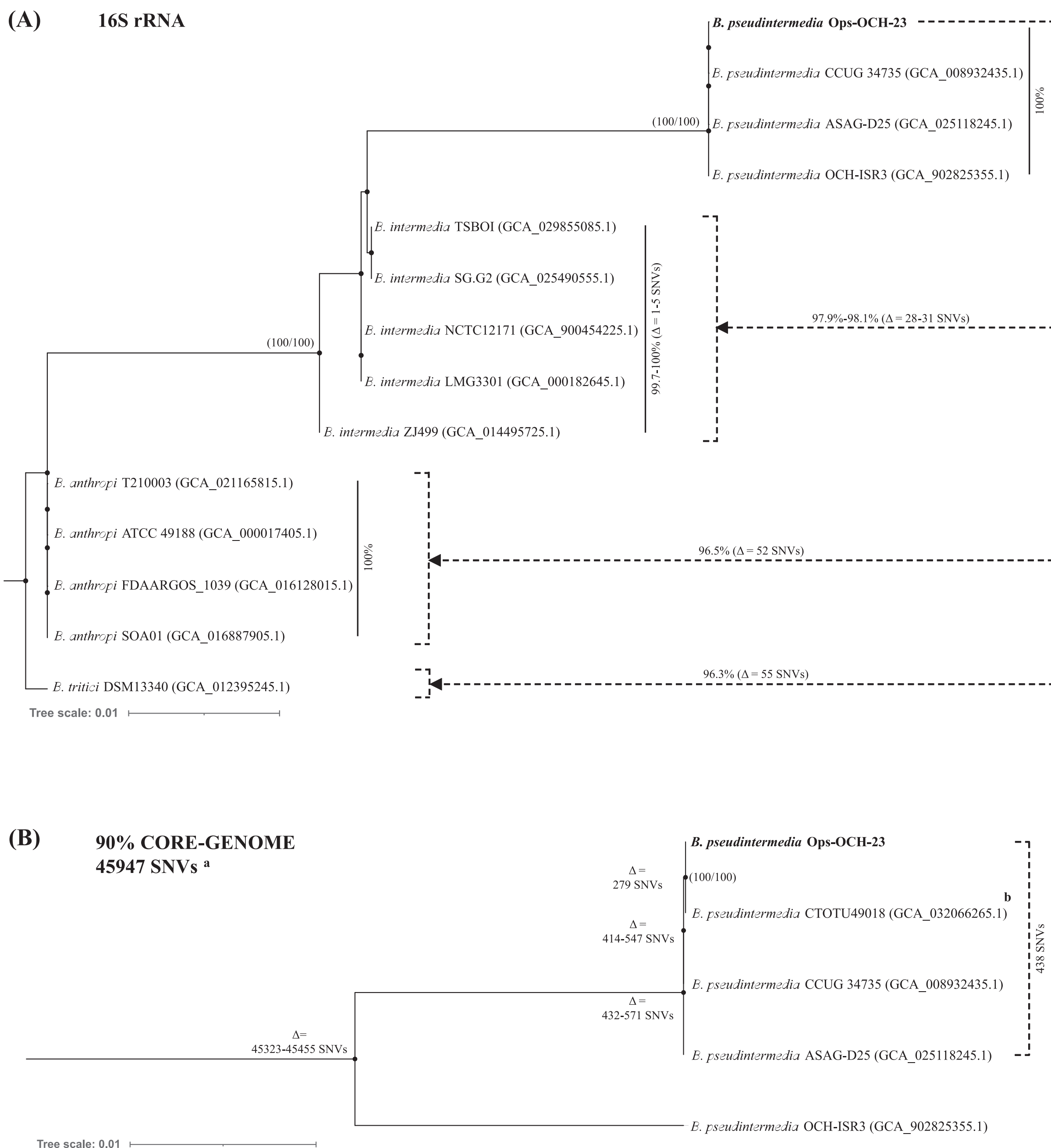

Fig. 1. (A) Phylogenetic tree based on the 16S rRNA gene sequence (1482-bp; $n = 14$). This tree illustrates the relationships between *B. pseudintermedia* and the three other species (*B. intermedia, B. anthropi* and *B. tritici*). (B) Core-genome phylogeny of *B. pseudintermedia* ($n = 5$). The reference sequence for this analysis was *B. pseudintermedia* ASAG-D25 and the sequence of *B. pseudintermedia* OCH-ISR3 was used as an outgroup. In both trees, bootstrap values are shown on nodes in parenthesis (SH-aLRT and UFBoot, respectively). Corresponding GenBank accession numbers are given in parentheses. The tree scale represents the average number of nucleotide substitutions per site. A delta (Δ) symbol indicates the number of single nucleotide variant (SNV) differences between each sequence/genome pair. Dash lines were used to compare specific strains.

[a] The core genome represents the maximum total coverage (90%) of the alignment among all five *B. pseudintermedia* genomes, which corresponded to 45,947 SNVs. [b] *B. pseudintermedia* CTOTU49018 was not considered for the 16S rRNA analysis due to the absence of a predicted sequence.

sequences into protein sequences. Further, OCH amino acid sequences from *B. intermedia* ($n = 2$), *B. tritici* ($n = 3$), *Brucella* spp. ($n = 1$) and *B. anthropi* ($n = 9$) were also downloaded from the NCBI website ($n = 15$; on 12 October 2023). Notably, for these 15 sequences, the corresponding genome data was not available. Finally, considering all the above OCH amino acid sequences ($n = 24$), a multiple sequence alignment was generated on Geneious Prime. Unless otherwise indicated, all software analyses described above were performed with default parameters.

2.6. Nucleotide sequence accession number

The complete genome sequence of Ops-OCH-23 is deposited in GenBank under the accession numbers CP137604.1 to CP137607.1 and under BioProject accession PRJNA1032464. The bla_{OCH} gene

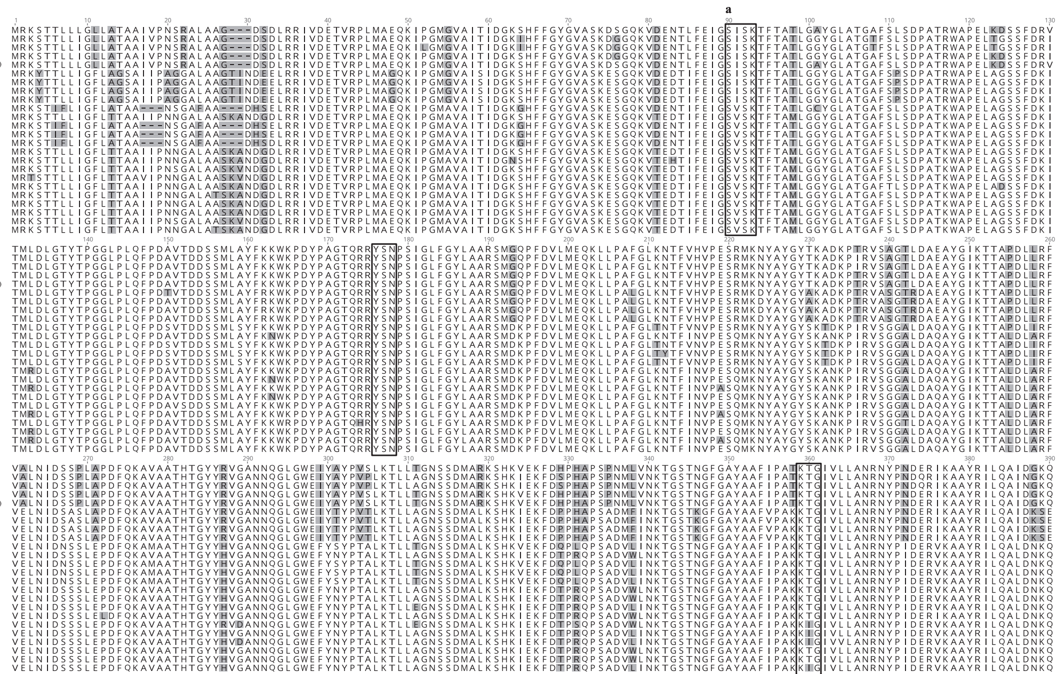

Fig. 2. Amino acid sequence alignment of OCH β-lactamase enzymes from different *Brucella* spp. (i.e., *B. pseudintermedia*, *B. intermedia*, *B. tritici*, and *B. anthropi*). Differences between each sequence are shown in gray. The specific serine active-site residue SXSK and YSN and KXG boxes specific for class C β-lactamases are indicated with a rectangle. Corresponding GenBank accession numbers are given in parentheses.
[a]Serine in position 90 is actually in position 64 according to the proposed structural alignment-based numbering of class C β-lactamases [22].

coding sequence of Ops-OCH-23 is deposited under accession number OR757115.1.

3. Results and discussion

Using the MALDI-TOF MS, Ops-OCH-23 was initially identified as *B. intermedia* (score 2.05). ASTs revealed that the strain was resistant to ESCs, piperacillin-tazobactam, aztreonam and colistin (Table 1).

Based on the TYGS platform, Ops-OCH-23 belonged to the species *B. intermedia*, whereas according to the rMLST it was *B. pseudintermedia* (94% homology). Moreover, ANIb showed a 99.31% match with the reference *B. pseudintermedia* ASAG-D25 strain [13]. These findings were corroborated using OrthoANIu [19], which revealed that Ops-OCH-23 exhibited a similarity relationship higher than the threshold for species delineation (95%) with the other *B. pseudintermedia* strains [20]: 99.57% with ASAG-D25, 99.84% with CTOTU49018 from urban environment (Germany, 2013; GenBank: GCA_032066265.1), 99.48% with CCUG 34735 from water (Sweden, 2019; GenBank: GCA_008932435.1), and 98.05% with OCH-ISR3 from a horse (Israel, 2020; GenBank: GCA_902,825,355.1).

Results of the 16S rRNA phylogenetic analysis are shown in Fig. 1A. Ops-OCH-23 shared 100% homology with the other three *B. pseudintermedia* strains (ASAG-D25, CCUG 34735, OCH-ISR3). In addition, Ops-OCH-23 was closely related to all *B. intermedia* strains with a homology of 97.9–98.1%; it also shared 96.5% and 96.3% homology with *B. anthropi* and *B. tritici*, respectively (Fig. 1A). Notably, the *B. pseudintermedia* CTOTU49018 strain was excluded because no 16S rRNA sequence was predicted.

To explore the relationship of Ops-OCH-23 within all available *B. pseudintermedia* genomes, a core-genome alignment (considering 90% of the total) was performed (Fig. 1B). As a result, Ops-OCH-23 was closest to *B. pseudintermedia* strain CTOTU49018 (279 ΔSNVs); moreover, Ops-OCH-23 differed from the reference strain *B. pseudintermedia* ASAG-D25 by 438 SNVs [13].

The hybrid assembly of Ops-OCH-23 generated a complete *de novo* genome with an average depth of 705x and a GC content of 57.6%. As already described for *B. pseudintermedia* ASAG-D25 [13], the genome of Ops-OCH-23 consisted of two circular chromosomes (chromosome 1 of 2,687,786-bp and chromosome 2 of 1,537,144-bp). Furthermore, it possessed two other circular *repABC* replicon-type plasmids (119,733-bp and 59,075-bp) not associated with ARGs.

Within chromosome 1, a bla_{OCH} gene encoding a class C β-lactamase was identified between base pair positions 769,028 and 770,191. The encoded OCH protein contained the specific serine (position 90) active site residue SXSK, as well as the sequences YSN (positions 176–178) and KXG (positions 359–361) specific for class C enzymes (Fig. 2) [21]. However, considering the proposed structural alignment-based numbering of class C β-lactamases, the above amino acid positions should be 64, 150–152, and 333–335, respectively [22]. Notably, no other *bla* genes were identified in the genome using CARD-RGI (default parameters) and ResFinder (nucleotide identity threshold: 30%).

According to the multiple sequence alignment, the OCH enzyme found in Ops-OCH-23 shared with the other reported OCH proteins a homology of: 95.9–100% with the OCHs from *B. pseudintermedia* (n = 4); 84.1–86.9% with those from *B. intermedia* (n = 6); 84.5–85.0% with those from *B. tritici* (n = 3), and 83.6–84.4% for the OCHs from *B. anthropi* (n = 9) (data not shown). We also noted that at position 91, all *B. pseudintermedia* and *B. intermedia* had an isoleucine, while all *B. tritici* and *B. anthropi* had a valine residue (Fig. 2). Furthermore, as for all AmpCs, upstream the bla_{OCH} gene, in an opposite orientation, the transcriptional regulator AmpR responsible for regulating the *ampC* gene was present [1].

4. Conclusion

Our work has led to the ID and characterisation of a new strain of *B. pseudintermedia* which was unexpectedly isolated from retailed *Z. morio* larvae. These insects are used massively in the pet

food industry or for recreational fishing [23]. Therefore, the finding of a rare ESC-R bacterial species in such larvae indicates that this source should be monitored as it may serve as a carrier of clinically relevant bacteria that could potentially lead to human disease.

Overall, our study provided a better understanding of *Brucella* spp. and the bla_{OCH} genes carried by some species of this clinically important bacterial genus. Further studies are recommended to investigate the hydrolytic kinetic activity of OCH enzymes against different classes of β-lactam antibiotics.

Author contributions: Conception and design (AE); acquisition of data (CK, CA, YE, EICM); analysis and interpretation of data (CK, EICM, AE); drafting the work (CK, AE); critical revision for important intellectual content (all authors); final approval of the version to be published (all authors).

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Competing interests: None.

Ethical approval: Not required.

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