Viability of meticillin-resistant *Staphylococcus aureus* after long-term storage on Dorset egg medium

Meticillin-resistant *Staphylococcus aureus* (MRSA) has emerged over the past decade as a significant bacterial pathogen associated with healthcare-associated infection (Otter & French, 2011). For this reason, it is important that clinical microbiology laboratories archive representative isolates for historical purposes, as well as for longitudinal studies, where such isolates may be required within the short, medium and long terms. Many clinical laboratories in the UK have traditionally used Dorset egg medium (DEM) as the storage medium of choice for the maintenance of their MRSA isolates. To date, there have been no reports in the literature detailing the fate of MRSA viability under storage conditions of MRSA isolates in DEM. Therefore, it was the aim of this study to determine the survival of clinical MRSA isolates in the medium to long terms (post 8–9 years of storage). Such survival data will be of interest to biomedical scientists with responsibility for their hospital MRSA collections, as well as to clinical microbiologists and curators of bacterial culture collections, so that they are aware of the survival dynamics of these organisms under such storage conditions.

Human clinical MRSA bacterial isolates (n=100) were selected randomly from the historical bacteriological archive of the Northern Ireland Health and Social Care Microbiology Repository (MicroARK), housed at the Northern Ireland Public Health Laboratory, Department of Bacteriology, Belfast City Hospital (www.microark.com). Isolates were dated from either 2008 or 2009 and had been stored on slopes in 2 ml sterile glass screw-cap bottles (Bijou bottles) on DEM. All isolates had been inoculated heavily either 7 or 8 years previously (2008–2009), following culture purification and confirmation on Columbia blood agar base (CM331; Oxoid), supplemented with defibrinated horse blood 5 % (v/v) (Oxoid). All isolates had been stored unopened, in the dark and at ambient (laboratory) temperature, for the intervening period, until the commencement of the current study. All isolates were inoculated onto freshly prepared nutrient agar (Oxoid CM0003) and were incubated aerobically at 37 °C for 24 to 48 h before examination. Plates showing no growth were incubated at ambient temperature for a further week. All subsequent cultures were checked for the presence of *Staphylococcus aureus* by (i) colour/appearance (*Staphylococcus aureus* straw/white colonies on nutrient agar), (ii) catalase appearance (*Staphylococcus aureus* catalase positive) and (iii) serological/antibody through use of the Staphaurex assay (Oxoid) appearance (*Staphylococcus aureus* yields a clumping/agglutination reaction).

Of the 100 archived isolates of MRSA examined, 28 yielded growth after 24 h. No further positives were obtained post 24 h initial incubation. Of these 28 cultures, 22 were subsequently identified as *Staphylococcus aureus*, with the remaining six isolates being surviving contaminants from the archived specimens. We do not have any data from our current study to help elucidate the reason for such a low recovery rate, but we speculate that some factors that have been responsible for the demise of the MRSA included dessication of the medium due to improper closure of the lids of the glass vials, as well as enzymatic degradation of the DEM by contaminating organisms.

DEM was first described in 1902, as a medium containing eggs for the cultivation of *Bacillus tuberculosis* (Dorset, 1902). It contains beef extract (0.3 % w/v), peptone (0.5 % w/v) and egg extract (from whole fresh eggs) (75 % v/v), with a pH of 7.2±0.3. More recently, it has been described as a successful medium for the storage of Salmonella enterica serovar Typhi (Matthews et al., 2011), Neisseria meningitidis (Wasas et al., 1999), Haemophilus influenzae type b (Wasas et al., 1999), Streptococcus pneumoniae (Wasas et al., 1998) and Escherichia coli (Yoh et al., 1991). To date, there have been no formal reports of its value in the short-, medium- or long-term storage of MRSA isolates.

Overall, this study demonstrated that medium- to long-term storage of 8 to 9 years of MRSA isolates on DEM at ambient temperature resulted in significant loss of culturability, where culturability dropped by 78%. It would have been interesting to examine the shorter-term survival of MRSA on DEM; however, over the last 5 years, due mainly to laboratory space constraints, many clinical laboratories are moving away from the traditional Dorset egg/agar slope storage systems to miniaturized storage systems usually at –80 °C, which require significantly smaller footprints than glass Bijou bottles.

In conclusion, biomedical scientists and other curators of bacteriological culture collections/repositories should be aware that storage of MRSA on DEM can lead to significant losses in isolate culturability of up to 78 % in the medium to long terms. Therefore, alternative storage mechanisms, including freeze-drying or frozen storage, should be put in place to protect the longevity of important MRSA culture collections (Vitko & Richardson, 2013).

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Abbreviations: DEM, Dorset egg medium; MRSA, meticillin-resistant Staphylococcus aureus.

References


