Evaluation of a rapid turbidimetry-based method for the detection of cefotaxime-resistant *Escherichia coli* and *Klebsiella* spp. from blood cultures

Evaluación de un método turbidimétrico rápido para la detección de Escherichia coli *y* Klebsiella *spp. resistentes a cefotaxima a partir de hemocultivos*

Early administration of appropriate empirical treatment for bloodstream infections (BSI) improves clinical outcomes.[1–3] Nevertheless, empiric broad-spectrum antibiotic therapy is often inadequate because the frequent involvement of multiresistant bacteria, among which third-generation cephalosporin-resistant Enterobacterales are of special concern due to their association with increased mortality.[4] Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS)-based assays provide reliable and timely information on bacterial susceptibility/resistance to antimicrobials.[5] Unfortunately, MALDI-TOF MS instruments are not available in all microbiology laboratories. Herein, we developed a simple turbidimetry-based method for detecting resistance to ceftriaxone (CRO) in *Escherichia coli* and *Klebisella* spp. from positive blood cultures (BC), which circumvents the use of MALDI-TOF MS instruments. A total of 112 consecutive patients (mean age 74 years; range 40–99; 62.5% male) admitted between November 2019 and June 2020 with BSI due to *E. coli* ($n = 77$) or *Klebsiella* spp. ($n = 35$; *K. pneumoniae*, $n = 32$; *K. oxytoca*, $n = 2$; *K. variicola*, $n = 1$) were included. BC bottles were incubated in the Bactec FX automated system (Becton Dickinson, New Jersey, USA). Direct identification of bacteria from BC was carried out using a MALDI-TOF MS protocol[6] (Supplementary material). Antimicrobial susceptibility testing (AST) was performed using the broth microdilution MicroScan NM44 panel (Beckman Coulter) and interpreted according to EUCAST guidelines.[7] A total of 20 (26%) *E. coli* and 10 (28.5%) *Klebsiella* spp. (in all cases *K. pneumoniae*) isolates were resistant to CRO. Antimicrobial resistance gene characterization of all CRO resistant isolates was carried out using the Antimicrobial Resistance (AMR) Direct Flow Chip (Máster Diagnóstica, Granada, Spain)[8] (Supplementary material). All CRO-resistant *E. coli* isolates and 9/10 CRO-resistant *K. pneumoniae* harboured a CTX-M type ESBL (extended spectrum beta-lactamase); in turn, 2/10 *K. pneumoniae* isolated harboured an OXA-48 type D carbapenemase. One CRO-resistant *K. pneumoniae* presumably harboured a plasmid-mediated AmpC according to the antimicrobial susceptibility profile (piperacillin–tazobactam, MIC $\leq$8 mg/L; cefepime, MIC $\leq$1 mg/L; CRO, MIC >32 mg/L; ertapenem, MIC $\leq$0.5 mg/L) and the lack of detection of any beta-lactamase resistance gene by the AMR chip assay.

The turbidimetry-based method for detection of CRO resistance in *E. coli* and *Klebsiella* spp. (Supplementary material) was conducted as follows. A volume of 50 μL of positive BC was incubated with 450 μL of brain heart infusion (BHI) broth (Oxoid Limited, Hampshire, UK) in the absence (control) and presence (test) of CRO at a final concentration of 2 mg/mL,[8] at 37 °C in an atmosphere with 5% of CO_2 (Heracell 240i CO_2 incubator, Thermo Fisher Scientific, Langenselbold, Germany) for 2 h. Next, control and test tubes were centrifuged for 3 min at 13,000 rpm and the resulting pellets were resuspended with 1 mL of sterile H_2O for subsequent turbidity measurement (Densicheck Plus Instrument, bioMerieux Inc., France). Median nephelometric turbidity units (NTU) value for the control tubes was 3.77 (range 0.86–4), compared with 0.27 (range 0–2.54) and 1.73 (0.27–4) [$P < 0.001$; Mann–Whitney *U*-test] for the test tubes corresponding to CRO-susceptible and CRO-resistant isolates, respectively. Differential NTU values resulting from the subtraction of NTU values in test tubes from that in control tubes for CRO-susceptible and CRO-resistant *E. coli* and *Klebsiella* spp. isolates are shown in Fig. 1. Receiver operating characteristic (ROC) curves determined that overall, a differential NTU value of 2.64 best discriminated (area under the curve of 0.93; $P < 0.001$) between CRO susceptible and resistant isolates, yielding a sensitivity of 96.7% (95% CI, 83.3–99.4; $P < 0.001$) a specificity of 88%, a positive predictive value of 74% and a negative predictive value of 99%; it correctly categorized 29 (96.7%) and 75 (91.5%) of CRO-resistant and CRO-susceptible isolates, respectively. One CTX-M type ESBL-producing *E. coli* was erroneously categorized as susceptible. As for CRO-susceptible isolates ($n = 80$), 75 (91.5%) were categorized as such by the turbidimetry-based method and 7 were misclassified as resistant. The Kappa correlation index between results obtained by the turbidimetry method and conventional microdilution AST was 0.829 (95% CI, 0.71–0.94). The feasibility of turbidimetry to reliable predict antimicrobial susceptibility directly from bacteria in grown BC was shown for the Alfred AST® system (Alifax, Padova, Italy).[9] In summary, the turbidimetry-based method described herein reliably predicts CRO resistance in *E. coli* and *Klebsiella* spp.; the method is cheap and accessible to all clinical microbiology laboratories. Nevertheless, due to the limited number of CRO-resistant isolates included, its validation requires further studies.

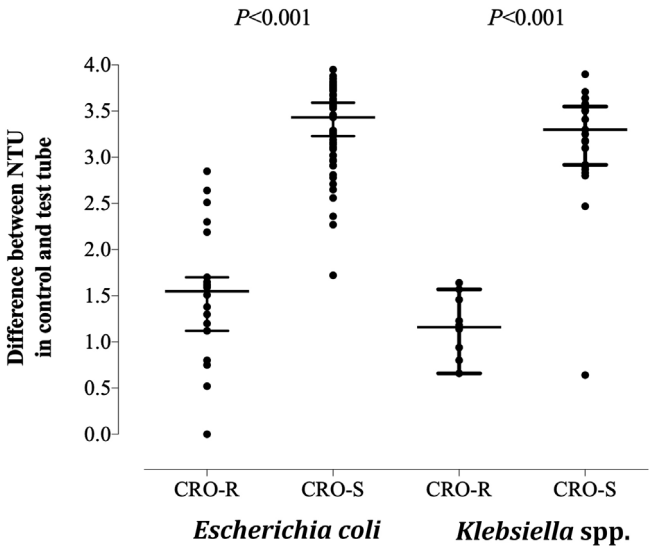

Fig. 1. Differential nephelometric turbidity units (NTU) values resulting from the subtraction of NTU values in test tubes (with ceftriaxone at 2 mg/mL) from that in control tubes (without ceftriaxone-CRO-) for CRO-susceptible and CRO-resistant *E. coli* and *Klebsiella* spp. recovered from blood cultures. *P* values for comparisons (Mann–Whitney *U* test) are shown. Statistical significance was set at *P* value <0.05.

Authors' contributions

DS and IT: methodology and data validation; DS, IT, JC and DN: study design and logistics; DN and IT: conceptualization, data analysis and manuscript writing.

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Ethical approval

The current study was approved by the Research Ethics Committee of Hospital Clínico Universitario INCLIVA (September, 2019).

Conflict of interest

The authors have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.eimce.2023.01.009.

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Methicillin-resistant *Staphylococcus aureus* bacteremia carrying the *mecC* gene

Bacteriemia por Staphylococcus aureus *resistente a meticilina portador del gen* mecC

In methicillin-resistant *Staphylococcus aureus (S. aureus)* (MRSA), resistance to β-lactams is due to alteration of penicillin-binding proteins (PBPs). This resistance mechanism arises from the acquisition of the *mecA* gene, encoding a PBP2a with low affinity for β-lactams.[1,2] In 2011, a new resistance gene was described, the *mecC* gene that encodes PBP2c. Its presence has spread[3] and for this reason its identification is essential to establish adequate antibiotic therapy.

We present the case of a patient with *mecC* MRSA bacteraemia.

This was a 70-year-old man with multiple pathologies, who attended the accident and emergency department due to poor pain control after a fall. He was diagnosed with dorsal fractures and presented with hyperbilirubinaemia with jaundice, and it was decided to admit him. His condition worsened and blood and urine cultures were taken. Empirical treatment with piperacillin-tazobactam was started and he was admitted to the Intensive Care Unit (ICU) with suspicion of septic shock.

In the Gram stain of the blood culture, Gram-positive cocci were observed in clusters that were identified by PCR (Xpert® MRSA/SA BC, Cepheid) as methicillin-susceptible *S. aureus* (MSSA), so cloxacillin was added. After performing culture and antibiogram following the EUCAST breakpoints,[4] resistance to β-lactams was observed. Immunochromatography was performed (CLEARVIEW™ PBP2a SA Culture Colony Test, Abbott) with a negative result for PBP2a. With suspicion of infection by *mecC* MRSA, a second commercial PCR was performed (FilmArray® Blood Culture Identification Panel, bioMérieux) with a positive result for the targets *mecA/C* and MREJ. Furthermore, by microdilution (MicroScan Pos Combo Panel Type 33, Beckman Coulter), MIC values for oxacillin and cefoxitin were 2 mg/l and >4 mg/l, respectively, and the rest of the families of antibiotics were interpreted as susceptible. The presence of the *mecC* gene was confirmed by ẗome PCRẅith specific molecular targets and sequencing. Antibiotic therapy was changed to daptomycin and levofloxacin but the patient died 14 days after admission.

Recent publications suggest that the appearance of *mecC* MRSA predates the use of antibiotics,[5] and that transmission to humans can occur through contact with animals.[3,6,7] In addition, colonisation, advanced age and underlying disease have also been associated with infection.[8] In our case, the patient confirmed contact with livestock and in the nasal sample for the study of resistant bacteria collected on his admission to the ICU, *mecC* MRSA was also isolated, so the patient was colonised and the infection developed.

In microbiological diagnosis, the discovery of the *mecC* gene with 70% nucleotide homology to the *mecA*gene, and PBP2c encoding with 62% amino acid sequence similarity to PBP2a means tests targeting the detection of the *mecA* gene and PBP2a misidentify a *mecC* MRSA isolate as MSSA.[2,9] Therefore, performing an antibiogram and using specific techniques is essential.[2] *mecC* MRSA accounts for less than 1% of all MRSA, and the majority

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