Improving turnaround time for identifying extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: experience with the BD Phoenix automated system

Infections by resistant organisms such as extended-spectrum β-lactamase (ESBL)-producing bacteria represent an increased risk of therapeutic failure and are associated with longer duration of hospital stay and higher hospital charges. Over the last decade, *Escherichia coli* and *Klebsiella pneumoniae* have been associated with epidemic clones harbouring virulence factors and multiresistance (Rodríguez-Baño & Pascual, 2008). Thus, it is necessary to rapidly identify ESBL-producing bacteria to control their dissemination and to select appropriate antimicrobial therapy (Pitout & Laupland, 2008), which is usually non-β-lactams such as imipenem, fluoroquinolones or cotrimoxazole.

Automated systems are widely used in clinical laboratories for species identification and antimicrobial susceptibility testing in order to decrease the in-laboratory turnaround time and to improve cost-effectiveness. Gram-negative susceptibility panels have been designed for the BD Phoenix (Becton Dickinson) system to detect ESBLs in *E. coli* and *K. pneumoniae*. These panels incorporate specific internal ESBL testing based on growth in the presence of cefpodoxime and the third-generation cephalosporin (3GC) compounds ceftriaxone, ceftazidime and cefotaxime and inhibition when combined with clavulanic acid to detect the production of ESBLs. Overall, this system has a high sensitivity (91.4–99.0 %) but a low specificity (false positive) of 45.5–52.0 % (Wiegand et al., 2007; Färber et al., 2008), so a phenotypic confirmatory test is recommended and the results of the BD Phoenix ESBL test should be used only as an initial screen (Fisher et al., 2009). The reduced specificity is due mostly to high-level AmpC-producing isolates misclassified as ESBL producers (Wiegand et al., 2007). Additional phenotypic confirmatory testing of ESBLs performed on screening-positive isolates delays reporting and is labour-intensive. Therefore, we intended to develop an algorithm to avoid systematic ESBL confirmatory testing.

For this purpose, MICs of 3GC and of amoxicillin–clavulanate (AMC) were used as confirmatory criteria of ESBL production detected by the BD Phoenix system. From July 2008 to June 2009, *E. coli* and *K. pneumoniae* detected as ESBL producers by the BD Phoenix system were prospectively tested for ESBL production by the combined discs (CD) method (NeoSenstabs) using three substrates, cefotaxime 30 μg, ceftazidime 30 μg and cefepime 30 μg, with and without clavulanic acid 10 μg, performed according to Clinical and Laboratory Standards Institute recommendations (CLSI, 2008). An increase in the inhibition zone diameter ≥5 mm in the presence of clavulanic acid was considered confirmation of ESBL production (Drieux et al., 2008). Genotypic characterization of the ESBLs was performed for randomized isolates (*n* = 17, 9 *E. coli* and 8 *K. pneumoniae*) by PCR to detect *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> and *bla*<sub>CTX-M</sub> genes. PCR for CTX-M groups in CTX-M-producing strains and DNA sequencing were also done as described previously (Rodríguez-Villalobos et al., 2006). All results of antimicrobial susceptibility testing performed on the BD NMIC/ID-51 panel and of the corresponding phenotypic confirmatory test were recorded. Isolates detected as ESBL-positive by the BD Phoenix system and having AMC MIC ≤16/8 μg ml<sup>−1</sup> were classified as ESBL producers by the CD method. Forty-four (83 %) of the 53 *K. pneumoniae* isolates with AMC MIC ≤16/8 μg ml<sup>−1</sup> were confirmed as ESBL producers by the CD test. Molecular analysis of the eight randomized isolates exhibited evidence of ESBLs CTX-M-1 (*n* = 6) or CTX-M-9 (*n* = 2). All of these isolates had MICs >16 μg ml<sup>−1</sup> for ceftazidime and/or ceftriaxone. None of the nine remaining isolates with MICs for 3GC ≤16 μg ml<sup>−1</sup> were confirmed as ESBL producers. These data indicate that the results of the BD Phoenix ESBL test are highly reliable (100 % concordance with the CD test) for isolates having AMC MIC ≤16/8 μg ml<sup>−1</sup> combined with ceftazidime and/or ceftriaxone MICs ≥16 μg ml<sup>−1</sup> for *E. coli* and >16 μg ml<sup>−1</sup> for *K. pneumoniae*.

Our study highlighted that high level of hydrolysis of 3GC combined with a clavulanic acid inhibitory effect are sufficient evidence of ESBL production for isolates detected to be ESBL-positive by the BD Phoenix system, and an interpretative algorithm for these isolates was proposed (Fig. 1). In order to facilitate treatment decisions and to detect all clinically important resistance regardless of its mechanism, 3GC...
breakpoints were recently revised (CLSI, 2010; EUCAST version 1.1 April 2010). Nevertheless, in many areas, ESBL detection is recommended or mandatory for infection control purposes.

In conclusion, for *E. coli* and *K. pneumoniae* reported as ESBL producers by the BD Phoenix system, an interpretative method based on AMC and 3GC MICs could be helpful for faster reporting of ESBL production and for cost saving by skipping additional confirmatory testing. For isolates having AMC MIC $>16/8 \mu g/m^l^2$, a CD test or molecular confirmation of ESBL production is necessary.

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Fig. 1. Algorithm for the confirmation of ESBL-producing *E. coli* and *K. pneumoniae*. AMC, Amoxicillin–clavulanic acid; 3GC, third-generation cephalosporins (ceftazidime or ceftriaxone).