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 ≤8 mg/L; cefepime, MIC ≤1 mg/L; CRO, MIC >32 mg/L; ertapenem, MIC ≤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.

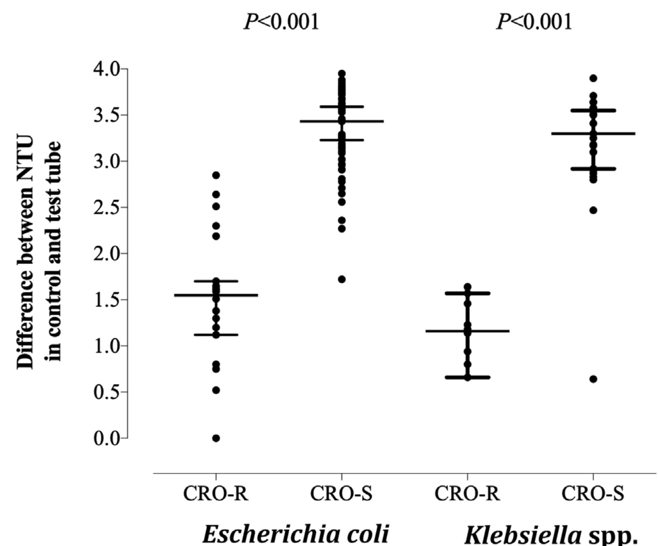

Fig. 1. Differential nephelometric turbidity units (NTU) values resulting from the subtraction of NTU values in test tubes (with ceftriaxone at 2 mg/mL) from that in control tubes (without ceftriaxone-CRO-) for CRO-susceptible and CRO-resistant *E. coli* and *Klebsiella* spp. recovered from blood cultures. *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.eimc.2023.01.006.

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

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

En *Staphylococcus aureus (S. aureus)* resistente a meticilina (SARM) la resistencia a betalactámicos se debe a la alteración de las proteínas de unión a la penicilina (PBP). Este mecanismo de resistencia es consecuencia de la adquisición del gen *mecA*, que codifica una PBP2a de baja afinidad a betalactámicos[1,2]. En 2011 se describió un nuevo gen de resistencia, el gen *mecC*, que codifica la PBP2c. Su presencia se ha extendido[3] y por ello su identificación es esencial para instaurar un tratamiento antibiótico adecuado.

Presentamos un caso de paciente con bacteriemia por SARM *mecC*.

Se trata de un varón de 70 años pluripatológico, que acude a urgencias por mal control del dolor tras una caída. Es diagnosticado de fracturas dorsales, presenta hiperbilirrubinemia con ictericia, y se decide su ingreso. Empeora y se extraen hemocultivos y urocultivo, se inicia tratamiento empírico con piperacilina-tazobactam y con sospecha de shock séptico es ingresado en la Unidad de Cuidados Intensivos (UCI).

En la tinción de Gram del hemocultivo, se observan cocos grampositivos en racimos que fueron identificados mediante PCR (Xpert® MRSA/SA BC, Cepheid) como *S. aureus* sensible a meticilina (SASM), por lo que se añadió cloxacilina. Tras la realización de cultivo y antibiograma siguiendo los puntos de corte de EUCAST[4], se observó resistencia a betalactámicos. Se realizó inmunocromatografía (CLEARVIEW™ PBP2a SA culture colony test, Abbott) con resultado negativo para la PBP2a. Con la sospecha de infección por SARM *mecC*, se realizó una segunda PCR comercial (Filmarray® Blood Culture Identification Panel, Biomerieux) con resultado positivo para las dianas *mecA/C* y MREJ. Además, mediante microdilución (MicroScan Pos Combo Panel Type 33, Beckman Coulter) los valores de CMI para oxacilina y cefoxitina fueron 2 mg/l y > 4 mg/l, respectivamente y el resto de las familias de antibióticos se interpretaron como sensibles. La presencia del gen *mecC* se confirmó mediante «PCR casera» con dianas moleculares específicas y secuenciación. Se modificó la antibioterapia a daptomicina y levofloxacino pero el paciente falleció tras 14 días de ingreso.

Recientes publicaciones sugieren que la aparición de SARM *mecC* es previa al uso de antibióticos[5], y que la trasmisión a humanos se puede producir por contacto con animales[3,6,7]. Además, la colonización, la edad avanzada y tener alguna enfermedad de base también se han asociado con infección[8]. En nuestro caso, el paciente afirmó su contacto con ganado y en la muestra nasal para estudio de bacterias resistentes recogida en su ingreso en UCI, también se aisló SARM *mecC* por lo que el paciente estaba colonizado y desarrolló la infección.

En el diagnóstico microbiológico, el descubrimiento del gen *mecC* con una homología de nucleótidos del 70% con el gen *mecA*, y la PBP2c que codifica con una similitud en la secuencia de aminoácidos del 62% con la PBP2a hace que las pruebas dirigidas a la detección del gen *mecA* y la PBP2a identifiquen erróneamente