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BRIEF REPORT

Extended-spectrum beta-lactamases screening agar with AmpC inhibition

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The serious increase in the prevalence of extended-spectrum beta-lactamases (ESBLs) worldwide creates a need for effective and easy to perform screening methods for detection [1]. The use of an ESBL screening agar would allow rapid recognition and isolation of ESBL-producing bacteria. The currently available screening agars have low specificity, mainly due to growth of species with inducible AmpC beta-lactamases [2, 3]. The inhibition of AmpC beta-lactamases by cloxacillin is used in ESBL confirmation tests [4]; however, to the best of our knowledge, cloxacillin for ESBL screening has not been described. We developed an ESBL screening agar (ESA), which contains cloxacillin to inhibit growth of AmpC-producing species and vancomycin to inhibit growth of *Enterococci*, and compared it with the commercially available, selective medium for screening of presumptive ESBL Enterobacteriaceae, namely, BLSE agar (AES Laboratory, France).

The ESA consists of two MacConkey agars: one containing ceftazidime 1.0 mg/l, and the other cefotaxime 1.0 mg/l, cloxacillin 400 mg/l, and vancomycin 64 mg/l. The BLSE agar is a commercial double-plate agar (MacConkey with ceftazidime 2 mg/l and Drigalski with cefotaxime 1.5 mg/l). The combined screening with cefotaxime and ceftazidime has been shown to yield the best sensitivity and specificity for ESBL detection [5]. The concentration of cefotaxime and ceftazidime in ESA is consistent with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) for screening of ESBL-producing Enterobacteriaceae [6] and with the guideline of the Dutch society for medical microbiology for screening and confirmation of ESBLs in Enterobacteriaceae (www.nvmm.nl). The inhibition of AmpC with cloxacillin depends on the concentration of cloxacillin in the agar (Fig. 1). Therefore, we evaluated the performance of ESA with different cloxacillin concentrations (400, 600, 800 and 1000 mg/l), whereby 400 mg/l yielded the most optimal sensitivity.

The ESA and the BLSE agar were evaluated with 208 Enterobacteriaceae isolates, 70 of them were ESBL-producers (37 *Escherichia coli*, 10 *Klebsiella* spp., 1 *Proteus mirabilis*, 20 *Enterobacter* spp., 2 *Citrobacter freundii*) and 138 were ESBL negative (43 *E. coli*, 17 *Klebsiella* spp., 9 *Proteus mirabilis*, 55 *Enterobacter* spp., 6 *Citrobacter freundii*, 4 *Morganella morganii*, 1 *Providencia* spp., 3 *Serratia* spp.). These isolates had previously been genotypically characterized with PCR and subsequent sequence analysis with SHV, TEM and CTX-M primers for the bla_{SHV}, bla_{TEM}, and $bla_{\text{CTX-M}}$ genes [7, 8]. The media were incubated in air at 37°C for 18 to 24 h. For negative cultures the incubation was prolonged for 48 h.

The sensitivity and specificity of the ESA and the BLSE agar tested with the 208 Enterobacteriaceae isolates were 100% (70/70) and 84.7% (117/138), respectively, for the ESA, and 100% (70/70) and 57.2% (79/138) for the BLSE

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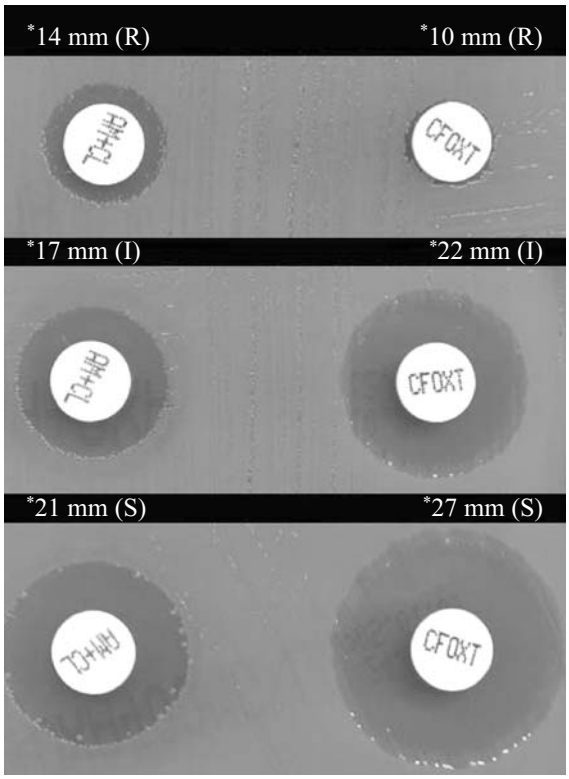

Fig. 1 AmpC inhibition with cloxacillin. *Citrobacter freundii*, AmpC positive, ESBL negative. The increase in the zone around AM+CL and CFOXT with addition of cloxacillin to the MH indicates the inhibition of AmpC. R, resistant; I, intermediate susceptible; S, susceptible; AM+CL, amoxicillin-clavulanate; CFOXT, cefoxitin

agar. The prolongation of incubation did not improve the sensitivity of the ESA or BLSE agars. The better performance of the ESA was mainly due to less false positive results due to AmpC-producing strains, especially *Enterobacter* spp. (false positive results for *Enterobacter* spp. with ESA was 29% or 16/55 and with BLSE was 89% or 49/55). The specificity of ESA for screening of ESBL-producing strains was significantly better than the specificity of BLSE agar, which reduced the number of unnecessary confirmations. A quick and easy to use screening method to facilitate the detection of ESBL-producing Enterobacteriaceae in clinical settings is very important for optimal therapy and early application of appropriate infection control measures.

The ESA is, however, intended as a screening tool; therefore, it is important to note that any growth on the plates should not be taken as definitive proof of ESBL production, which can only be achieved by use of appropriate confirmatory tests. Despite the limitations of this preliminary study, our results show that ESA is a sensitive and convenient method to screen for ESBL-producing organisms. Further evaluation should be made with clinical specimens originating directly from human carries.

References

1. Paterson DL, Bonomo RA (2005) Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 18(4):657–686. doi:10.1128/CMR.18.4.657-686.2005
2. Glupczynski Y, Berhin C, Bauraing C, Bogaerts P (2007) Evaluation of a new selective chromogenic agar medium for detection of extended-spectrum beta-lactamase-producing Enterobacteriaceae. J Clin Microbiol 45(2):501–505. doi:10.1128/JCM.02221-06
3. Sturenburg E, Sobottka I, Laufs R, Mack D (2005) Evaluation of a new screen agar plate for detection and presumptive identification of Enterobacteriaceae producing extended-spectrum beta-lactamases. Diagn Microbiol Infect Dis 51(1):51–55. doi:10.1016/j.diagmicrobio.2004.08.009
4. Drieux L, Brossier F, Sougakoff W, Jarlier V (2008) Phenotypic detection of extended-spectrum beta-lactamase production in Enterobacteriaceae: review and bench guide. Clin Microbiol Infect 14 (Suppl 1):90–103. doi:10.1111/j.1469-0691.2007.01846.x
5. Hope R, Potz NA, Warner M, Fagan EJ, Arnold E, Livermore DM (2007) Efficacy of practised screening methods for detection of cephalosporin-resistant Enterobacteriaceae. J Antimicrob Chemother 59(1):110–113. doi:10.1093/jac/dkl431
6. Clinical and Laboratory Standards Institute (2007) Performance standards for antimicrobial susceptibility testing, seventeenth informational supplement. M100-S17. CLSI, Wayne, PA, USA
7. Al Naiemi N, Bart A, de Jong MD, Vandenbroucke-Grauls CM, Rietra PJ, Debets-Ossenkopp YJ, Wever PC, Spanjaard L, Bos AJ, Duim B (2006) Widely distributed and predominant CTX-M extended- spectrum beta-lactamases in Amsterdam, The Netherlands. J Clin Microbiol 44(8):3012–3014. doi:10.1128/JCM.01112-06
8. Al Naiemi N, Duim B, Savelkoul PH, Spanjaard L, de Jonge E, Bart A, Vandenbroucke-Grauls CM, de Jong MD (2005) Widespread transfer of resistance genes between bacterial species in an intensive care unit: implications for hospital epidemiology. J Clin Microbiol 43(9):4862–4864. doi:10.1128/JCM.43.9.4862-4864.2005