Diagnostic value of pediatric blood culture bottles for acute postoperative endophthalmitis

Tatiana Tanaka,1,* Bruno Fortaleza de Aquino Ferreira,1 Luiza Manhezi Shin de Oliveira,1 Juliana Mika Kato,1 Thais Sabato Romano Di Gioia,1 Flavia Rossi,1 Yoshitaka Nakashima,1 Sergio Luis Gianotti Pimentel,1 Joyce Hisae Yamamoto,1 Joao Nobrega de Almeida Junior1

1Departamento de Oftalmologia (LIM 33), Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, BR. 2Divisao Laboratorio Central (LIM 03), Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, BR.

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*Corresponding author: E-mail: tatiana.tanaka@hc.fm.usp.br

** INTRODUCTION

Endophthalmitis is a serious intraocular infectious disease associated with elective surgical procedures (75 to 80%), ocular trauma (3.3 to 17%) and endogenous infections (5-15%) (1-3). Suspected cases are initially treated with intravitreal injection of broad-spectrum antibiotics (vancomycin and ceftazidime) (4).

The use of sample cultures is essential to confirming endophthalmitis etiology. Several conditions such as ocular inflammation from noninfectious uveitis, fungal endophthalmitis, and toxic anterior segment syndrome may mimic clinical presentation of endophthalmitis, but bacterial cultures are negative in these cases (5). Identification of the pathogen in cases of endophthalmitis may improve treatment by the early introduction of targeted antibiotics.

Despite advances in molecular assays for detecting pathogens, microbial culture is still the current reference method for the etiological diagnosis of endophthalmitis. Conventional culture methods (CM) use solid or broth media including thioglycolate. However, rates of identification increase when blood culture bottles (BCBs) are used (3,6-9).

The present study aimed to report our own experience using pediatric BCBs (PBCBs) and conventional media for vitreous sample culture in acute postoperative endophthalmitis.

** METHODS

Fifty-four cases of clinically suspected acute postoperative endophthalmitis, attended at the Department of Ophthalmology, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, BR, between January 2010 and December 2015, were retrospectively included. This
study was approved by the Institutional Ethics Committee (CAAE: 36514614.4.0000.0068).

Undiluted vitreous samples (200 to 500 mL) were collected by pars plana vitrectomy or vitreous tap after local antisepsis, under local anesthesia and before intravitreal administration of antibiotics.

From January 2010 to December 2011, samples were cultivated in CM (thioglycolate) for 5 days at 35°C. From January 2012 to December 2015, samples were inoculated in PBCBs (BACTEC Plus Aerobic/F, BD Diagnostics, USA) and incubated in automated machines for up to 5 days. Positive samples from CM or PBCBs were later inoculated in sheep blood and chocolate agar and incubated for 48 hours under a 5% CO₂ atmosphere. Identification of causative agents and antibiotic sensitivity tests were performed by VITEK 2 (BioMérieux, France).

The yields of positive cultures with CM and with PBCBs were compared by using McNemar’s test, and the results were considered statistically significant if the p-value was less than 5% (p<0.05).

### RESULTS

Vitreous samples from 54 patients with endophthalmitis were analyzed. They were associated with phacoemulsification (n=21; 38.9%), trabeculectomy (n=11; 20.4%), extracapsular cataract extraction (n=6; 11.1%), phacoemulsification combined with trabeculectomy (n=5; 9.3%), pars plana vitrectomy (n=4; 7.4%), intravitreal bevacizumab injection (n=4; 7.4%), congenital cataract surgery (n=2; 3.7%) and phacoemulsification combined with pars plana vitrectomy (n=1; 1.8%).

Thirty-five percent (7 out of 20 cases) of CM and 64.7% (22 out of 34 cases) of PBCB cultures were positive (p=0.034) (Table 1). Isolated agents from the 29 positive cultures were Staphylococcus epidermidis (n=7; 24.2%), Streptococcus viridans (n=6; 20.9%), Staphylococcus aureus (n=3; 10.4%), Haemophilus influenzae (n=3; 10.4%), coagulase-negative Staphylococcus (n=2; 6.9%), Streptococcus pneumoniae (n=1; 3.4%), Enterococcus faecalis (n=1; 3.4%), Pseudomonas aeruginosa (n=1; 3.4%), Klebsiella oxytoca (n=1; 3.4%), Serratia marcescens (n=1; 3.4%), Staphylococcus lugdunensis (n=1; 3.4%), unspecific gram-positive bacilli (n=1; 3.4%) and Enterobacter cloacae (n=1; 3.4%). Seventy-six percent of the isolates were gram-positive bacteria, mainly Staphylococcus spp. and Streptococcus spp. (n=20; 68.9%). Agents isolated from conventional media or in PBCBs according to the associated procedure are described in Table 2.

### DISCUSSION

Endophthalmitis is a rare and devastating complication of ocular surgeries. Rapid identification of the pathogen with adequate treatment may impact visual prognosis. Conventional culture uses solid or broth media; however, PBCBs confer several advantages. Therefore, we demonstrate our experience with using PBCBs for endophthalmitis.

Conventional methods include the use of blood agar, chocolate agar, Sabouraud agar and thioglycolate broth. They require immediate incubation (not available at all ophthalmologic centers), and endophthalmitis positivity varies widely in the literature, ranging from 24 to 72% (3,6-8,10-17). These low sensitivities can be explained by various factors such as the small volume of specimens, the use of antibiotics before the collection of clinical material and the presence of fastidious microorganisms causing endophthalmitis (18).

On the other hand, BCBS confer the possibility of storage at room temperature, microorganism growth with small volume samples, ease of inoculation and low risk of contamination during transport. The use of BCBS is a good alternative in cases of endophthalmitis in areas with limited access to a microbiology laboratory. BCBS also allows the growth of fastidious pathogens (which grow better in atmospheres with high CO₂ tension) and contain resin that can adsorb antibiotics if the patient has already received them (19). BCBS have already been accepted as a diagnostic tool for small samples such as blood in pediatric practice, synovial fluid, pleural fluid and peritoneal fluid (19). Kratz et al. have also used BCBS to test for infectious keratitis and had promising results. Indeed, in some endophthalmitis studies, PBCBS were used (3). Studies using undiluted vitreous samples and BCBS showed average positivity varying from 61% to 100% (3,6-9,19-22). In contrast, Rachitskaya et al. (21) had lower positivity (31.7%) than these values when they used BCBS, likely due to the use of diluted vitreous.

| Isolated agents                  | CM (n=7) | PBCB (n=22) | Total  |
|----------------------------------|---------|-------------|--------|
| Staphylococcus epidermidis       | 2       | 5           | 7 (24.2%) |
| Streptococcus viridans          | 1       | 5           | 6 (20.9%) |
| Staphylococcus aureus           | -       | 3           | 3 (10.4%) |
| Haemophilus influenzae          | -       | 3           | 3 (10.4%) |
| Coagulase-negative Staphylococcus | 2      | -           | 2 (6.9%)  |
| Staphylococcus lugdunensis      | -       | 1           | 1 (3.4%)  |
| Streptococcus pneumoniae        | -       | 1           | 1 (3.4%)  |
| Enterococcus faecalis           | -       | 1           | 1 (3.4%)  |
| Enterobacter cloacae            | 1       | -           | 1 (3.4%)  |
| Pseudomonas aeruginosa          | 1       | -           | 1 (3.4%)  |
| Klebsiella oxytoca              | -       | 1           | 1 (3.4%)  |
| Serratia marcescens             | -       | 1           | 1 (3.4%)  |
| Unspecific gram-positive bacilli | -       | 1           | 1 (3.4%)  |
Chiquet et al. compared diluted with undiluted vitreous samples using conventional culture methods and suggested that diluted samples were as effective as undiluted samples for microbiological diagnosis of endophthalmitis; however, they also commented that the small number of positive cultures could preclude improving the understanding of the impact of dilution on culture sensitivity (23).

Comparative studies of CM and BCB positivities were carried out in six studies (3,6-9,22); five of them demonstrated a higher positivity with BCBs than with conventional methods (Figure 1). Yospaiboon et al. had a cohort of 27 patients and reported low growth rates overall, 51.9% positivity in BCBs and 25.9% in the traditional method; as discussed by the authors, these results

| Procedure                      | Conventional media, n (positive cases, %) | Isolated agent (n) | PBCB , n (positive cases, %) | Isolated agent (n) |
|--------------------------------|------------------------------------------|--------------------|-------------------------------|-------------------|
| Phacoemulsification            | 9 (4, 44.4%)                             | Staphylococcus epidermidis (1) | 12 (8, 66.7%)               | Staphylococcus epidermidis (3) |
|                               |                                          | Coagulase-negative Staphylococcus (1) |                           |                   |
|                               |                                          | Pseudomonas aeruginosa (1) |                           |                   |
|                               |                                          | Enterobacter cloacae (1) |                           |                   |
| Extracapsular cataract extraction | 2 (1, 50.0%)                             | Staphylococcus epidermidis (1) | 4 (3, 75.0%)               | Staphylococcus aureus (1) |
|                               |                                          |                           |                             | Haemophilus influenzae (1) |
|                               |                                          |                           |                             | Klebsiella oxytoca (1) |
| Pars plana vitrectomy         | 4 (1, 25.0%)                             | Coagulase-negative Staphylococcus (1) | 0 (0)                      |                   |
| Trabeculectomy                | 3 (0, 0%)                                |                           | 8 (7, 87.5%)               |                   |

Figure 1 - Positivity (%) of vitreous sample cultures of patients with endophthalmitis using the conventional method (gray) and pediatric blood culture bottles (black) in the medical literature and including the present study.

Table 2 - Isolated agents according to procedure and use of conventional media or pediatric blood culture bottles (PBCBs).
are likely due to the limited volume of samples (0.1-0.2 mL) and previous use of antibiotic therapy (6). Similar to the present study, Kim et al. used PBCBs and CM at different times, reporting a higher positivity with PBCBs (35% versus 64.7%; p=0.034). These results are in agreement with previous studies and reinforce the advantages of using PBCBs as an alternative to CM for the etiologic diagnosis of acute postoperative endophthalmitis (3,6,9,10). Figure 1 summarizes the main studies using CM and BCB/PBCBs, including the present study.

The low number of samples for each method and the different periods of inclusion are the main limitations of the present study. Additionally, although the use of PBCBs has several advantages over conventional culture, in cases where anaerobic pathogens are suspected, anaerobic BCBs or broth medium (e.g., thioglycolate broth) should be used (21). Nevertheless, these are the first case series of the advantages of PBCBs produced in Brazil and adding to the international literature. The use of PBCBs should be recommended for microbiological diagnosis of endophthalmitis and is especially suitable for office settings and remote clinics.

■ CONCLUSION

PBCBs confer a higher positivity than CM in cultures of vitreous samples of clinically suspected infectious endophthalmitis.

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■ AUTHOR CONTRIBUTIONS

Tanaka T helped to design the study, collected samples, collected microbiology data, and drafted and reviewed the manuscript. Ferreira BFA, Kato JM and Oliveira LMS collected microbiology data. Gioia TSR, Rossi F, Pimentel SLG and Nakashima Y drafted and reviewed the manuscript. Yamamoto JH and Almeida Junior JN helped to design the study, conducted the statistical analysis, and drafted and reviewed the manuscript.

■ REFERENCES

1. Jackson TL, Erykin SJ, Graham EM, Stanford MR. Endogenous bacterial endophthalmitis: a 17-year prospective series and review of 267 reported cases. Surv Ophthalmol. 2003;48(4):403-23. https://doi.org/10.1016/S0039-6257(03)00054-7
2. Taban M, Behrens A, Newcomb RL, Nobe MY, Saedi G, Sweet PM, et al. Acute endophthalmitis following cataract surgery: a systematic review of the literature. Arch Ophthalmol. 2005;123(5):613-20. https://doi.org/10.1001/archopht.123.5.613
3. Kratz A, Levy J, Belfair N, Weinstein O, Klemperer I, Lifshitz T. Broth culture yield vs traditional approach in the work-up of endophthalmitis. Am J Ophthalmol. 2006;141(6):1022-6. https://doi.org/10.1016/j.ajo.2006.01.076
4. Results of the Endophthalmitis Vitrectomy Study. A randomized trial of immediate vitrectomy and of intravenous antibiotics for the treatment of postoperative bacterial endophthalmitis. Arch Ophthalmol. 1995;113(12):1479-96. https://doi.org/10.1001/archopht.1995.0110012009001
5. Gam IM, Ugahary LC, van Dissel JT, Feron E, Peperkamp E, Veekneer M, et al. Intravitreal dexamethasone as adjuvant in the treatment of postoperative endophthalmitis: a prospective randomized trial. Graefes Arch Clin Exp Ophthalmol. 2005;243(12):1200-5. https://doi.org/10.1007/s00417-005-0133-1
6. Yospaiboon Y, Sareet S, Pasadhika S. Blood culture and conventional media for vitreous culture in infectious endophthalmitis. J Med Assoc Thai. 2005;88(5):639-42.
7. Tan HS, Ghyzcy-Carlberg EA, Spanjaard L, de Smet MD. The additional value of blood culture bottles in the diagnosis of endophthalmitis. Eye (Lond). 2011;25(8):1069-73. https://doi.org/10.1038/eye.2011.142
8. Kim KJH, Kwon HY, Park SW, Byon IS, Lee JE, Oum BS, et al. The effectiveness of pediatric blood culture bottle in endophthalmitis. J Korean Ophthalmol Soc. 2015;56(9):1365-70. https://doi.org/10.3334/jkos.2015.56.9.1365
9. Thariya P, Yospaiboon Y, Sinawat S, Sanguansak T, Boonmitchucharoen C, Laovirojanakul W. Blood culture bottles are superior to conventional media for vitreous culture. Clin Exp Ophthalmol. 2016;44(6):488-91. https://doi.org/10.1111/coe.12707
10. Chab AR, Freitss D, Scarpi MJ, Guidugli T. [Laboratory findings in endophthalmitis]. Arq Bras Oftalmol. 1997;60(3):250-7. https://doi.org/10.9353/abof.1997.0056
11. Kunimoto DY, Das T, Sharma S, Jalali S, Majji AB, Gopinathan U, et al. Microbiologic spectrum and susceptibility of isolates: part II. Posttraumatic endophthalmitis. Endophthalmitis Research Group. Am J Ophthalmol. 1999;128(2):242-4. https://doi.org/10.1016/S0002-9399(99)00113-0
12. Anand AR, Madhavan HN, Theres KL. Use of polymerase chain reaction (PCR) and DNA probe hybridization to determine the Gram reaction of the infecting bacterium in the intraocular fluids of patients with endophthalmitis. J Infect. 2000;41(3):221-6. https://doi.org/10.1053/jinf.2000.0731
13. Lohmann CP, Lunde HC, Reissel U. Improved detection of microorganisms by polymerase chain reaction in delayed endophthalmitis after cataract surgery. Ophthalmology. 2000;107(6):1047-51. https://doi.org/10.1016/S0161-6420(00)00083-X
14. Uesugui E, Cypel-Gomes MC, Atique D, Goulart DG, Galluccio FR, Nishiwaki-Dantas MC, et al. [Laboratory identification of the most frequent ocular pathogens and their in vitro sensitivity to antibiotics]. Arq Bras Oftalmol. 2002;65(3):339-42. https://doi.org/10.1590/S0004-27492002000300011
15. Saraiva FF, Costa PG, Inomata DL, Preti RC, Helal Jr J, Nakashima Y.Perfil clinico dos pacientes portadores de endoflitie internados no Hospital das Clinicas de Sao Paulo. Rev Bras Oftalmol. 2007;66(3):169-74.
16. Melo GB, Bispo PJ, Regatieri CV, Yu MC, Pighatari AC, Hoefling-Lima AL. Incidence of endophthalmitis after cataract surgery (2002-2008) at a Brazilian university-hospital. Arq Bras Oftalmol. 2010;73(6):505-7. https://doi.org/10.1590/S0161-6420201000000007
17. Bispo PJ, Hoefling-Lima AL, Pighatari AC. Molecular biology applied to the laboratory diagnosis of bacterial endophthalmitis. Arq Bras Oftalmol. 2009;72(5):734-40. https://doi.org/10.1590/S0161-64202009090000028
18. Church DL, Davies HD, Cadrain G, Trevenen CL. Comparative study of three different BACTEC culture media for the detection of bacteremia in ambulatory and hospitalized children. Can J Infect Dis. 1998;9(2):77-82. https://doi.org/10.1155/1998/063898
19. Joondhe BC, Flynn HW Jr, Miller D, Joondhe HC. A new culture method for infectious endophthalmitis. Arch Ophthalmol. 1989;107(9):1334-7. https://doi.org/10.1001/archopht.1989.01070020404044
20. Eser I, Kapran Z, Altan T, Eren H, Yilmaz OF. The use of blood culture bottles in endophthalmitis. Retina. 2007;27(4):971-3. https://doi.org/10.1097/IAE.0b013e31820fe6f4
21. Rachitskaya AV, Flynn HW Jr, Wong J, Kuriyan AE, Miller DL. A 10-year study of membrane filter system versus blood culture bottles in culturing vitreomicrocassette vitreous in infectious endophthalmitis. Am J Ophthalmol. 2013;156(2):349-354.e2. https://doi.org/10.1016/j.ajo.2013.03.040
22. Kehrmann J, Chapot V, Buer J, Rating P, Bornfeld N, Steinemann J. Diagnostic performance of blood culture bottles for vitreous culture compared to conventional microbiological cultures in patients with suspected endophthalmitis. Eur J Clin Microbiol Infect Dis. 2018;37(5):889-95. https://doi.org/10.1007/s10096-017-3182-6
23. Chiuet C, Maurin M, Thuret G, Benoist Y, Cordui PL, Creuzot-Garcher C, et al. Analysis of diluted vitreous samples from vitreous is useful in eyes with severe acute postoperative endophthalmitis. Ophthalmology. 2009;116(12):2437-41.e1. https://doi.org/10.1016/j.ophtha.2009.06.007