Role of Liquid Culture Media in the Laboratory Diagnosis of Microbial Keratitis

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• PURPOSE: **To determine whether liquid culture media are helpful in the diagnosis of infectious keratitis.**
• DESIGN: **Retrospective noncomparative case series.**
• SUBJECTS AND METHODS: **This is a retrospective review of microbiology records of 114 corneal scraping samples from infectious keratitis patients. Samples were processed by corneal smear microscopy (potassium hydroxide with calcofluor white and Gram stains) and culture examination (5% sheep blood agar, sheep blood chocolate agar, Sabouraud dextrose agar, brain heart infusion, thioglycolate broth, and Robertson's cooked meat broth. Cases where at least 1 liquid medium was taken were included in the study and all cases were required to have significant growth in culture as per the institutional criteria. Results of smear examination and culture growth were analyzed.**
• RESULTS: **Out of 114 cases, 44 (38.59%) were bacterial, 62 (54.38%) fungal, and 8 (7.01%) were mixed (bacteria + fungus) infection. Thirty-eight out of 44 cases of bacterial keratitis (86.36%) were diagnosed by solid media alone (criterion 1) and 6 of 44 (13.63%) required liquid media for diagnosis ($P < .001$). In fungal keratitis, 61 of 62 cases (98.38%) were diagnosed using solid media alone (criterion 1) while 1 case required liquid media for diagnosis. In mixed infection, none of the cases required liquid media for diagnosis of fungal component; however, all 8 cases required liquid media for establishing bacterial component.**
• CONCLUSIONS: **Liquid culture media increase the chance of isolation of bacteria in pure bacterial and/or mixed infection; however, their role in isolating fungus is limited. Owing to overlap in clinical diagnosis of bacterial and fungal keratitis, we recommend inclusion of both solid and liquid culture media in the laboratory diagnosis of nonviral keratitis. (Am J Ophthalmol 2013;156: 745–751. © 2013 by Elsevier Inc. All rights reserved.)**

INFECTIOUS KERATITIS IS AN IMPORTANT CLINICAL entity that can potentially threaten vision. It is defined as corneal epithelial defect associated with stromal infiltrate caused by various micro-organisms, the most common and important being bacteria and fungi. It is one of the leading causes of blindness in developing countries.[1] Accurate and rapid identification of the micro-organism is required for successful treatment of the disease.[2–5] Smear and culture examination of the corneal sample is considered the gold standard for identification of the offending agent, which helps in guiding appropriate therapy to the patient.[6] The American Academy of Ophthalmology recommends blood agar, chocolate agar, Sabouraud dextrose agar, and thioglycolate broth as standard media to be included.[7] Although the recommendation is to collect the corneal scraping on both solid and liquid media, there is an ongoing debate in the literature on the actual clinical relevance of using multiple culture media. Added to that, there seems to be reluctance among ophthalmologists to treat microbial keratitis patients based on laboratory investigation results. Nearly half of the corneal ulcer patients in southern California were treated empirically without culture examination.[8] Noncompliance to the recommended guidelines was said to be attributable to the high success rate of empirical treatment (>90%), cost of culture examination, and common failure of culture to identify the cause.[8] To balance between the recommended guidelines and actual community practice, many authors have recommended modified culture techniques in microbial keratitis. Schonheyder and associates suggested that the broth culture technique may replace the standard technique of direct plate culture.[9] Others have concluded that blood or chocolate agar alone is a reasonable alternative for standard culture methods.[10]

Our institutional protocol, apart from direct microscopic examination of corneal scrapings stained with potassium hydroxide with calcofluor white and Gram stain, consists of inclusion of blood agar, chocolate agar, Sabouraud dextrose agar, brain heart infusion broth, thioglycolate broth, Robertson's cooked meat broth, and non-nutrient agar for the investigation of nonviral corneal ulcers. We believe that this repertoire allows detection of bacteria (aerobic/anaerobic), fungi, or parasites associated with nonviral keratitis. However, we are aware that it is not always possible to inoculate corneal samples in all these media and liquid media are either inoculated towards the end or are sacrificed. This prompted us to determine retrospectively whether liquid culture media were helpful in the diagnosis of infectious keratitis, and we report the results in this communication.

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SUBJECTS AND METHODS

MICROBIOLOGY RECORDS OF 114 PATIENTS SEEN IN THE cornea service of the L.V. Prasad Eye Institute between January 1, and June 30, 2011 were retrospectively analyzed. The retrospective evaluation of microbiology records was approved by the ethics committee of the institute (Hyderabad Eye Research Foundation; Ethics Ref No. LEC 04-13-38; LVPEI-B-35-2013). These patients were diagnosed to have infectious keratitis and had been subjected to corneal smear microscopy (stained with potassium hydroxide with calcofluor white and Gram stain) and culture examination of the corneal scrapings. Type of media and the sequence of inoculation included 5% sheep blood agar, 5% sheep blood chocolate agar, Sabouraud dextrose agar (SDA), brain heart infusion (BHI), thioglycolate broth (THIO), Robertson's cooked meat broth (RCM), and non-nutrient agar (NNA). The corneal scraping sample was obtained on a slit-lamp microscope with no. 15 blade attached to a Bard Parker handle after instillation of 1 drop of topical proparacaine (0.5%). Care was taken to collect the scraping directly from the corneal infiltrate without coming in contact with the conjunctiva and lids.

All media were incubated at 36°C ± 1°C except chocolate agar (5% CO_2 at 36°C ± 1°C) and SDA (27°C). Positivity of growth in different media and results of smear examination were noted. Table 1 shows the distribution of type of media that were taken for the 114 cases. Cases where at least 1 liquid medium was taken were included in the study and all cases included in the study were required to have significant growth in culture. Significance of the culture was based on: confluent growth (10 or more colonies) on the inoculum of 1 solid medium (criterion 1), and/or growth in more than 1 medium (criterion 2), and/or growth in 1 medium consistent with direct smear result (criterion 3). All media were observed daily for growth for 2 weeks. Time taken for growth was noted. Identification of bacterial colonies was done after consideration of colony characteristics, Gram reaction, morphology, and results of biochemical tests. API system (bioMérieux, Paris, France) was used for bacterial identification. Fungal species were identified by observing the rate of growth, color, consistency, texture, and characteristic microscopic features. Statistical analysis was done using online statistical software (QuickCalcs, GraphPad software Inc, San Diego, California, USA). Fisher exact test was used to calculate the *P* value and $P < .05$ was considered to be statistically significant.

RESULTS

APPLYING THE CRITERIA FOR SIGNIFICANCE OF THE culture results, 44 of 114 cases (38.59%) were bacterial, 62 (54.38%) were fungal, and 8 (7.01%) were mixed bacterial and fungal. Among the 44 bacterial cases analyzed, 38 (86.36%) showed confluent growth on at least 1 solid medium. In 32 of 42 cases (76.19%), growth was seen in blood agar, in 28 of 41 (68.29%) growth was seen in chocolate agar, and in 22 of 44 cases (50.00%) growth was seen in both media. The average duration for growth in blood and chocolate agar was 2 days (range 1-7) and 1 day (range 1-5), respectively. Whereas BHI showed growth in 26 of 42 cases (61.90%), thioglycolate was positive in 20 of 38 (52.63%) and RCM in 21 of 29 (72.41%). The average time for growth in liquid media was 2 days. *Staphylococcus* species was the most common microorganism isolated, followed by *Streptococcus pneumoniae* (Table 2). Criterion 1 was applicable in 38 of 44 cases (86.36%) that showed confluent growth in solid media. Samples from 2 cases (4.54%) showed growth in 2 different liquid media and were diagnosed by criterion 2 while in 4 cases (9.09%) criterion 3 was applied as the culture in 1 liquid medium was consistent with smear results of the corneal scraping. Among these 6 bacteria, 4 showed growth in RCM, 3 in BHI, and 2 in THIO (Table 3). Thus, 6 of 44 (13.63%) samples of bacterial keratitis required liquid media in order to establish the diagnosis. Applicability of criterion 1 was significantly higher than criteria 2 and 3 combined ($P < .001$, Fisher exact test).

Among the 62 cases of fungal keratitis, culture was positive in solid media in 61 of 62 cases (98.38%), blood agar was positive in 51 of 55 (92.72%), chocolate agar in 50 of 59 (84.74%), and SDA in 47 of 59 (79.66%). The average duration for growth was 3 days (range 1-11 days) in blood/chocolate agar and 2 days (1-5 days) in SDA. Fungal growth was seen in liquid media in 52 of 62 cases (83.87%), BHI was positive in 42 of 58 (72.41%), thioglycolate broth in 16 of 49 (32.65%), and RCM in 25 of 45 (55.55%). The average time for growth in all 3 liquid media was 2 days. Unidentified dematiaceous fungus was the most common fungus, followed by *Aspergillus flavus* (Table 2). Sixty-one out of 62 samples (98.38%) were diagnosed by criterion 1. None of the cases was diagnosed using criterion 2 and only 1 case (1.61%) fell within criterion 3 for diagnosis using liquid media.

TABLE 1. Types of Media Used for Inoculating Corneal Scrapings of 114 Patients With Nonviral Microbial Keratitis

Media Inoculated	Bacterial Keratitis N = 44	Fungal Keratitis N = 62	Mixed Infection N = 8
Blood agar	42	55	8
Chocolate agar	41	59	8
Sabouraud dextrose agar	42	59	8
Brain heart infusion broth	42	58	8
Thioglycolate broth	38	49	7
Robertson's cooked meat broth	29	45	5