INTRODUCTION

The National Collection of Type Cultures (NCTC) is one of the world’s longest-running collections of medically important bacteria. The strains in the collection are provided to the biomedical science community for both research and as control strains. The purity of each strain is assessed visually by growing the bacteria of interest, on non-selective solid agar and checking for contaminants after an extended incubation. Common contaminants including coagulase-negative Staphylococci (CNS) organisms and other organisms that compose the normal skin flora can cause secondary contamination as these can be introduced by the operator. Currently, NCTC uses Baird Parker Agar (BPA) (Fig. 1) to detect the presence of Staphylococcus spp. before and after a strain is freeze-dried. BPA is commonly used to isolate and identify Staphylococcus in microbiology showing colonies with a black morphology and a zone of clearing. An alternate to BPA is Mannitol Salt Agar (MSA) (Fig. 2) which is selective for Staphylococcus due to the high salt concentration and able to differentiate coagulase-positive Staphylococcus (CPS) from CNS through its ability to ferment mannitol. Blood Agar (BA) is used routinely at NCTC to identify aerobic contaminants.

AIMS

The aims of this project were to:
1. Determine if the use of BPA is an optimal method of detecting contamination in NCTC strains, or if an alternative method can be implemented
2. To appraise the cost-effectiveness of using BPA in NCTC compared to an alternate method

METHOD

In this study, 92 NCTC strains underwent testing for contamination. The strains selected consist of those undergoing quality control checks as part of routine work, (n=24) as well as those previously failed due to known CNS contamination (n=68).

A freeze-dried ampoule of each strain was opened and reconstituted with nutrient broth. The broth was plated out onto the following agars and incubated aerobically at 37°C:
- Baird Parker Agar (BPA) (Oxoid) (Fig. 1)
- Mannitol Salt Agar (MSA) (Oxoid) (Fig. 2)
- Blood Agar (BA) (Oxoid) (Fig. 3)

The plates were checked for growth after 24 hours and again after seven days. All growth observed was identified using Matrix Assisted Laser Desorption Ionisation – Time of Flight (MALDI-TOF) (Bruker). (Fig. 4).

RESULTS

Review of Non-Conformances

Over the past five years, 125 non-conformances were raised due to contamination.

The most common organisms identified as contaminants are skin flora: Micrococcus and Staphylococcus spp., which accounted for 17% of all contaminated batches. No contamination was caused by CPS (Fig. 5).

Only 1% of the CNS contamination grew on BPA, the remaining were detected in the blood purity plate.

The other organisms causing 83% of contamination were primarily Micrococcus spp. as well as Bacillus spp. and Gram-negative rods.

Cost-effectiveness of using BPA in NCTC

Since 2010, NCTC has spent £1,914.56 on BPA plates (average of £213 per year). This figure is based on two plates used per strain (Fig. 6).

In addition, the estimated cost of labour is £7.11 per strain. This figure is based on the assumption of an EO-grade Microbiologist working on each plate for 20 minutes (labelling, sub-culturing, reading and recording results).

As part of the investigation, previously contaminated strains were used to see if contamination would be observed on BPA. In Fig. 8 (a, b and c), NCTC 7180 was contaminated with Micrococcus luteus. Following initial growth, contamination was seen only on BA and not on BPA after one day of growth.

The contaminant was picked up on BPA after seven days, where it was also present on BA. MSA grew only the contaminant; Bacillus licheniformis. BPA grew the target organism and small white colonies were also present which were identified as a Bacillus (Fig. 8 (d and e)).

DISCUSSION

Effectiveness of BPA

The majority of contamination is due to Micrococcus spp. This organism does not grow on BPA. BPA was only able to pick up 1% of the contamination observed in NCTC. The remaining was identified on the blood purity plate. This shows that BPA is not effective. The specificity of BPA was low at 67%. Many other organisms beside Staphylococcus grew on the agar such as Klebsiella sp., Bacillus sp. and Enterococcus sp.

BPA vs. MSA

The alternate agar plate proposed, MSA, was less specific and accurate than BPA. As a result, this agar plate will not be implemented in NCTC.

Alternate methods of detecting contamination

The majority of contamination observed in NCTC strains is detected by culture on BA. MALDI-TOF is routinely used to authenticate NCTC strains and can also be used to identify contaminants.

Further study

The use of selective and/or chromogenic media chosen on a strain by strain basis could also be evaluated as a method of detecting contamination.

CONCLUSIONS

- NCTC will no longer use Baird-Parker Agar in the purity testing of the strains in the collection
- NCTC will continue to use the blood agar purity plate and 24-hour broth for non-O2 organisms
- The increasing use of the MALDI-TOF procedure to identify contaminants has provided faster and more reliable results

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REFERENCES