http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (3): 422-427, 2008
ISSN 1028-8880

Epidemiology and Antibacterial Susceptibility Patterns of Bloodstream Infections, 2001-2004: An Experience with BACTEC 9240 in Southern Iran

Aziz Japoni, Shohreh Farshad, Abdolvahab Alborzi, Mehdi Kalani,
Nouradin Rafaatpour, Barat Oboodi and Bahman Pourabbas
Prof. Alborzi Clinical Microbiology Research Center,
Shiraz University of Medical Sciences, Shiraz, Fars, Iran

Abstract: This study was conducted to determine the prevalence of bacteria recovered from bloodstream samples by Bactec 9240 at our hospital wards and to evaluate their antibacterial susceptibility patterns. During January 2001 through December 2004, 9407 referred blood samples in Bactec bottles from admitted patients at three main wards, neonates, pediatrics and adults at Nemazee Hospital, affiliated to Shiraz University of Medical Sciences in Shiraz were processed. Positive cultures were purified and identified according to standard methods. Sensitivity of bacteria to different antibiotics was determined by Kirby-Bauer disk diffusion method. *Staphylococcus aureus* 132(25%), *Escherichia coli* 64(12.1%) and *Pseudomonas aeruginosa* 52(9.8%) were the most pathogenic bacteria which were recovered from the blood samples. Pathogenic microorganisms were isolated from blood samples of 305 (57.8%) at pediatrics, from 181 (34.2%) at adults and from 42 (8%) at neonates wards. The highest antibiotics activities against gram positive isolates observed for vancomycin (98.4%), chloromphenicol (86.4%) and ciprofloxacin (77.4%), while in gram negative bacteria imipenem (96.1%), ciprofloxacin (83%) and amikacin (77.9%), were effective antibiotics. Frequency of isolated bacteria at pediatrics compared to adults and neonates wards were approximately two and seven folds high, respectively which indicates special attention should be paid to pediatrics patients both in prevention and treatment aspects. Vancomycin and imipenim are the effective antibiotics and could cover majority of gram positive and negative bacteria. Therefore, combined administrations of these antibiotics seems mandatory for empirical therapy.

Key words: Bactec 9240, bloodstream infections, disk diffusion method, vancomycin, imipenim

INTRODUCTION

Rapid and reliable detection of microorganisms from blood is one of the most critical functions of a diagnostic microbiology laboratory.

The BACTEC 9240 (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) system was established in our center in 1999. This instrument is a continuous monitoring blood culture system that uses internal, fluorescent-CO_2 sensors. It accommodates up to 240 blood culture bottles and serves as an incubator, agitator and detection system. Each bottle contains a fluorescent-CO_2 sensor and the sensors are monitored on a continuous basis (every 10 min). This system is noninvasive and automated blood culture systems with continuous monitoring have introduced technology that reduces the time needed to detect positive blood cultures as well as decreases specimen handling (Nolte *et al*., 1993; Rohner *et al*., 1997). On such a system, computer algorithms analyze the rate and amount of CO_2 increase, which corresponds to an increase in fluorescence, thereby enabling the instrument to recognize a positive culture. When inoculated blood culture bottles are incubated and tested in the BACTEC instrument, microorganisms are detected as they enter the logarithmic phase of growth. Different media have been developed to enhance recovery and detection of positive cultures with this instrument. BACTEC plus aerobic/F and plus anaerobic/F media, which contain mixed resins, have been shown to be superior in their ability to support the growth of positive blood cultures (Rohner *et al*., 1997; Wilson *et al*., 2001). It has been suggested that enhanced recovery in resin-containing BACTEC media is due to lysis of leukocytes and subsequent release of viable, phagocytized microorganisms (Rohner *et al*., 1997). The manufacturer of plus media states that to maximize the

Corresponding Author: Aziz Japoni, Prof. Alborzi Clinical Microbiology Research Center, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz, Iran Tel: +98 711 647 0705 Fax: +98 711 6287071

number of positive blood cultures detected, it is recommended that blind Gram stains and/or subcultures be performed.

The purpose of this study was to report our four year experiences with BACTEC 9240 system regarding the epidemiology of recovered bacteria and to report antibacterial susceptibility patterns of the isolated bacteria. The data generated from this study could help the clinicians to treat patients more appropriately.

MATERIALS AND METHODS

Place of study: This study was conducted during January 2001 to December 2004 at Nemazee Hospital, affiliated to Shiraz University of Medical Sciences, Shiraz, Southern Iran. This hospital is tertiary care facility with 1000 beds and located in Fars Province. Normal or complicated patients from Fars, Hormozgan, Boushehr, Khouzestan and Kohkiloyeh-Boyerahamd provinces are referred daily or admitted in this hospital.

Patients and sampling: Suspicious patients to blood infections which were admitted at three main hospital wards including: neonates, pediatrics and adults were studied. Ten and three milliliter blood samples from adult and pediatric/neonate patients under supervision of specialized physician were taken and inoculated to BACTEC bottles peds plus/F or adult plus aerobic/F aseptically. An indication for patient blood infection was confirmed by the specialized physician in each ward.

Processing of specimens: The bottles were incubated in BACTEC system as recommended by manufacturer for 7 consecutive days. At the end of 7 days, the negative bottles were removed from the instrument and subcultured as mentioned below for positive bottles. During seven day incubation, when system alerts for positive results, 3 to 5 drops of blood culture samples with 1 mL sterile syringe were inoculated on blood and chocolate agars containing 5% whole sheep blood and incubated aerobically overnight. The pure culture were then stained by Gram's method. For data analysis, the following criteria were established. An isolate was defined as an organism recovered from a blood culture bottle. A culture that was detected by BACTEC 9240 and confirmed to be positive by both Gram stain and subculture was considered to be a true positive. A culture that was instrument negative and negative upon terminal subculture was considered to be a true negative. Any culture that was instrument negative but positive upon terminal subculture was considered to be a false negative. Further identification of bacteria in positive cultures was carried out according to standard biochemical tests and subculturing to appropriate media.

Antibacterial susceptibility testing: Sensitivity of identified bacteria to different antibiotics was determined according to standard disk diffusion (Kirby-Bauer) method using Mast Co (Mast Co, Merseyside, UK) or Difco (BBL, USA) disks and was interpreted as recommended by National Committee for Clinical Laboratory Standard (Approved standard M7-A5. Wayne, PA: NCCLS, 2003). *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were used as controls for antibiotic susceptibility determination.

RESULTS

Totally 9407 blood samples in BACTEC bottles have been received and processed. Numbers of received specimen, frequency of culture positive specimens during a four year period screening is shown in Table 1.

Frequency and percent of bacteria isolated from blood samples based on wards are shown in Table 2. Coagulase Negative Staphylococci (CNS), *Bacillus* sp., *Diphthroid* and *Micrococcus* sp. were common contaminants of patients' samples. Therefore, results are reported for all isolated bacteria except for potential contaminants (Table 2). *S. aureus*, *E. coli* and *P. aeruginosa* were the most pathogenic bacteria isolated from the blood samples. The pediatrics with 305 (57.8%), adults with 181 (34.2%) and neonates wards with 42 (8%) were sequential for incidence of the pathogenic microorgamisms. Antibacterial susceptibility patterns of Gram negative and Gram positive bacteria are shown in Table 3 and 4. Vancomycin and ciprofloxacin were the most active antibiotics against gram positive and negative bacteria *in vitro*. Brucellosis as an endemic disease has been observed in some reigns of the country which needs specific antibiotic therapy; therefore, sensitivity of these bacteria to antibiotics is shown separately (Table 5). *Brucella* sp. were highly sensitive to imipenem, rifampin and chloramphenicol.

Table 1: No. of specimens and positive culture isolated from BACTEC 9240 during year 2001-2004

Year	No. of specimens	No. of positive culture (%)
2001	1615	138 (8.5)
2002	2200	156 (7.1)
2003	2229	288 (12.9)
2004	3363	548 (16.2)
Total	9407	1130 (12.1)

Table 2: Frequency of true pathogenic bacteria isolated from patients with BACTEC 9240

Bacteria	Frequency (%) in neonates ward	Frequency (%) in pediatrics ward	Frequency (%) in adults ward	Total frequency (%)
Staphylococcus aureus	11 (26.2)	66 (21.6)	55 (30.4)	132 (25)
E. coli	4 (9.5)	29 (9.5)	31 (17.1)	64 (12.1)
Pseudomonas aeruginosa	4 (9.5)	29 (9.5)	19 (10.5)	52 (9.8)
Enterobacter sp.	8 (19)	25 (8.2)	17 (9.4)	50 (9.4)
Acinetobacter sp.	1 (2.4)	31 (10.2)	8 (4.4)	40 (7.6)
Streptococcus viridans	2 (4.8)	30 (9.8)	2 (1.1)	34 (6.4)
Enterococcus sp.	6 (14.2)	10 (3.3)	13 (7.2)	29 (5.5)
Klebsiella sp.	5 (12)	11 (3.6)	13 (7.2)	29 (5.5)
Brucella sp.	-	16 (5.2)	12 (6.6)	28 (5.3)
Streptococcus pneumoniae	-	19 (6.2)	1 (0.6)	20 (3.8)
Salmonella sp.	-	7 (2.3)	4 (2.2)	11 (2.1)
Haemophilus inflnenzae.	-	8 (2.4)	-	8 (1.5)
**Candida* sp.	-	3 (1)	3 (1.6)	6 (1.2)
Proteus sp.	-	3 (1)	2 (1.1)	5 (0.9)
Oligella sp.	-	4 (1.4)	-	4 (0.7)
Streptococcus sp.	-	3 (0.9)	-	3 (0.6)
Serratia sp.	1 (2.4)	1 (0.3)	1 (0.6)	3 (0.6)
Gram positive anaerobic org	-	2 (0.6)	-	2 (0.4)
Gram negative rod	-	2 (0.6)	-	2 (0.4)
Fusobacter	-	1 (0.3)	-	1 (0.2)
Neissieria menigitidis	-	1 (0.3)	-	1 (0.2)
β hemolytic sterp group B	-	1 (0.3)	-	1 (0.2)
Listeria monocytogrenes	-	1 (0.3)	-	1 (0.2)
Campylobacter sp.	-	1 (0.3)	-	1 (0.2)
Hafnia	-	1 (0.3)	-	1 (0.2)
Total	42 (100)	305 (100)	181 (100)	528 (100)

*: It is fungus isolated by BACTEC system

Table 3: Antibacterial susceptibility patterns of Gram negative bacteria isolated from patients with BACTEC 9240

	Bacteria						
Antibiotics	*E. coli*	*Enterobacter* sp.	*Klebsiella* sp.	*Salmonella* sp.	*Acinetobacter* sp.	*Pseudomonas* sp.	Total
Imipenem							
Sensitive	43 (95.5)	25 (100)	10 (83.3)	19 (100)	ND	ND	97 (96.1)
Resistant	2 (4.5)	0	2 (16.7)	0	-	-	4 (3.9)
Intermediate	0	0	0	0	-	-	0
Ciprofloxacin							
Sensitive	39 (75)	ND	8 (72.7)	17 (100)	25 (83)	33 (89.2)	122 (83)
Resistant	8 (16.8)	-	1 (9.1)	0	5 (17)	4 (10.8)	18 (12.2)
Intermediate	5 (9.6)	-	2 (18.2)	0	-	0	7 (4.8)
Amikacin							
Sensitive	38 (86.4)	32 (84)	5 (71.4)	ND	16 (55)	36 (100)	127 (77.9)
Resistant	4 (9.1)	4 (11)	2 (28.6)	-	21 (41)	0	31 (19)
Intermediate	2 (4.5)	2 (5)	0	-	1 (4)	0	5 (3.1)
Gentamicin							
Sensitive	43 (70.5)	ND	13 (76.5)	16 (89)	20 (54)	27 (75.5)	119 (70)
Resistant	11 (18)	-	4 (23.5)	2 (11)	17 (46)	10 (24.3)	44 (25.9)
Intermediate	7 (11.5)	-	0	0		0	7 (4.1)
Ceftazidime							
Sensitive	35 (76)	17 (44)	8 (72.7)	17 (94)	17 (55)	23 (71.8)	117 (66.1)
Resistant	11 (24)	21 (54)	3 (27.3)	1 (6)	12 (39)	9 (28.2)	57 (32.2)
Intermediate	0	1 (2)	0	0	2 (6)	0	3 (1.7)
Cephalexin							
Sensitive	12 (40)	ND	5 (35.7)	16 (100)	24 (58)	ND	57 (56.5)
Resistant	16 (53.3)	-	4 (28.6)	0	16 (39)	-	36 (35.6)
Intermediate	2 (6.7)	-	5 (35.7)	0	1 (3)	-	8 (7.9)
Cefuroxime							
Sensitive	20 (54)	17 (53)	7 (63.6)	ND	ND	0	44 (42.3)
Resistant	14 (37.9)	14 (44)	2 (18.2)	-	-	24 (100)	54 (51.9)
Intermediate	3 (8.1)	1 (3)	2 (18.2)	-	-	0	6 (5.8)
Co-trimoxazole							
Sensitive	11 (22)	19 (61)	11 (73.7)	7 (78)	18 (62)	2 (6.2)	68 (41)
Resistant	38 (76)	12 (39)	4 (26.6)	1 (11)	11 (38)	30 (93.8)	96 (57.8)
Intermediate	1 (2)	0	0	1 (11)		0	2 (1.2)
Ampicillin							
Sensitive	6 (26)	ND	6 (54.5)	ND	ND	ND	12 (35.3)
Resistant	17 (74)	-	4 (36.4)	-	-	-	21 (63.6)
Intermediate	0	-	1 (9.1)	-	-	-	1 (1.1)

ND: Not Determined; Values in parenthesis show percentage

Table 4: Antibacterial susceptibility patterns of Gram positive bacteria isolated from patients with BACTEC 9240

	Bacteria					
Antibiotics	Coagulase negative *Staphylococci*	*Staphylococcus aureus*	*Streptococcus viridans*	*Streptococcus pneumoniae*	*Enterococcus* sp.	Total
Vancomycin						
Sensitive	439 (99)	127 (100)	31 (97)	19 (100)	18 (78)	634 (98.4)
Resistant	4 (1)	0	1 (3)	0	5 (22)	10 (1.6)
Intermediate	0	0	0	0	0	0
Chloramphenicol						
Sensitive	336 (85.9)	77 (87.5)	25 (93)	13 (81.2)	ND	451 (86.4)
Resistant	46 (11.8)	6 (6.8)	2 (7)	3 (17.8)		57 (10.9)
Intermediate	9 (2.3)	5 (5.7)	0	0		14 (2.7)
Ciprofloxacin						
Sensitive	361 (78.8)	65 (87.3)	13 (62)	8 (100)	13 (54)	460 (77.4)
Resistant	97 (21.2)	17 (20.5)	6 (29)	0	8 (33)	128 (21.5)
Intermediate	0	1 (1.2)	2 (9)	0	3 (13)	6 (1)
Cephalexin						
Sensitive	319 (77.8)	58 (71.6)	ND	13 (100)	ND	390 (77.4)
Resistant	78 (19)	21 (25.9)		0		99 (19.6)
Intermediate	13 (3.3)	2 (2.5)		0		15 (3)
Clindamycin						
Sensitive	274 (55.4)	92 (80.7)	ND	12 (92.3)	ND	378 (66)
Resistant	170 (38.1)	21 (18.4)		1 (7.7)		192 (33.5)
Intermediate	2 (0.5)	1 (0.9)		0		3 (0.5)
Gentamicin						
Sensitive	287 (62.1)	82 (67.8)	6 (21)	2 (13.3)	3 (12)	380 (58.4)
Resistant	169 (36.6)	37 (30.6)	22 (79)	13 (86.7)	20 (80)	261 (40.1)
Intermediate	6 (1.3)	2 (1.6)	0	0	2 (8)	10 (1.5)
Co-trimoxazole						
Sensitive	156 (36.6)	77 (87.5)	6 (22)	4 (25)	11 (42)	254 (41.2)
Resistant	265 (62.2)	41 (34)	21 (78)	10 (62.5)	13 (50)	350 (56.8)
Intermediate	5 (1.2)	3 (2.5)	0	2 (12.5)	2 (8)	12 (2)
Ampicillin						
Sensitive	28 (16)	ND	ND	ND	ND	28 (16)
Resistant	145 (82.8)					145 (82.2)
Intermediate	2 (1.2)					2 (1.1)

ND: Not Determined

Table 5: Antibiotic susceptibility of *Brucella* sp. recovered from BACTEC 9240

Antibiotics	No. of sensitive (%)	No. of resistant (%)	No. of intermediate (%)	Total
Imipenem•	10 (100)	-	-	10
Rifampin•	6 (100)	-	-	6
Chloramphenicol•	5 (100)	-	-	5
Gentamicin	9 (90)	1 (10)	-	10
Ciprofloxacin	9 (90)	1 (10)	-	10
Amikacin	5 (83.3)	1 (16.70	-	6
Ceftriaxone	3 (75)	1 (25)	-	4
Cefotaxime	5 (71.4)	2 (28.6)	-	7
Cefoxitin	2 (66.7)	1 (33.3)	-	3
Ampicillin	4 (66.7)	2 (33.3)	-	6
Co-trimoxazole	5 (55.5)	4 (44.5)	-	9
Cefuroxime®	2 (40)	-	3 (60)	5
Ceftazidime®	2 (33.3)	4 (66.7)	-	6
Cephalexin®	1 (20)	4 (80)	-	5

•: Highly effective antibiotics, respectively: 1-Imipenem, 2-Rifampin, 3-Chloramphenicol. ®: Less effective antibiotics, respectively: 1-Cephalexin, 2-Ceftazidime, 3- Cefuroxime

DISCUSSION

Due to constant evolving antimicrobial resistant patterns which results in present global public health problem, there is the necessity for constant antimicrobial sensitivity surveillance. This will help clinicians provide safe and effective empirical therapies, develop rational prescription programs and make policy decisions and finally assess the effectiveness of all.

During the study, the annual numbers of specimens and positive cultures have been increased rapidly (Table 1). This could happen due to the introduction of this equipped system to the physicians in hospitals, health centers and private sectors in our regain. It is well known that isolation of microorganisms is a golden standard for accurate detection of etiological agents of infectious diseases (Liu *et al*., 2005; Jervoe-Storm *et al*., 2005). Furthermore, early detection of bloodstream infections could prevent implantation of microorganisms into vital organs such as brain, heart or kidneys (Pirker *et al*., 2006; Alp *et al*., 2006; Safdar *et al*., 2005).

The number of positive cultures in the pediatrics ward is high as compared with adults and neonates wards. This is partly due to number of beds in pediatrics ward which admit a wide range of patients with ages ranging from 6 months to 16 years old. Moreover, children at pre or primary school ages may have not received an appropriate care regarding their hygiene as compared with

adults. Besides, neonates' hygiene are continuously supported and monitored by their mothers.

Ampicillin, co-trimoxazole gentamicin and cefuroxime were less active against our isolates. This is in agreement with the reports of other studies (Mamishi *et al*., 2005; El Kholy *et al*., 2003). They showed that due to low activity of these antibiotics against bloodstream infections, it should not be administrated for treatment, prophylactic and empirical purposes of the bloodstream infections. It is documented that due to emerging resistance, the efficacy of antibiotics is decreasing gradually particularly those administrated for bloodstream infections (Cordero *et al*., 1999; Tantracheewathorn *et al*., 2007). These data support the hypothesis that determination of antibiotic sensitivity patterns in periodic intervals is mandatory in each region for choosing appropriate antibiotic therapy (Turnidge, 2003). Overall, present results indicate that imipenem and vancomycine are highly active against Gram negative and positive bacteria. These results are in concordance with other reports (Raja, 2007; Zapantis *et al*., 2005). However, it should not be expected that this activity continues for a longtime, as we observed during the last two years in our hospital (unpublished data) or in other centers (Swoboda *et al*., 2007), Vancomycin Resistant Entrococci (VRE) were emerged in bacteria isolated from the patients with urinary tract infections. These findings suggest that survey of antibiotics sensitivity would be preferable if performed in two years intervals.

One of the advantages of BACTEC system is to recover intracellular fastidious bacteria such as *Brucella* sp. This could help to treat brucella infected patients with appropriate antibiotics such as rifampin or chloramphenicol and cease of administration of inappropriate antibiotics such as cephalosporins. Detection of brucella infection in our regions is epidemiologically valuable especially in rural areas where consumptions of unpasteurized milk and dairy products is still noticeable (Roushan *et al*., 2005; Refai, 2002). Accurate detection and complete treatment of the human brucellosis could reduce frequency of morbidity or chronic infection cases which have clinical and economic benefits (Fallatah *et al*., 2005; Ruiz-Mesa *et al*., 2005; Lim *et al*., 2005). Besides, it is evident that BACTEC 9240 can successfully recover *Brucella melitentsis* in synovial fluid within 5-7 day incubation period (Yagupsky *et al*., 2001).

CONCLUSION

Present findings reveal the etiology of infectious diseases of the blood. The bacteria with high frequency recovered from blood samples at pediatrics ward indicate that special attention should be paid to our pediatrics patients both in prevention and treatment aspects. Vancomycin and imipenem were the most active antibiotics which could cover the majority of gram positive and negative bacteria. Therefore, an administration of the combination of these antibiotics seems mandatory for empirical therapy when an infection with fastidious intracellular bacteria such as *Brucella* sp. is ruled out.

ACKNOWLEDGMENT

This study was financially supported by Professor Alborzi Clinical Microbiology Research Center (grant 80-15).

REFERENCES

Alp, E., D. Esel, O. Yildiz, A. Voss, W. Melcher and M. Doganay, 2006. Genotypic analysis of Acinetobacter bloodstream infection isolates in a Turkish University Hospital. Scand. J. Infect. Dis., 38: 335-340.

Cordero, L., M. Sananes and L.W. Ayers, 1999. Bloodstream infections in a neonatal intensive-care unit: 12 years' experience with an antibiotic control program. Infect. Control Hosp. Epidemiol., 20: 242-246.

El Kholy, A., H. Baseem, G.S. Hall, G.W. Procop and D.L. Longworth, 2003. Antimicrobial resistance in Cairo, Egypt 1999-2000: A survey of five hospitals. J. Antimicrob. Chemother., 51: 625-630.

Fallatah, S.M., A.J. Oduloju, S.N. Al-Dusari and Y.M. Fakunle, 2005. Human brucellosis in Northern Saudi Arabia. Saudi. Med. J., 26: 1562-1566.

Jervoe-Storm, P.M., M. Koltzscher, W. Fal, A. Dorfler and S. Jepsen, 2005. Comparison of culture and real-time PCR for detection and quantification of five putative periodontopathogenic bacteria in subgingival plaque samples. J. Clin. Periodontol., 32: 778-783.

Lim, H.S., Y.S. Min and S.H. Lee, 2005. Investigation of a series of brucellosis cases in Gyeongsangbuk-do during 2003-2004. J. Prev. Med. Pub. Health, 38: 482-488.

Liu, A.Y., M.J. Jiang, Y.P. Yin and J.F. Sun, 2005. Detection of pathogens causing genital ulcer disease by multiplex polymerase chain reaction. Chin. Med. Sci. J., 20: 273-275.

Mamishi, S., B. Pourakbari, M.H. Ashtiani and F.B. Hashemi, 2005. Frequency of isolation and antimicrobial susceptibility of bacteria isolated from bloodstream infections at Children's Medical Center, Tehran, Iran, 1996-2000. Int. J. Antimicrob. Agents, 26: 373-379.

Nolte, F.S., J.M. Williams, R.C. Jerris, J.A. Morello, C.D. Leitch, S. Matushek, L.D. Schwabe, F. Dorigan and F.E. Kocka, 1993. Multicenter clinical evaluation of a continuous monitoring blood culture system using a fluorescent-sensor technology (BACTEC 9240). J. Clin. Microbiol., 31: 552-557.

NCCLS (National Committee for Clinical Laboratory Standards), 2003. Methods for disk antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard M7-A5.Wayne, PA: NCCLS.

Pirker, M.E., E. Colhounand and P. Puri, 2006. Renal scarring in familial vesicoureteral reflux: Is prevention possible? J. Urol., 176: 1842-1846.

Raja, N.S., 2007. Microbiology of diabetic foot infections in a teaching hospital in Malaysia: A retrospective study of 194 cases. J. Microbiol. Immunol. Infect., 40: 39-44.

Refai, M., 2002. Incidence and control of brucellosis in the Near East region. Vet. Microbiol., 90: 81-110.

Rohner, P., B. Pepey and R. Auckenthaler, 1997. Advantage of combining resin with lytic BACTEC blood culture media. J. Clin. Microbiol., 35: 2634-2638.

Roushan, M.R., S.A. Ahmadi, S.M. Gangi, N. Janmohammadi and M.J. Amiri, 2005. Childhood brucellosis in Babol, Iran. Trop. Doct., 35: 229-231.

Ruiz-Mesa, J.D., J. Sanchez-Gonzalez, J.M. Reguera, L. Martin, S. Lopez-Palmero and J.D. Colmenero, 2005. Rose Bengal test: Diagnostic yield and use for the rapid diagnosis of human brucellosis in emergency departments in endemic areas. Clin. Microbiol. Infect., 11: 221-225.

Safdar, N., C.J. Crnich and D.G. Maki, 2005. The pathogenesis of ventilator-associated pneumonia: Its relevance to developing effective strategies for prevention. Respir. Care., 50: 725-741.

Swoboda, S., T. Hoppe-Tichy and H.K. Geiss, 2007. Septic shock due to vancomycin-resistant enterococci infection: 26. Tigecycline Monotherapy. Anaesthesist., 56: 169-174.

Tantracheewathorn, T., N. Vititpatarapak and U. Phumisantiphong, 2007. Epidemiologic study of nosocomial bacterial infection of pediatric patients at BMA Medical College and Vajira Hospital. J. Med. Assoc. Thai., 90: 258-265.

Turnidge, J., 2003. Impact of antibiotic resistance on the treatment of sepsis. Scand. J. Infect. Dis., 35: 677-682.

Wilson, M.L., S. Mirrett, F.T. Meredith, M.P. Weinstein, V. Scotto and L.B. Reller, 2001. Controlled clinical comparison of BACTEC plus anaerobic/F to standard anaerobic/F as the anaerobic companion bottle to plus aerobic/F medium for culturing blood from adults. J. Clin. Microbiol., 39: 983-989.

Yagupsky, P., N. Peled and J. Press, 2001. Use of BACTEC 9240 blood culture system for detection of *Brucella melitensis* in synovial fluid. J. Clin. Microbiol., 39: 738-739.

Zapantis, A., M.K. Lacy, R.T. Horvat, D. Grauer, B.J. Barnes, B. O'Neal and R. Couldry, 2005. Nationwide antibiogram analysis using NCCLS M39-A guidelines. J. Clin. Microbiol., 43 (6): 2629-2634.