Materials and methods

Stools: from inpatients (>2) suffering from antimicrobial- or chemotherapy-associated diarrhea. Between Jul 2013 and Dec 2013 retrospectively 95 positive stools (kept at -80°C) and prospectively 161 stools were tested.

Inoculations: The prospective 161 stools were inoculated manually using a 10 µl loop. The retrospective 95 positive stools were inoculated using a 30 µl loop on BD™ Innovia. To obtain liquid stools they were prediluted minimally with physiologic serum.

Cultures: Chrom ID (bioMérieux, Lyon, France) and CHROMagar® (bioMérieux, Lyon, France) and CHROMagar® C.difficile (CHROMagar®, Paris, France).

ColoniesTM is a registered brand name of CHROMagar®, Paris, France when supplied ready prepared (24 h anaerobic incubation at 35°C).

Reading: All cultures were read with a binocular stereomicroscope, with the lightbeam through the Petridish under a certain angle.

Identification: MALDI-ToF MS biocycler (Bruker Daltonik GmbH, Bremen, Germany) was used to confirm the C. difficile colonies.

Ribotyping: DNA were extracted with chelex and 16S - 238S rRNA intergenic spacer regions were amplified using primers as described by Barbut et al. (1). Amplon size were analysed by capillary electrophoresis using an automatic sequencer (ABI 3100 Automated DNA Sequencer) and GeneMapper Analysis (Applied Biosystems, Inc.). A 35-500 bp ROX ladder (ABI) was used as internal marker. Profiles were analysed by comparison with those of reference strains from the European collection (Brazier classification, BR…) and with our own database, UCL…

Results

Between Jul 2013 and Dec 2013, we inoculated retrospectively 95 positive stool samples and all of them grew on both chromogenic plates. Prospectively we analysed 161 routine samples. We isolated C.difficile in 51 (31.7%) samples on chromID and 54 (33.5%) on CHROMagar®. All isolated C.difficile colonies on the CHROMagar® plate were fluorescent and larger than on chromID. We detected 44 colonies with the MALDI-TOF MS (Bruker). Two C. Hethawy were black and looked very much. We were able to confirm one of them with MALDI-TOF MS. Some ribotypes (BR014, BR020) of C. difficile, stayed uncolored after 24 h but became coloured within 48 h. Other colonies were black but did not look like C. difficile.

We tested at least one strain from the 23 most frequent ribotypes in Belgium in 2012. All strains gave fluorescence under ultraviolet illumination. Although the fluorescence diffuses rather quickly in the agar plate that we used.

Discussion

When aiming at a quicker diagnosis of CDI to reduce the incubation time is crucial. Both Chrom ID (bioMérieux, Lyon, France) and CHROMagar® C.difficile (CHROMagar®, Paris France) allow isolation of C. difficile from stools after 24 h anaerobic incubation at 35°C. In both media endogen flora is reduced to a minimum. Manually inoculation is still superior since the isolation is not always successful. Following the streptomycin homogenise the stool sample making comparison more rigorous. The medium allows a major reduction of the incubation period (24h). An important point of attention is that, after 24h incubation, the biochemical identification of the strain must be determined by a molecular biology method instead of an immunoassay. On the Chrom ID medium, C. difficile grows as very small colonies which are coloured black. The colonies of C. difficile are bigger after 24 h on the CHROMagar®. The Chrom ID medium has to be used with precaution, since some ribotypes stay uncoloured on the plate while other bacteria coloured black and were not identified as C. difficile. Biocycler reading can solve this problem. CHROMagar® can more easily be recognised on the CHROMagar® medium by an experienced eye. Case of doubt ultraviolet illumination can solve this problem. The CHROMagar® is an excellent new 24 h C. difficile detection medium.

Abstract

Objective: Toxigenic culture remains one of the most sensitive diagnostic method for C. difficile infection and is usually considered as a gold standard in diagnostic method evaluations. However, it is very slow as compared with other rapid tests like immunoassay.

Methods: Two commercial media, the CHROMagar® C.difficile (CHROMagar®, Paris France) and C.difficile broth (bioMérieux, Lyon, France), were compared in a retrospective and prospective study. A suspension of defrosted stools was inoculated manually with 10µl loop or was processed with a 24-h loop on BD™ Innovia. All media were incubated in anaerobic conditions at 35°C. Plates were read after 48h incubation using the BD™ Innovia which is an automated species processor.

Results: Between Jul 2013 and Dec 2013, we inoculated retrospectively 95 positive stool samples and all of them gave on both chromogenic plates. Prospectively we analysed 161 routine samples. We isolated C.difficile in 51 (31.7%) samples on chromID and 54 (33.5%) on CHROMagar®. All isolated C.difficile colonies on the CHROMagar® plate were fluorescent and larger than on chromID. We detected 44 colonies with the MALDI-TOF MS (Bruker). Two C. Hethawy were black and looked very much. We were able to confirm one of them with MALDI-TOF MS. Some ribotypes (BR014, BR020) of C. difficile, stayed uncolored after 24 h but became coloured within 48 h. Other colonies were black but did not look like C. difficile.

Conclusion: The new fluorescent CHROMagar® C.difficile is an excellent medium for the detection of C. difficile in stool samples after 24 h. Larger colonies make identification easier. Even after automated inoculation CHROMagar® was demonstrated to be the most sensitive and allows a major reduction of the incubation period. On chromID, both colonists were preferentially coloured on C. difficile and colonies of certain ribotypes were coloured but nevertheless easily recognised by their typical appearance using a binocular.