

Bacterial Agents Isolated from Blood Cultures of Patients Referred to Tabriz Imam Reza Hospital in 2010

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ABSTRACT

Bacteremias are recognized globally as a major cause of morbidity and mortality in hospitalized patients. Since bacteremia is a potentially life-threatening condition, appropriate examination and definition of the causative pathogen is crucial. The aim of this study was to survey of Bacterial agents isolated from blood cultures of patients referred to Tabriz Imam Reza Hospital. This descriptive study - cross over records and information in Tabriz Imam Reza Hospital was conducted. Patients that referred to this hospital investigate by a physician for blood culture testing in the 12-month period. Methods of this study include; patient's records examination and data on blood culture results were obtained. In the 12-month period, the number of 1339 blood samples had been referred to this center. In total 180 cases (13.44%) contamination was observed. Highest percentage of infection associated with *Staphylococcus aureus* 56 cases (4.18%). Many other bacteria including *E. coli*, 29 cases (2.16%), *Staphylococcus epidermis*, 26 cases (1.94%), *Enterobacter aeruginosa*, 52 cases (3.88%), *Pseudomonas*, 8 cases (0.59%), alpha-hemolytic *Streptococcus*, 7 cases (0.52%), *Streptococcus pneumoniae*, 2 cases (0.14%), respectively. Obtained data showed that prevalence rate in Tabriz hospitalized patients is high and must be taken measures in this field.

Key words: bacterial agents, blood samples, culture Medias, Tabriz.

Introduction

Bacteremias are recognized globally as a major cause of morbidity and mortality in hospitalized patients [2]. Furthermore, bacteremias most commonly appear concomitantly with other serious infections such as urinary tract infections, endocarditis, kidney and bowel infections. Bacteria may also occur in the blood after trauma or surgery (especially of visceral organs), after suppression of the immunological system, and upon obstetrical complications [10]. Since bacteremia is a potentially life-threatening condition, appropriate examination and definition of the causative pathogen is crucial. The finding of bacteria in blood smears must be interpreted carefully. Thorough examination of

peripheral blood smears may be very valuable in the early diagnosis of bacteremia [36], and appropriate information on the causative agent should be available to the clinician as soon as possible [17]. A few methods for rapid identification of pathogenic bacteria in blood have been described in earlier studies. These were based on a fluorescence in situ hybridization assay [15,28,32] using fluorescent-labeled oligonucleotide probes [17], analysis of the 16S rRNA gene [6,35], direct identification of bacteria using the Vitek system [7] or the polymerase chain reaction (PCR) [19,20,23]. Most of the methods described to date are unspecific and use 16S "universal" primers [31]. Prevalent bacteria that might cause bacteremia have been reported. These include: coagulase-negative staphylococci (CoNS),

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mainly *Staphylococcus epidermidis*, which can cause bacteremia despite its existence in the normal human microflora [12]. Bacteremia caused by *Pseudomonas aeruginosa* [18] and *Acinetobacter baumannii* [3] is associated with high mortality rates, especially in hospitalized patients. *Staphylococcus aureus* and *Klebsiella pneumoniae* have long been known as agents that can cause nosocomial bacteremia [5]. Staphylococcal species are commonly isolated from blood cultures. Among these species, *Staphylococcus aureus*, especially methicillin-resistant *S. aureus* is the most virulent and is considered among the most important pathogens isolated from blood cultures in hospitals around the world [8]. *Staphylococcus epidermidis* and other coagulase-negative staphylococci (CoNSs) are common members of the normal flora of skin and recognized as frequent contaminants in blood cultures [1,16,33]. However, the incidence of infections caused by CoNS has increased throughout the world [11,22]. Blood cultures are considered to be one of the most significant sample types that microbiology laboratory process [13]. It was found that no more than 5 days of incubation were required for continuously monitoring blood-culture systems [29]. Recently, several studies have reported that 3 or 4 days of incubation may be sufficient for some continuously monitoring automated blood-culture systems [4,9,14,30]. There have been controversies about suitable incubation periods for blood-culture bottles in regard to recovery rates of bacteria, and labor-and/or cost-effectiveness. It is also unclear whether facultative anaerobic bacteria grow preferentially in anaerobic blood-culture (AN) bottles or in aerobic blood-culture (AE) bottles [26].

Materials and methods

This descriptive study - cross over records and information in Tabriz Imam Reza Hospital was conducted. Patients that referred to this hospital investigate by a physician for blood culture testing in the 12-month period. Methods of this study include; patient's records examination and data on blood culture results were obtained. Blood samples were collected from hospitalized patients at a medical center which is located in Tabriz Imam Reza Hospital. Each blood sample was divided and transferred into two bottles, one containing medium for aerobic growth and the other for anaerobic growth. Aliquots were taken for PCR identification immediately after the blood sample was transferred to the bottle. Cultures were incubated in the BacT/ALERT microbial detection system (BioMérieux, Durham, NC, USA). Cultures found to contain bacterial growth in the bottles are regarded as positive cultures and were further processed for identification by conventional microbial methods,

including Gram staining, seeding in different kinds of growth medium, and using an API test system (BioMérieux, Marcy l'Etoile, France). Aliquots from the positive blood samples were taken for PCR identification. Samples in which no bacterial growth is detected are discarded after 7 days of incubation and are regarded as negative. After PCR Sequence analysis was performed at microbiology laboratory (Tabriz, Iran, Islamic Azad University), in order to confirm the sequence authenticity of the PCR products. Moreover, the product sequences were checked for their specificity using nucleotide-nucleotide NCBI Blast (Blastn) for all microorganisms and the 'in silico simulation of molecular biology experiments' software.

Results:

In the 12-month period, the number of 1339 blood samples had been referred to this center. In total 180 cases (13.44%) contamination was observed. Highest percentage of infection associated with *Staphylococcus aureus* 56 cases (4.18%). Many other bacteria including *E. coli*, 29 cases (2.16%), *Staphylococcus epidermidis*, 26 cases (1.94%), *Enterobacter aeruginosa*, 52 cases (3.88%), *Pseudomonas*, 8 cases (0.59%), alpha-hemolytic *Streptococcus*, 7 cases (0.52%), *Streptococcus pneumoniae*, 2 cases (0.14%), respectively (Table 1).

Discussion:

The findings of this study indicate the prevalence of bacterial agents in patients referred to Imam Reza Hospital, Tabriz in 2010. The highest percentage of infection in patients referred from the date 2009.10.25 to 2009.11.21 with 27 cases (2.01%). The lowest rate of infection since 2009.5.23 to 2009.5.18 with 6 cases (0.44%) have been reported. In Iran reports on the prevalence of bacterial agents showed high rates of infection is bacterial factors. Including the prevalence of these factors in Hamedan in 1998 and 1999 (16.8%), in Sanandaj in 2004 (17.6%), Tehrans Shahid Beheshti Hospital in 1992 to 1996 (29.2%) have been reported. According to available figures regarding the prevalence of bacterial agents in different parts of the country, is determined that the prevalence of bacterial agents in patients referred to Tabriz Imam Reza Hospital in 2010 is higher than other studies. Bacterial agents can easily be transferred from person to person or through contaminated water and food. Fortunately, the prevalence that obtained from patients in this study undergoing lower than the figures reported in similar studies in the country. But whatever more comprehensive health measures should be performs to reducing transmission and pathogenicity of these bacterial factors. In one other study that carried out

Table 1: Types of isolated bacteria from blood cultures and PCR.

Genus	Count (percent)
Staphylococcus aureus	56 (31.12)
Enterobacter aeruginosa	52 (28.88)
Escherichia coli	29 (16.12)
Staphylococcus epidermis	26 (14.44)
Pseudomonas	8 (4.44)
alpha-hemolytic Streptococcus	7 (3.88)
Streptococcus pneumoniae	2 (1.12)
Total	180 (100%)

by Lindholm and Sarkkinen, 2004; Lyytikäinen et al., 2002 revealed that a total of 3071 positive blood cultures were collected during study, only 891 positive blood cultures (29.8%) resembled a significant finding that matches medical findings. This percentage of positive significant blood cultures is about the same during some decades and was shown in previous works (25,27). The other positive blood cultures (69.2%) contained external contaminants, especially coagulase-negative staphylococci (CoNS) and cultures that appeared only once in the patient without any relative symptoms caused due to bacteremia. *S. epidermidis* was found in 168 blood cultures (18.8% of the significant samples). A total of 174 blood cultures were found to be positive and significant (19.5%) for *S. aureus*. *K. pneumoniae* rods were found in 164 significant positive blood cultures (18.4%). *P. aeruginosa* and *A. baumannii* grew in 108 and 102 positive cultures, respectively. Various microorganisms, including yeasts, were found in small numbers in the remaining 19.8% significant blood cultures. This screening was carried out using traditional microbial detection methods. It should be mentioned that the prevalent species isolated in blood cultures in other countries may be different. For example, in Finland, *E. coli*, CoNS, *S. aureus*, *S. pneumoniae*, and *Enterococcus faecalis* are the most prevalent [21,24]. Also in other study that accomplished by Takashi Saito et al., 2003 demonstrated that of 6229 samples, 731 (11.7%) were positive for bacteria or yeast [34]. With comparison of different results revealed that contamination rate is high in Tabriz area and must be taken measures in this field.

References

- Bates, D.W., L. Goldman, T.H. Lee, 1991. Contaminant blood cultures and resource utilization. The true consequences of false-positive results. *JAMA*, 265: 365-369.
- Bearman, G.M.L., R.P. Wenzel, 2005. Bacteremias: a leading cause of death. *Arch. Med. Res.*, 36: 646-659.
- Bergogne-Berezin, E., K.J. Towner, 1996. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical and epidemiological features. *Clin. Microbiol. Rev.*, 9: 148-165.

- Bourbeau, P.P., J.K. Pohlman, 2001. Three days of incubation may be sufficient for routine blood cultures with BacT/Alert FAN blood culture bottles. *J Clin Microbiol.*, 39: 2079-82.
- Cağatay, A.A., P.E. Ozcan, L. Gulec, N. Ince, S. Tugrul, H. Ozsut, N. Cakar, F. Esen, H. Eraksoy, S. Calangu, 2007. Risk factors for mortality of nosocomial bacteraemia in intensive care units. *Med. Princ. Pract.*, 16: 187-192.
- Christensen, J.E., J.A. Stencil, K.D. Reed, 2003. Rapid identification of bacteria from positive blood cultures by terminal restriction fragment length polymorphism profile analysis of the 16S rRNA gene. *J. Clin. Microbiol.*, 41: 3790-3800.
- De Cueto, M., E. Ceballos, L. Martinez-Martinez, E.J. Perea, A. Pascual, 2004. Use of positive blood cultures for direct identification and susceptibility testing with the Vitek 2 system. *J. Clin. Microbiol.*, 42: 3734-3738.
- Deresinski, S., 2005. Methicillin-resistant *Staphylococcus aureus*: an evolutionary, epidemiologic, and therapeutic odyssey. *Clin Infect Dis.*, 40: 562-573.
- Doern, G.V., A.B. Brueggemann, W.M. Dunne, S.G. Jenkins, D.C. Halstead, J.C. McLaughlin, 1997. Four-day incubation period for blood culture bottles processed with the Difco ESP blood culture system. *J. Clin. Microbiol.*, 35: 1290-2.
- Edwards, J.D., 1993. Management of septic shock. *Br. Med. J.*, 306: 1661-1664.
- Freney, J., Y. Brun, M. Bes, H. Meugnier, F. Grimont, P.A. Grimont, C. Nervi, J. Fleurette, 1988. *Staphylococcus lugdunensis* sp. nov., two species from human clinical specimens. *Int J Syst Bacteriol.*, 38: 168-172.
- Fujita, S.I., Y. Senda, T. Iwagami, T. Hashimoto, 2005. Rapid identification of staphylococcal strains from positive-testing blood culture bottles by internal transcribed spacer PCR followed by microchip gel electrophoresis. *J. Clin. Microbiol.*, 43: 1149-1157.
- Gill, V.J., D.P. Fedorko, F.G. Witebsky, 2000. The clinician and the microbiology laboratory. In: Mandell GL, Bennett JE, Dolin R, editors. *Mandell, Douglas, and Bennett's principles and practice of infectious diseases*. 5th Ed. Philadelphia: Churchill Livingstone, pp: 184-221.

14. Han, X.Y., A.L. Truant, 1999. The detection of positive blood cultures by the AccuMed ESP-384 system: the clinical significance of 3-day testing. *Diagn Microbiol Infect Dis.*, 33: 1-6.
15. Hartmann, H., H. Stender, A. Schafer, I.B. Autenrieth, V.A.J. Kempf, 2005. Rapid identification of *Staphylococcus aureus* in blood cultures by a combination of fluorescence in situ hybridization using peptide nucleic acid probes and flow cytometry. *J. Clin. Microbiol.*, 43: 4855-4857.
16. Herwaldt, L.A., M. Geiss, C. Kao, M.A. Pfaller, 1996. The positive predictive value of isolating coagulase-negative staphylococci from blood cultures. *Clin Infect Dis.*, 22: 14-20.
17. Jansen, G.J., M. Mooibroek, J. Idema, H.J.M. Harmsen, G.W. Welling, J.E. Degener, 2000. Rapid identification of bacteria in blood cultures by using fluorescently labeled oligonucleotide probes. *J. Clin. Microbiol.*, 38: 814-817.
18. Kang, C.I., S.H. Kim, H.B. Kim, S.W. Park, Y.J. Choe, M.D. Oh, E.C. Kim, K.W. Choe, 2003. *Pseudomonas aeruginosa* bacteremia: risk factors for mortality and influence of delayed receipt of effective antimicrobial therapy on clinical outcome. *Clin. Infect. Dis.*, 37: 745-751.
19. Kurupati, P., C. Chow, G. Kumarasinghe, C.L. Poh, 2004. Rapid detection of *Klebsiella pneumoniae* from blood culture bottles by real-time PCR. *J. Clin. Microbiol.*, 42: 1337-1340.
20. Laforgia, N., B. Coppola, R. Carbone, A. Grassi, A. Mautone, A. Iolascon, 1997. Rapid detection of neonatal sepsis using polymerase chain reaction. *Acta Paediatr.*, 86: 1097-1099.
21. Lindholm, L., H. Sarkkinen, 2004. Direct identification of gram-positive cocci from routine blood cultures by using AccuProbe tests. *J. Clin. Microbiol.*, 42: 5609-5613.
22. Loiez, C., F. Wallet, P. Pischedda, E. Renaux, E. Senneville, N. Mehdi, R.J. Courcol, 2007. First case of osteomyelitis caused by "Staphylococcus pettenkoferi". *J Clin Microbiol.*, 45: 1069-1071.
23. Louie, L., J. Goodfellow, P. Mathieu, A. Glatt, M. Louie, A.E. Simor, 2002. Rapid detection of methicillin-resistant staphylococci from blood culture bottles by using a multiplex PCR assay. *J. Clin. Microbiol.*, 40: 2786-2790.
24. Lyytikäinen, O., J. Lumio, H. Sarkkinen, E. Kolho, A. Kostiala, P. Ruutu, 2002. Hospital Infection Surveillance Team. Nosocomial bloodstream infections in Finnish hospitals during 1999–2000. *Clin. Infect. Dis.*, 35, E14–E19.
25. Marchaim, D., R. Zaidenstein, T. Lazarovitch, Y. Karpuch, T. Ziv, M. Weinberger, 2008. Epidemiology of bacteremia episodes in a single center: increase in Gram-negative isolates, antibiotics resistance, and patient age. *Eur. J. Clin. Microbiol. Infect. Dis.*, 27: 1045-1051.
26. Murray, P.R., P. Tenover, D. Tenover, 1992. Critical assessment of blood culture techniques: analysis of recovery of obligate and facultative anaerobes, strict aerobic bacteria, and fungi in aerobic and anaerobic blood culture bottles. *J Clin Microbiol.*, 30: 1462–8.
27. Nitzan, Y., M. Maayan, M. Drucker, 1980. Microorganisms isolated from blood and cerebrospinal fluid in a general hospital. *Isr. J. Med. Sci.*, 16: 503-509.
28. Peters, R.P.H., van M.A. Agtmael, Simoons-A.M. Smit, S.A. Danner, 2006. Vandembroucke-Grauls, C.M.J.E., Savelkoul, P.H.M. Rapid identification of pathogens in blood cultures with a modified fluorescence in situ hybridization assay. *J. Clin. Microbiol.*, 44: 4186-4188.
29. Reisner, B.S., G.L. Woods, R.B. Jr Thomson, D.H. Larone, L.S. Garcia, R.Y. Shimizu, 1999. Specimen processing. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover RH, editors. *Manual of clinical microbiology*. 7th Ed. Washington, DC: American Society for Microbiology, pp: 64-104.
30. Reisner, B.S., G.L. Woods, 1999. Times to detection of bacteria and yeasts in BACTEC 9240 blood culture bottles. *J Clin Microbiol.*, 37: 2024-6.
31. Rothman, R.E., M.D. Majmudar, G.D. Kelen, G. Madico, C.A. Gaydos, T. Walker, T.C. Quinn, 2002. Detection of bacteremia in emergency department patients at risk for infective endocarditis using universal 16S rRNA primers in a decontaminated polymerase chain reaction assay. *J. Infect. Dis.*, 186: 1677-1681.
32. Sogaard, M., H. Stender, H.C. Schonheyder, 2005. Direct identification of major blood culture pathogens, including *Pseudomonas aeruginosa* and *Escherichia coli*, by a panel of fluorescence in situ hybridization assays using peptide nucleic acid probes. *J. Clin. Microbiol.*, 43: 1947-1949.
33. Souvenir, D., D.E. Anderson, S. Palpant, H. Mroch, S. Askin, J. Anderson, J. Claridge, J. Eiland, C. Malone, M.W. Garrison, P. Watson, D.M. Campbell, 1998. Blood cultures positive for coagulase-negative staphylococci: antisepsis, pseudobacteremia, and therapy of patients. *J Clin Microbiol.*, 36: 1923-1926.
34. Takashi Saito, Kazuyoshi Senda, Shunji Takakura, Naoko Fujihara, Toyochiro Kudo, Yoshitsugu Inuma, Mitsune Tanimoto, Satoshi Ichiyama, 2003. Detection of bacteria and fungi in BacT/Alert standard blood-culture bottles. *J Infect Chemother.*, 9: 227-232.

35. Turenne, C.Y., E. Witwicki, Hoban, D.J. J.A. Karlowsky, A.M. Kabani, 2000. Rapid identification of bacteria from positive blood cultures by fluorescence-based PCRsingle-strand conformation polymorphism analysis of the 16S rRNA gene. *J. Clin. Microbiol.*, 38: 513-520.
36. Van der Meer, W., J.M.M. Verwiël, C.E.M. Gidding, M. de Metz, M.H. de Keijzer, 2002. Bacteria in blood smears: overwhelming sepsis or trivial contamination. *Acta Haematol.*, 107: 220-223.