Revisiting blood agar for the isolation of *Neisseria gonorrhoeae*

Sir,

Neisseria gonorrhoeae is an obligate intracellular bacterium infecting humans, causing cervicitis, urethritis, pharyngitis, and proctitis. If untreated, complications such as infertility, ectopic pregnancy, and pelvic inflammatory disease may result in women, while prostatitis, urethral strictures, and epididymitis may result in men.[1] Dissemination occurs in 0.5%–3% of gonococcal infections, typically presenting with fever, dermatological manifestations, polyarthralgia, and tenosynovitis.[2] Conventionally, chocolate agar or selective media containing antimicrobial agents, such as New York City agar or modified Thayer-Martin agar, have been used for the isolation of *N. gonorrhoeae* from clinical specimens. Since *N. gonorrhoeae* lacks hemolysin, it can not directly release factor V (nicotinamide adenine dinucleotide) from red blood cells (RBCs) due to which blood agar is not traditionally used for its primary isolation. Lysed RBCs in chocolate agar readily provide this essential coenzyme due to which it is preferred for *N. gonorrhoeae* isolation. However, some strains of *N. gonorrhoeae* can grow on commercially available sheep blood agar, although growth is often slower and less prominent compared to chocolate agar.[3] Recently, a case of bacteremia due to *N. gonorrhoeae* was described from Korea, wherein aerobic culture in the presence of 5% CO_2 resulted in bacterial isolation on blood agar and chocolate agar following 2 days of incubation, suggesting a comparable growth rate on both agar types.[4]

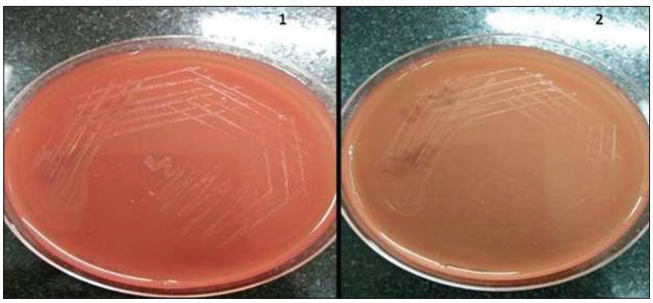

Figure 1: (1) Blood agar and (2) chocolate agar plates showing colonies of *Neisseria gonorrhoeae* after 48 h incubation

We aimed to study the effectiveness of blood agar for the isolation of *N. gonorrhoeae* compared to chocolate agar. A total of 1405 cervical swabs and 125 urethral swabs were received at our laboratory from October 2015 to October 2017. All samples and two World Health Organization reference strains C and F were inoculated on blood agar and chocolate agar plates in parallel followed by incubation at 37°C with 5% CO_2. Eight urethral swabs and two cervical swab samples demonstrated growth after 48 h, revealing 1–2 mm size, opaque, round, smooth with grayish-white colonies on both blood and chocolate agar [Figure 1]. The appearance of colonies was 24 h earlier in blood agar during primary isolation, and the size and number of colonies in both media were comparable. Identification was done using conventional biochemical tests and confirmed by matrix-assisted laser desorption time of flight by Bruker Daltonics, Germany and MALDI Biotyper 3.0 software (Bruker Daltonics, Billerica, MA, USA). All ten clinical isolates and two reference isolates were confirmed to be *N. gonorrhoeae*.Antimicrobial susceptibility testing was done as per the Clinical and Laboratory Standards Institute, 2014 guidelines.[5] Identical results of susceptibility were obtained from the isolates from blood agar, and chocolate agar indicating that choice of media did not affect the susceptibility pattern.

Our study highlights the role of blood agar; likely the most commonly used enriched media in clinical microbiology laboratories, for bacterial culture, specifically for the isolation of *N. gonorrhoeae*. Blood agar is cheap and readily available in most settings making it a good substitute for chocolate agar. In resource limited areas where gonococcal isolation is often not attempted due to the lack of selective media or non-selective enriched media like chocolate agar, using blood agar which is routinely used even for other samples can help in the prompt diagnosis of this infection. The preparation of in-house blood agar necessitates the maintenance of animal house facilities with an adequate number of healthy sheep, which is a difficult endeavor in small establishments. Thus, in laboratories where blood agar is being routinely purchased or where low sample load for *N. gonorrhoeae* recovery is expected, using blood agar alone may be recommended under appropriate temperature and CO_2 conditions. Although the use of selective media or chocolate agar cannot be replaced by blood agar based on this brief report, an evaluation of blood agar in a larger number of samples may provide more conclusive evidence for substitutive value in isolating N. gonorrhoeae. With greater availability of culture isolates, more robust drug susceptibility profiles of *N. gonorrhoeae* and drug resistance trends can be conducted for our region.

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Conflicts of interest

There are no conflicts of interest.

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