Sensitivity of in-house polymerase chain reaction for detecting hepatitis B-DNA in HbsAg positive sera

Amin A. Aqel, DVM, PhD.

Hepatitis B virus (HBV) infection is a global health problem, and more than 350 million people are chronic carriers of the virus.[1,2] The infection is associated with a wide spectrum of clinical manifestations, ranging from acute or fulminant hepatitis to various forms of chronic infection, including asymptomatic carrier, chronic hepatitis, cirrhosis, and hepatocellular carcinoma.[2] Although serological and genotypic classifications of HBV have been well documented,[3] sensitive detection and quantification of HBV genomic DNA seems to constitute a promising method to monitor HBV infections and the efficacy of antiviral treatment.[4] The routine HBV serology includes tests for detecting HBV surface antigen (HbsAg), HBV envelope-antigen (HbeAg), or surface-antibody (anti-Hbs), envelope-antibody (anti-Hbe), and HBV core antibodies (anti-HbcIgG and anti-HbcIgM).

In this study we evaluate primarily the sensitivity and suitability of our in house polymerase chain reaction (PCR) to detect HBV-DNA in patients' serum with different immunological and enzymatic status. One hundred and twenty serum samples included in this study, were collected from deferent private laboratories in Amman, Jordan from December, 2005 to April, 2006 according to the human ethics, approved by the local human ethics committee. All samples were tested for HBV by serological and molecular methods. We used the commercial kit SURASE B-96 (TMB) (General Biological Corp. Hsin Chu, Taiwan), as a general biological enzyme immunoassay (EIA) for the qualitative test of HbsAg and HbeAg status. As a result, 88 samples were diagnosed as HbsAg (+), 20 samples HbsAg-HbeAg (+), and 12 samples were HbsAg (-). For our in-house PCR, HBV-DNA was extracted using DNAzol® BD (Molecular Research Center, Incorporation, Cincinnati, OH, USA) reagents[4] according to the manufacture's instructions, and finally 5 µl of the extracted DNA solution was taken for the amplification. The primers were constructed to target a part of the S-region DNA sequence encoding for the HbsAg. To yield a product of 255 bp, we used [5'CAAGGTATGTTGCCCGTTTG-3'] as forward primer and [5'-AAAGCCCTGCGAACCACTGA-3'] as reverse primer. Polymerase chain reaction was performed for 35 cycles in a DNA thermal cycler (Techne) for 30 seconds at 94°C (denaturation), and for 30 seconds at 55°C (for primer annealing), and for one minute at 72°C (for primer extension). All runs included duplicates of negative water control, positive control, and external standard control. Aliquots of the amplification products were analyzed by agarose gel (2%) electrophoresis, and the products were stained with ethidium bromide and visualized under ultraviolet light. Sixty-six of the 88 HbsAg (+) samples, and all 20 samples of HbsAg-HbeAg (+) revealed PCR products of 255bp. Whereas, none of the 12 HbsAg (-) had any PCR product (**Table 1**). The relationship between HBV antigens status, alanine transaminase (ALT) and serum HBV DNA level were as follows: 66 from 88 of HbsAg (+) samples had moderate ALT levels, all 20 of HbsAg-HbeAg (+) samples had high ALT levels, and 12 HbsAg (-) samples had normal ALT levels. Only the HbsAg-HbeAg (+) group with elevated ALT levels showed the most detectable DNA by using our in-house PCR (**Table 1**).

Our comparison of HBV antigens status and serum HBV-DNA level, confirmed that high HBV-DNA detection are associated with HbsAg and HbeAg (+). Increasing serum ALT means more viral replication in the liver with increasing viral load in the blood.[5] The chance to detect HBV-DNA from sera with positive immunological markers and elevated ALT is high. Seventy-five percent from all HbsAg (+), and 100% of HbsAg, and HbeAg (+) samples were revealed HBV-DNA by our in-house PCR. In some cases, antiviral therapy and very low viral load (below 2500 HBV DNA copies/ml) (data not shown) will impair the successive detection by PCR,[6] and that may explain the failure of our PCR to detect HBV-DNA in 25% of the HbsAg (+) samples. The HbsAg/HbeAg status does not assess patient's viral load and their ability to transmit the disease, whereas, PCR detection of

Table 1 - The relationship between the HBV antigens status, ALT comparison, and serum HBV DNA level.

Antigen status	Number of Samples	Proportion of DNA (+) by PCR (%)	ALT
HbsAg positive	88	66/88 (75)	60-70 U/L
HbeAg/HbsAg positive	20	20/20 (100)	More than 100 U/L
HbsAg negative	12	0/12 (0)	Normal (up to 30 U/L)
Total serum samples	120		

HBV - Hepatitis B virus, DNA - deoxyribonucleic acid, ALT - alanine transaminase, HbsAg - Hepatitis B surface antigen, HbeAg - Hepatitis B envelope antigen, PCR - polymerase chain reaction, U/L - Units per litre

HBV-DNA in blood or other biological samples can assess the viral load and possible disease transmission. Our data indicates that PCR combined with other immunological techniques are the most sensitive methods for HBV-DNA detection. These methods can also be used in the clinical laboratories to detect the disease progression and to help in the treatment follow up. Not all PCR negative results reflect the absence of HBV in patient's serum, in the diagnostic laboratories, it is recommended to perform other molecular methods such as nested, multiplex or real time PCR, southern blot analysis or even using DNA sequencing to achieve higher sensitivity in samples with low viral load and in case any HBV markers are positive. We constructed this simple and rapid PCR method for HBV detection with approximately 3-4 working hours. Small and middle laboratories, which maybe affected by the cost of the other molecular techniques may perform the immunological markers for HBV detection combined with our in-house PCR for rapid and easy survey of samples for HBV.

In conclusion, the HBV-DNA detection by our in-house PCR depends on HBV immunological markers status and patient's liver enzymes elevation.

Acknowledgments. *We thank Mr. Walid Aljalad for his kind help to prepare this manuscript and for Mr. Hamza Nasr for his collection of samples.*

Received 31st October 2007. Accepted 16th February 2008.

From the Department of Medical Technology, Faculty of Allied Medical Sciences, Zarqa Private University, Zarqa, Jordan. Address correspondence and reprint requests to: Assistant Professor Amin A. Aqel, Department of Medical Technology, Zarqa Private University, Faculty of Allied Medical Sciences, PO Box 2000, Zarqa 13110, Jordan. Tel. +962 (5) 3821100 Ext. 1803. E- Mail: aminaq@med.uoa.gr

References

1. Merat SH, Malekzadeh R, Rezvan H, Khatibian M. Hepatitis B in Iran. ***Arch Iranian Med*** 2000; 3: 192-201.
2. Ameen R, Sanad N, Al-Shemmari S, Siddique I, Chowdhury RI, Al-Hamdan S, et al. Prevalence of viral markers among first-time Arab blood donors in Kuwait. ***Transfusion*** 2005; 45: 1973-1980.
3. Kao JH, Wu NH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes and the response to interferon therapy. ***J Hepatol*** 2000; 33: 998-1002.
4. Almeida RP, Cardoso DD. Detection of HBV DNA by nested-PCR in a HBsAg and anti-HBc negative blood bank donor. ***J Clin Virol*** 2006; 36: 231-234.
5. Kuhns MC, Kleinman SH, McNamara AL, Rawal B, Glynn S, Busch MP. Lack of correlation between HBsAg and HBV DNA levels in blood donors who test positive for HBsAg and anti-HBc: implications for future HBV screening policy. ***Transfusion*** 2004; 44: 1332-1339.
6. Dreier J, Kröger M, Diekmann J, Götting C, Kleesiek K. Low-level viraemia of hepatitis B virus in an anti-HBc- and anti-HBs-positive blood donor. ***Transfus Med*** 2004; 14: 97-103.

Microbial yield in febrile sickle cell disease patients with acute painful episode from a University Hospital in the Sultanate of Oman

Asem A. Alghzaly, *MSc, MRCP,*
Salam S. Alkindi, *MD, FRCP(I),*
Anil V. Pathare, *MD, FIMSA.*

Patients with sickle cell disease (SCD) are particularly prone to develop infections. This is due to several factors including functional aspleenia, impaired phagocytic function, or a defect in activation of alternate complement pathway. Before the use of prophylactic penicillin, invasive pneumococcal disease was the most common cause followed by bacteremia due to Haemophilus type b, accounting for 10-25% of episodes.[1] Bacteremia in older patients is more likely due to *Escherichia coli* and other gram-negative organism, in urinary tract infections.[2] Between December 2004 to May 2007, over a period of 2 and half years, we studied SCD patients with acute painful episode, who at the

Table 1 - Positive microbiological cultures in febrile sickle cell disease patients (n=182).

Pt. No	Age/ Gender	H/o Fever	Organism	Remarks
Blood cultures n=4 culture positive episodes				
1	26/F	-1 week	Salmonella species	Bilateral psoas abscesses. Aspirated pus grew same organism.
2	20/F	-1 week	*Klebsiella pneumonia*	
3	20/M	-6 days	Anaerobes/ Bacteroides species	RT. ankle osteomyelitis with aspiration showing same organism.
4	24/F	+1 day	Salmonella species	
Urine cultures n=8				
5	23/F	+ 1 day	*Klebsiella pneumoniae*	
6	27/F	+ 1 day	*Klebsiella pneumoniae*	
7	30/F	+ 1 day	*Coliform bacillus*	
8	18/F	+ 1 day	*Klebsiella pneumoniae*	
9	21/M	+ 3 days	*Klebsiella pneumoniae*	
10	16/F	+ 1 day	*Pseudomonas aeruginosa*	
11	22/F	+ 1 day	*Escherichia coli*	
12	35/F	+ 2 day	*Escherichia coli*	

RT - right, H/o - history of, F - female, M - male

time of admission or later had a temperature of 38^{0}C or more. The aim of this study was to find the incidence and nature of microbiological isolates in SCD patients with acute painful episode. Our SCD management protocol makes it mandatory to culture blood, urine, and throat swabs, before initiating empiric antibiotic therapy for such patients. None of the patients had received antibiotics before admission except for 2 patients who had received antibiotics in other hospitals before being transferred. A total of 182 patients with 249 episodes, 85 in males, and 97 in females, were studied. Their ages ranged from 13 to 48 years with a mean (±SD) of 22.8 ± 6.3 years. Seventeen males and 14 females had 2 admissions each, whereas, 7 males and 6 females had 3 admissions each, and 3 males, and one female had 4 admissions each. Fever was present in 12 (4.8%) episodes at the time of presentation, whereas in the remaining 237 (95.2%) episodes, it occurred thereafter, within a mean (±SD) of 2.2 ± 1.5 days. Amongst a total of 249 admissions, there were 4 (1.6%) positive blood and 8 (3.2%) positive urine cultures with all showing gram-negative organisms. Interestingly, there was no association between positive urine and blood culture.

Acute pain is the presenting symptom of SCD in more than 25% of patients, being the most frequent symptom after the age of 2 years, with over 90% of patients experiencing pain once in their lifetime.[2] Approximately half of the episodes present with objective clinical signs such as fever and other constitutional disturbances. Although acute painful episodes can be precipitated by infections, fever is often present even in the absence of demonstrable infection. In a study from Jamaica, most patients with SCD with fever > 39^{0}C had no evidence of bacterial infection and the fever was assumed to be attributable to viral or atypical organisms.[3] There is now ample evidence indicative of an ongoing inflammatory state (between painful crisis) in SCD involving neutrophil and monocyte activation and an abnormality of cytokine regulated neutrophil function, which may compromise the host defenses against certain microorganisms.[4] This is often mediated by the release of various inflammatory cytokines leading to pyrexia. Our study demonstrated an incidence of microbiologically confirmed blood positive bacterial cultures in only 1.6%, and urine positive bacterial cultures in only 3.2% episodes. Importantly, all the positive blood and urine cultures grew gram-negative organisms, concordant with other studies, which also demonstrated gram-negative organisms in most of them.[1,2,5] Interestingly, most of the patients who grew organisms in blood had been febrile for an average of one week, whereas, all patients who grew organisms in urine did so after admission usually on the first day after becoming febrile (**Table 1**). Secondly, as all patients grew gram-negative organisms, empiric therapy should be broad spectrum, especially with the aim to cover the common gram-negative pathogens, until a definitive sensitivity pattern is available. Thirdly, although there were almost an equal number of males:females (85M:97F) in this study, females predominated in the patients in whom blood were positive, namely in 3/4 (75%), and urine culture were positive in 7/8 (87.5%). In the case of urinary tract infections, it is likely that the underlying anatomy may have been responsible for the high incidence of urine culture positivity, but we are unaware of any gender bias that predisposes to infection. Thus, in managing SCD patients with painful crisis who develops fever, it is imperative to diligently search for an underlying bacteremia in cases with prolonged fever with multiple blood cultures before starting broad spectrum antibiotic prophylaxis, especially with a gram negative cover. In a patient who develops fever after hospitalization, it is usually rewarding to culture urine with appropriate aseptic techniques to optimize the chances of finding the underlying cause for fever. Lastly, inspite of the best efforts, in approximately 95% of these cases, no demonstrable infective cause of infection was found.

Acknowledgments. *We gratefully appreciate the help of the laboratory personnel of the microbiology department at Sultan Qaboos University Hospital. We also thank the hospital administration for the use of hospital material for this study.*

Received 16th November 2005. Accepted 18th February 2008

From the Department of Hematology, Sultan Qaboos University Hospital, Muscat, Oman. Address correspondence and reprint requests to: Asst. Professor Salam Alkindi, Consultant Hematologist and Head, Department of Hematology, College of Medicine and Health Sciences, Sultan Qaboos University, PO Box 35, Muscat 123, Sultanate of Oman. Tel. +968 (2) 4415736. Fax: +968 (2) 4413419. E-mail: sskindi@yahoo.com

References

1. Zarkowsky HS, Gallagher D, Gill FM, Wang WC, Falletta JM, Lande WM, et al. Bacteremia in sickle hemoglobinopathies. ***J Pediatr*** 1986; 109: 579-585.
2. Gill FM, Sleeper LA, Weiner SJ, Brown AK, Bellevue R, Grover R, et al. Clinical events in the first decade in a cohort of infants with sickle cell disease. Cooperative Study of Sickle Cell Disease. ***Blood*** 1995; 86: 776-783.
3. Wierenga KJJ, Hambleton IR, Wilson RM, Alexander H, Serjeant BE, Serjeant GR. Significance of fever in Jamaican patients with homozygous sickle cell disease. ***Arch Dis Child*** 2001; 84: 156-159.
4. Pathare AV, Al-Kindi SS, Daar S, Dennison D. "Cytokines in Sickle Cell Disease". ***Hematology*** 2003; 8: 329-337.
5. Serjeant G. Mortality from sickle cell disease in Africa. ***BMJ*** 2005; 330: 432-433.

A hole of human immunodeficiency virus (HIV/AIDS) and hunger in rural Nigerian communities

Ogboi S. Johnbull, FMLS, MPH.

At the beginning of 1980's, just when it seemed that medical science has tamed the most dangerous microbes, a new infectious disease known as human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) arose to haunt humanity. Over 2 decades, the HIV/AIDS death toll has surpassed many plagues that swept across the world that cannot be forgotten easily. In Sub-Saharan Africa, HIV/AIDS is the leading cause of adult mortality. Almost 30 million people in this area of the world living with HIV/AIDS will have died by the year 2020, in addition to 13.7 million already claimed by the epidemic.[1] More than those affected are women.[1] In Nigeria with a population of 3.8 million already affected with HIV/AIDS, its burden is clearly evident and its effect on health and socioeconomic development especially in the agricultural sector can easily be imagined.[2] Even with the national prevalence reducing from 50-4.4% in 2005,[2] the pandemic continues to cause wide-reaching impact in our rural communities. The HIV/AIDS epidemic is becoming higher in rural communities than in urban cities as the epidemic is maturing, the gap between urban and rural prevalence is becoming narrower. The growing prevalence of the HIV/AIDS epidemic is adding to the escalation of the hunger problem in Africa, especially in rural communities in an alarming way. And if the epidemic continues, we are wondering how one of the millennium development goals, which is poverty eradication, will be eliminated as the agricultural sector in Nigeria relies mostly on the rural areas for agricultural produce. Human immunodeficiency virus/AIDS is dramatically reducing households earning power by killing young adults, the key producers, and earners in the farm as the non-farm economies.[1] Our farm productions in our rural communities are likely to decline as farmers become ill and unable to work. Indeed, the national food supplies in stricken countries like ours may decline; leading to a price rise. Further, expenditure will be diverted away from other uses to the care and treatment of those infected. The effect of HIV/AIDS on households' in our rural communities is that they are not only eating fewer meals and consuming poorer foods, but are investing less in the health of the surviving member. The greatest percentage of farmers in our rural communities are women who have a range of responsibilities associated with feeding the family, fetch water, cooking, and collecting fire wood, all these are compromised as people become ill. The already poor are becoming poorer, and a new groups of poor are being created across our rural communities. Since the rural communities are playing a big host to HIV/AIDS as people with HIV/AIDS are migrating to their communities to die,[3] it is expected that families affected are likely to sell productive assets either to uphold their expenditures on the care of infected household members. Also, access to food by poor afflicted families will decline significantly and good nutrition is essential in reducing the transition from HIV infection to the full effects of AIDS as poorly fed people will succumb much more quickly.

Presently, in our rural communities the expanding number of orphans (0.9% of Nigeria' total population)[2,4] who are returning from the cities and the elderly that do not have any financial support from family members are creating a precarious situation for the elderly, and the orphaned children who lack care, and education (data not available) are likely to be subjected to a lifetime of poverty and hunger. Also, HIV/AIDS is stretching our compassion and our resources to the breaking point and beyond as we are now having a new structure "Orphan-headed household." In our rural communities, HIV/AIDS is spreading at such a high pace and many children are being withdrawn from school and pulled into farming with concerns that the rapid mortality of adults is leaving children without anyone to teach them how to farm in which future generations might lack agricultural skills in farming.[3] What this means is that hungry young people will seek means of earning incomes and many will be pushed into prostitution and other high risk activities, which will turn exacerbate the spread of disease. The effect of HIV/AIDS on our rural communities can result in labor changes leading to shortage of household labor, hired labor due to mortality, lack of cash leading to less land being farmed, under farming, more child labor, and decline in marketed output for crop processors. Also, loss of farm-specific knowledge as a result of premature mortality curtails the period for role modeling and knowledge transfer leading to less appropriate farming practices and the inexperienced needing training. Losses in agricultural productivity will result in lower demands for farm inputs from rural products and subsequent lower demand for farm inputs from the rural non-farm sectors.

Human immunodeficiency virus/AIDS is killing those we rely on to grow crops, and farm produce in our rural communities, and various households, particularly those with multiple adults, may break down as an adult dies or migrates in search of work. As often said, "the cost of prevention will be far less than the cost of ignoring prevention" therefore, technology and resource management in the area of agriculture in our society can not ignore the ways in which HIV/AIDS decimates families, communities in our rural areas of Nigeria, as

there is going to be a dramatic reduction in the size and productivity of the agricultural labor force, loss of knowledge among household and communities.

In conclusion, it is increasingly recognized that the impact of HIV/AIDS in our rural communities is far reaching, affecting individual families, cultures, and values, so the United Nations Food and Agricultural Organization and partners need to consider the resources and needs of a new group of poor people and how to assist the HIV/AIDS - afflicted rural population to mitigate the effects of the disease. Education and outreach need to be adjusted to take account of the effects of the disease on rural indigenous and knowledge systems, where new forms of capacity building are needed to reach the poor and most marginalized.

Received 11th September 2007. Accepted 23rd February 2008.

From the Department of Community Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Address correspondence and reprint requests to: Dr. Ogboi S. Johnbull, Department of Community Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Tel. +234 (802) 3098078. E-mail: ogboijb@yahoo.com

References

1. Health, Dignity, and Development: What will it take? UN Millennium Project Task Force on water, Sanitation, Final Report. Abridged Edition, New York: Stockholm International Water Institute; 2004 April; p. 5-7. Report No.: ISBN 91-974183-2.
2. Federal Ministry of Health. Department of Public Health. National AIDS/STD Control Programme Technical Report. FMOH. April 2006. p. 7-10.
3. Johnbull OS. Rural community care in HIV/AIDS management in Nigeria. ***Medical Education Resource Africa (MERA, African Health)*** 2003; 26: 9-10.
4. Uniting the world against AIDS: Country Situation Analysis. 2008 February 06. Available from: URL: http://www.unaids.org/en/CountryResponses/Countries/nigeria.asp.

Emergence of multi and pan-drug resistance *Acinetobacter baumannii* carrying $bla_{\text{OXA-type}}$-carbapenemase genes among burn patients in Tehran, Iran

Morrovat Taherikalani, *MSc,* ***Gelavizh Etemadi,*** *MPh, MD,* ***Keramat N. Geliani,*** *PhD,* ***Bahram Fatollahzadeh,*** *PhD,* ***Setareh Soroush,*** *MSc,* ***Mohammad M. Feizabadi,*** *PhD.*

Burns provide a suitable site for bacterial multiplication primarily due to loss of skin integrity. Strains of *Acinetobacter baumannii* frequently cause infections in burns units, and display increasing drug resistance to the broad antimicrobial agents.[1] Carbapenems (for example, imipenem and meropenem) have become the drugs of choice against *Acinetobacter* infections in many centers however, they are being compromised by the emergence of carbapenem-hydrolyzing ß-lactamase (carbapenemase) of molecular classes B, and D. The enzymes in class D (OXA enzymes) have emerged as the main carbapenemase in the world.[2] The OXA enzymes (encoded by bla_{OXA} genes) can be sub-classified into 8 distinct subgroups of which OXA-23-like, OXA-24-like, OXA-51-like, and OXA-58 have been identified in *Acinetobacter* spp.[2] As burn victims are particularly susceptible to infections, detection of multi drug resistant (MDR) *A. baumannii* producing OXA-carbapenemase is essential to aid infection control, and to prevent the dissemination of these organisms. The purpose of this study was to determine the prevalence of MDR *A. baumannii* isolated from burns patients at 2 hospitals of Tehran, and to detect bla_{OXA}-type carbapenemase among them.

The prospective study included 42 *A. baumannii*, non-replicative isolates from burn wounds (n=38), and blood samples (n=4) in the burns unit from 2 distinct hospitals of Tehran, Iran. Informed consent was obtained from all patients. All isolates used in this study were cultured from the burn patients. The samples taken from patients who were hospitalized in the wards other than burns unit were excluded from this study. The specimens from out-patients were also excluded. *A. baumannii* was identified using standard laboratory methods.[3] Susceptibility of isolates to 18 different antibiotics was determined using disk diffusion method.[4] Isolates showing an intermediate level of susceptibility were classified as resistant. We defined *A. baumannii* as being MDR when displaying resistance to imipenem, and at least to one antimicrobials to which the organism is historically know to be sensitive. If any *A. baumannii* strains showed resistance to all antibiotics tested, it was defined as pan-drug resistant.

A multiplex polymerase chain reaction (PCR) assay was used to detect 4 subgroups of OXA-carbapenemase genes ($bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$, and $bla_{\text{OXA-58-like}}$) using the primers and conditions described by Woodford et al.[2] The type strains NCTC 13304, NCTC 13302, NCTC 12156, and NCTC 13305 containing the above bla_{OXA} genes were purchased from the Health Protection Agency (London, United Kingdom), and used as positive controls. The susceptibility of isolates to different antibiotics was as follows: colistin 83.3% (n=35),

Disclosure: This project was supported by a grant from Tehran University of Medical Science.

Table 1 - Distribution of OXA likes metalobetalactamse among 24 isolates of Acinetobacter baumanii with MDR, and pan-drug resistant phenotypes isolated from burn patients at 2 hospitals in Tehran.

Positive target genes	MDR (N=24)	Pan-MDR (N=9)
	n (%)	
$bla_{OXA-51-like}$ + $bla_{OXA-23-like}$	3 (12.5)	3 (33.3)
$bla_{OXA-51-like}$ + $bla_{OXA-24-like}$	2 (8.3)	1 (11.1)
$bla_{OXA-51-like}$ + $bla_{OXA-58-like}$	1 (4.1)	0
$bla_{OXA-51-like}$ + $bla_{OXA-23-like}$ + $bla_{OXA-58-like}$	3 (12.5)	3 (33.3)
$bla_{OXA-51-like}$ + $bla_{OXA-23-like}$ + $bla_{OXA-24-like}$ + $bla_{OXA-58-like}$	2 (8.3)	1 (11.1)
$bla_{OXA-51-like}$ + $bla_{OXA-23-like}$ + $bla_{OXA-24-like}$	1 (4.1)	1 (11.1)
$bla_{OXA-51-like}$ (only)	12 (50)	0
Total	**24 (100)**	**9 (100)**

MDR - multi drug resistant

tigecycline 76.1% (n=32), meropenem 42.8% (n=18), imipenem 40.4% (n=17), piperacillin-tazobactam 35.7% (n=15), amikacin 28.5% (n=12), levofloxacin 19% (n=8), ticarcillin-clavulanic acid 14.2% (n=6), ciprofloxacin 14.2% (n=6), gentamicin 11.9% (n=5), ampicillin-sulbactam 9.5% (n=4), tobramycin 9.5% (n=4), netilmicin 9.5% (n=4), ceftriaxone 7.1% (n=3), cefotaxime 7.1% (n=3), ceftazidime 7.1% (n=3). All isolates were resistant to ampicillin and cefotetan. The prevalence of isolates with MDR were 57.1% (n=24) and pan-drug resistance were 21.4% (n=9). Of 9 isolates within pan-drug resistant phenotype, 2 (22.5%) were susceptible to tigecycline and colistin. Colistin was more effective against MDR isolates (70.8%) than tigecycline (62.5%). All isolates were positive for $bla_{OXA-51-like}$ genes. Fifty percent (n=12) of all carbapenem resistance isolates of *A. baumannii* (n=24) including MDR and pan-drug resistance strains possessed at least 2 genes encoding OXA-type enzymes. The co-existence of different $bla_{OXA-types}$ were found in 12 (28.5%) of isolates. Carbapenem resistant isolates containing 3 or 4 carbapenemase genes were also identified (**Table 1**). Our results show that, genes encoding OXA-51-like exists among all *A. baumannii* strains including susceptible (n=18; 42.8%), and resistant strains, however, other $bla_{OXA-type}$ carbapenemase were found only in multi and pan-drug resistant strains. It appears that $bla_{OXA-51-like}$ do not play a major role as a sole source of carbapenemase among the Iranian strains. This may be due to lack of insertion sequence ISAba15. This IS element is located adjacent to the $bla_{OXA-51-like}$, and functions as a promoter for this gene. Isolate containing ISAba1 at the up stream of $bla_{OXA-51-like}$ are resistant to imipenem. In the absence of ISAba1 as a promoter, isolates carrying $bla_{OXA-51-like}$ remain susceptible carbapenems. However, detection of $bla_{OXA-51-like}$ can be used as a simple method for rapid identification of *A. baumannii* in clinical samples.[2,5] With the current distribution of different $bla_{OXA-like}$ genes among the Iranian isolates of *Acinetobacter,* colistin, and tigecycline look to be the choices of treatment. However, neither of them has been permitted for prescription in Iran.

In conclusion, multi and pan-drug resistance isolates of *A. baumannii*, particularly those carrying $bla_{OXA-like}$ genes, constitutes a therapeutic problem, and raises concerns for burn patients. The prevention of resistance lies on judicious use of antibiotics, and adherence to infection control guidelines, particularly hand hygiene. Tigecycline and colistin seem to be good alternatives against MDR *Acinetobacter* in Iran. Thus, correct identification of *A. baumannii*, and careful susceptibility testing prior to antibiotic use may help in the prevention, and treatment of *Acinetobacter* infections.

Further studies are required to determine the exact sequence of $bla_{OXA-like}$ gene$_s$. Typing of isolates by a molecular technique such as pulsed field gel electrophoresis will help to find any epidemiological links between the burn patients.

Acknowledgment. *The authors would like to thank Haudi Dehdashti of Georgetown University for editing the manuscript.*

Received 21st August 2007. Accepted 12th February 2008.

From the Departments of Microbiology (Taherikalani, Fatollahzadeh, Soroush, Feizabadi), School of Medicine and Epidemiology, and Biostatistics (Geliani), School of Public Health, Medical Sciences University of Tehran and the Department of Infectious Diseases (Etemadi), Chamran Hospital, Tehran, Iran. Address correspondence and reprint requests to: Dr. Mohammad Feizabadi, Associate Professor in Microbiology, School of Medicine, Medical Sciences, University of Tehran, Iran. Tel/Fax. +98 (21) 8895810. E-mail: mfeizabadi@tums.ac.ir

References

1. Agnihotri N, Gupta V, Joshi RM. Aerobic bacterial isolates from burn wound infections and their antibiograms-a five-year study. ***Burns*** 2004; 30: 241-243.
2. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. ***Int J Antimicrob Agents*** 2006; 27: 351-353.
3. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC Jr, editors. Color atlas and textbook of diagnostic microbiology. 5th ed. Philadelphia (PA): Lippincott; 1997. p. 253-320.
4. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 15th informational supplement. M100-515. PA: Clinical and Laboratory Standards Institute; 2005.
5. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of Acinetobacter baumannii by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. ***J Clin Microbiol*** 2006; 44: 2974-2976.