Comparison of Mono and Biphasic Culture Media in Isolating Bacteria from Blood and evaluation Bu-Ali Hospital Lab Quality

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ABSTRACT

Blood Culture is an important diagnostic method in infectious diseases and has positive results in 30%- 50% and even to 80% of cases due to sample volume. In Bu-Ali Hospital, Tehran, it decreases to 2-3%. In this survey, quality of hospital lab and difference between Mono and Biphasic culture media in isolating bacteria from blood of patients suggestive of sepsis were evaluated.106 (48 F + 58 M) newly admitted patients with impression of sepsis as SIRS (Systemic Inflammatory Response Syndrome) (36˚C >OT>38.3˚C , tachycardia more than 100/min, leukocytosis with shift to left or leucopenia) with infectious source were sampled for culture (5ml blood 3 times in 1 Biphasic and 2 Monophasic media) in the infectious ward. One Monophasic Media in hospital lab and the two other (1 Monophasic + 1 Biphasic Media) in Reference Laboratory of Iran, Research Center were handled. Media were quality-controlled at beginning and in the middle of study by NCCLS (National Committee for Clinical Lab Standard) with ATCC (American Type Culture Collection) samples. Sampling, transfer, and handling were all in standard conditions usually used in hospital. Results were compared by Fisher Exact Test. Clinical diagnosis were bacterial in 84 (79%), and nonbacterial in 22 (21%) patients at admission. 57 (54%) patients had not used antibiotics in the past 72 hours. In Monophasic Media of hospital lab 2 (1.9%) positive cultures (S. epidermidis) one with history of Erythromycin use were reported. In both Monophasic and Biphasic Media in reference lab 3 (2.8%) positive cultures (2 S.epidermidis, 1 E. coli) were reported equally, one with history of Erythromycin use. Growth Index in both Monophasic and Biphasic Media were standard in quality control. Qualities of Mono and Biphasic Media in growing bacteria were alike and Biphasic Media had no superiority to Monophasic Media in routine bacterial isolation. Positive culture in both labs had no significant statistical difference. So, negative results are not due to media and laboratorial fields, and it is needed to educate and evaluate two other fields: Sampling and Transferring. Also, we may have more positive cultures by increasing blood samples from 5 to 20 ml which can be compared in next studies.

Keywords: Blood Culture; Culture Media; Monophasic; Biphasic.

INTRODUCTION

Bacteria are important microorganisms causing infectious disease. Indeed to raising individual and public health care facilities, one of the best ways of eradication is rational use of antibiotics which needs to isolate and identify pathogen microorganisms and determine their antibiotic sensitivity [1].

After more than 100 years of using blood culture [2] hence factors can result in false negative and false positive results in three fields: Sampling, Transport, and Culture media processing in the laboratory [3]. Blood culture is essential in identifying bacterium responsible for bacteremia, sepsis, infection of native and prosthetic valves, suppurative thrombophlebitis, mycotic aneurysm and infection of vascular grafts [2]. Pathogens may be missed if their growth requirements are not met, for this reason selective culture techniques are employed. Pathogens represented in small numbers may be killed by metabolic acids or other products of non-pathogens or antibodies [4].

Sampling by disinfecting veni-puncture site with Alcohol 70% and Bethadine after 2-3 minutes with sterile needles decrease false positive culture of skin flora contamination in

febrile period results 80-90% positive with two samples. Ideally, specimen should be transported to lab in 30 minutes post collection. Specimen processing, election of culture media (nutrients, inhibitors, other bacteria) should be noticed [5]. To improve the yield and the detection time, procedures have developed as lyses centrifugation and increasing concentration [6], combining resin to culture media with more microorganism isolation mainly for patients receiving antimicrobial therapy [7], backup broth media also called supplemental or enrichment broth [5], using biphasic methods to avoid subcultures and false positive results with increasing rate of positivity and reducing detection time [8] with different results. Incorporating resins in blood culture media can shorten detection time too [5]. Usually 2-3 blood samples are sufficient to achieve the optimum blood culture sensitivity to 90%. Volume of specimen is also important usually 10-20 ml as ratio of 1/4 sample to culture media volume [1, 5].

In our hospital lab we have positive blood culture in only 3% of samples, unable even in isolating fastidious bacteria which makes this important diagnostic test useless.

In this survey to find the factors affecting the blood culture results in laboratorial processing field, quality of hospital lab and comparison of Mono and Biphasic Media in isolating bacteria in Reference laboratory of Iran, Research Center are studied.

MATERIALS AND METHODS

In this study which was conducted on a cross-sectional basis , all patients in infectious clinic and emergency wards presenting with sepsis as SIRS (oral temperature more than 38.3˚C or lower than 36 ˚C, tachycardia more than 100/min, leukocytosis with shift to left or leucopenia (4000>WBC>10,000) with infectious source requiring admission in infectious disease ward for diagnosis and treatment were studied in 2003.Three separate 5 ml blood samples were collected (ratio of 1/4 to culture media volume of 20 ml). Sampling was from three different veins of upper extremities with 20 minutes intervals. The proposed veni-puncture site was disinfected with alcohol 70% and Povidon-Iodine for 1-3 minutes to avoid contamination of blood sample with skin flora.

The first sample was inoculated in hospital Monophasic Media, the second sample in Biphasic and the third one in Monophasic reference lab media. Monophasic (BHIB; Brain Heart Infusion Broth) Culture media were obtained from Padtan Company and Biphasic Culture Media from Barafshan Company (both from Iran) with approval from Reference Laboratory of Iran, Research Center . In this study the quality of two Monophasic and Biphasic Culture Media in reference lab and results of Monophasic cultures of reference and hospital lab were compared. Culture media were in room temperature out of refrigerator for 15 minutes before inoculation. Inoculated media were kept in wards incubator in 35-37˚C until transporting to laboratory. First sample in hospital lab , second and third one in Reference Laboratory of Iran Research Center were processed. Samples were observed for turbidity, hemolysis, gas formation or colony forming on precipitated blood layer which are evidence of microorganism growth in the first 6-18 hours. Positive samples in the first 6-18 hours and negative samples after 18 hours were sub cultured in Mac Conkey, Blood Agar and Chocolate Agar (CO25-10%) media and incubated in 35 ˚C temperature. Culture media were observed for growth evidence daily, if positive re-sub-cultured, and if negative, they were blind sub-cultured at the 3rd and 7th days and results reported in the 14th day.

Quality control of culture media was evaluated two times: first, in the beginning and the second, in mean (50%) step of survey as NCCLS (National Committee for Clinical Lab Standard) with ATCC (American Type Culture Collection) samples in Reference Laboratory of Iran, Research Center. For statistical analysis of findings the Fisher Exact Test was applied.

RESULTS

In this study, 106 adult patients (more than 14 years old) (48 female + 58 male) with sepsis impression were studied. Clinical diagnosis were bacterial in 84(79%), and nonbacterial in 22(21%) patients at admission. 49(46%) patients had used antibiotics before admission including: oral Ampicillin, Amoxycillin, Trimetoprime- Sulfametoxazole, Gentamicine, Erythromycin and Penicillin procaine 24-72 hours before admission. (Table 1)

There was 3(2.8%) positive culture in refer- ence lab Monophasic Media (2 S.epidermidis +1 E.coli), one with history of

Table 1. Number of patients with and without history of Antibiotic use.

History of antibiotic	Number	Percent
Used	49	46%
Not used	57	54%
Total	106	100%

Erythromycin use; 2 = 1.9% positive culture in hospital lab Monophasic Media (2 S.epidermidis) one with history of Erythromycin use. S. epidermidis identified as Coagulase negative, Novobiocin sensitive strain. There was no statistical difference in positive cultures of Monophasic Media of two laboratories by Fisher Exact Test and laboratorial handling had no effect on results.

3(2.8%) positive cultures (2 S. epidermidis + 1 E. coli) were detected in Biphasic Media at reference laboratory as Monophasic Media. So, efficacy of two lab Monophasic and Biphasic Media was similar (Table 2 & 3).

Table 2. Number and percent of positive cultures in Mono and Biphasic Media in two laboratories

Cul. Med	Hos-Mon No %	Ref-Mon No %	Ref-Biph No %
Pos	2 (1.9%)	3 (2.8%)	3 (2.8%)
Neg	104 (%98.1)	103 (97.2%)	103 (97.2%)
Total	106 (100%)	106 (100%)	106 (100%)

Cul.Med:Culture Media,
Hos-Mon:Hospital Monophasic Media,
Ref-Mon:Refference Monophasic Media,
Ref-Biph:Refference Biphasic Media.

In quality control growth evidence of C. albicans in three media with bottles of 1.5×10^1 CFU/ml organism; S. pyogenes and E. coli with bottles of $1.5x10^2$ CFU/ml were observed presenting reliable blood culture media and showed no different quality.

Table 3. Number of culture positive patients with and without history of antibiotic use.

History of Antibiotic	Positive Culture	
	Used	Not used
Hospital Lab Monophasic	1/49	1/57
Referrence Lab Monophasic	1/49	2/57
Referrence Lab Biphasic	1/49	2/57

There was no statistical difference in number of positive blood cultures of two groups of patients with and without history of antibiotic use by Fisher Exact Test.

E. coli was isolated from blood of a patient with pyelonephritis compatible with clinic. S. epidermidis was isolated from one patient with mortal neutropenia suffering pyelonephritis incompatible with clinic, and the other one with pneumonia recovered with antibiotics ineffective on S. epidermidis representing false positive results due to contamination.

DISCUSSION

Mono and Biphasic Media in Referrence Laboratory had equal quality in growing microorganisms (3 positive blood cultures out of 106 samples : <3%) which were not compatible with other studies showing more effective biphasic media [1, 2, 5, 6] especially, in some slow growing bacteria as Brucella [9] and 1.9% false positive cultures due to contamination (2 positive culture of S. epidermidis from 106 samples) was lower than other studies as 5% [10] and 2-3% positive cultures has significant difference with other observations as 30-50% with 10-20 ml sample volume [3] even to 80% in other studies [1]. Another study in hospitals of Sari (a city in the north of Iran) showed 43.4% positive cultures and 21.7% contamination [11].

As used Mono and Biphasic Media were standard and had equal quality with no difference in isolating bacteria and biphasic media had no priority to monophasic media to increase positive samples, the laboratorial processing factor could not be a cause of negative cultures in this study, while it was expected to have more positive cultures in biphasic media, because there is no need for sub-culturing which decreases false negative and false positive results due to nonstandard conditions. [8]

There was no significant statistical difference in results of monophasic media of reference and hospital laboratories, and quality of two labs was equal and negative results relies on two steps before laboratorial processing including sampling and transport.

Sample volume has an important role. In this study 5 ml blood sample was used in 20 ml media with fraction of 1/4 of culture media

volume, which can be an important factor in decreasing positive cultures, while in other studies sample volume was 10-20 ml with more positive results[1, 3, 5].

Low rate of contamination (2 positive blood cultures of S. epidermidis in 106 samples) in this study as 1.9% with standard culture media represents errors in sampling [12] and transport ; and causing false negative results for contamination in comparison to other surveys as 5%, too [10].

History of antibiotic use had no effect on results in contrast to other observations [1, 3, and 5] of course our results cannot be really interpreted because of small sample size.

It represents that increasing blood sample and culture media volume along with better education and control of standard sampling can results in true positive cultures which can be studied in other surveys, also it is not advised to use biphasic media instead of monophasic media to increase detection of usual bacteria.

ACKNOWLEGEMENTS

This work was supported by Medical Faculty of Shahid Beheshti Medical University and with helps of Bu –Ali Hospital Laboratory staff.

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