Detection of mecC methicillin-resistant Staphylococcus aureus with a semi-selective enrichment broth

C. Ekenberg*, K. Boye, K. Schønning, H. Westh and G. Lisby

Department of Clinical Microbiology, Copenhagen University Hospital, Hvidovre, Denmark

*Corresponding author. Fax: +45-3862-3357; E-mail: christina.ekenberg@regionh.dk

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Sir,

We have with interest read the recent article by Paterson et al.1 describing the prevalence of mecC-carrying methicillin-resistant Staphylococcus aureus (MRSA) in England. In that study, the prevalence of mecC MRSA was 0.45% of all MRSA. In contrast, in a recent study mecC-positive MRSA constituted 2.8% of all MRSA in Denmark in 2011.2 This difference may be caused by a different screening methodology. Direct plating on chromogenic selective agar plates may miss low inocula of mecC MRSA2 as these agar media have been optimized for the growth of mecA MRSA.

In Denmark, for the detection of MRSA, we traditionally use semi-selective enrichment broth containing 3.5 mg/L cefoxitin, 20 mg/L aztreonam and 2.5% NaCl4 (SSI Diagnostica, Denmark) before plating on chromogenic selective agar. This broth is optimized for the detection of 20 cfu mecA-positive MRSA. However, at the time of development the sensitivity of this broth for the detection of mecC-positive MRSA had not been established.

These considerations prompted us to investigate whether the use of this semi-selective enrichment broth enabled the reliable detection of low inocula of mecC MRSA.

Six mecC-encoding MRSA, isolated between August 2010 and November 2013 from six patients, were selected. Typing indicated that the spa types of the six isolates were t843 (4), t373 and t1978 and that there were two sequence types (STs) by multilocus sequence typing: ST130 and ST1245.

After overnight incubation on 5% Danish blood agar (DBA; SSI Diagnostica, Denmark), the colonies were suspended in 0.9% NaCl solution and adjusted to a turbidity equivalent to that of a 1.0 McFarland standard (~3 × 10⁶ cfu/mL). After serial dilution of the suspension in sterile water, tubes with semi-selective broth were inoculated with ~1280, 320, 80 and 20 cfu. The same inoculum was plated on DBA and on Brilliance™ MRSA 2 Agar (Oxoid, UK). The semi-selective enrichment broths and plates were incubated at 37°C for 22 h, and the growth was recorded by counting the number of bacterial colonies on the plates with the intended inocula of 80 and 20 cfu. Colony counting was repeated after incubation for an additional 26 h. Total nucleic acid was extracted from 200 µL of each semi-selective broth on a MagNA Pure 96 (Roche, Switzerland). An in-house multiplex PCR targeting mecA, mecC, nuc and femA (K. Boye, H. Westh, K. Schønning, D. Özdemir and G. Lisby, unpublished data) was performed on the LightCycler 480 platform (Roche, Switzerland) for all semi-selective broths after 22 h of growth.

The real-time PCR performed on all semi-selective enrichment broths after incubation for 22 h had similar amplification curves, with mecC Ct detection values close to 18 (corresponding to 10⁶ cfu/mL) regardless of the initial inoculum (1280 cfu, average 17.36 Ct; 320 cfu, average 17.86 Ct; 80 cfu, average 18.22 Ct; and 20 cfu, average 18.93 Ct).

Direct plating on DBA of an intended inoculum of 20 cfu yielded on average 19 cfu (range 13–23 cfu) after 22 h. In contrast, the cfu count on Brilliance™ MRSA 2 Agar was only 4 cfu (range 1–13 cfu), i.e. 4.8-fold lower than on DBA. Similarly, a 4.3-fold difference was found with the 80 cfu inoculum.

In conclusion, the semi-selective enrichment broth routinely used in Denmark does not inhibit the growth of mecC MRSA even at 20 cfu. It thus performs equally well for mecC and mecA MRSA. As expected, mecC MRSA grew poorly on Brilliance™ MRSA 2 Agar. Future studies are warranted to show whether or not other types of semi-selective enrichment broth for the detection of MRSA perform as well as the one used in this study.

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Transparency declarations
None to declare.

References