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RESEARCH ARTICLE

MULTIDRUG-RESISTANT ACINETOBACTERBAUMANNII IN ADJARA REGION.

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Keyword:-

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Abstract

Background:- Carbapenem resistance in *A. baumannii* is now an emerging issue worldwide. Hospital outbreaks have been described from various geographic areas and this organism has become endemic in some of them. Emergence and spread of multidrug-resistant (MDR) *Acinetobacter baumannii* in nosocomial settings has become a serious concern in clinical practice. *Acinetobacter baumannii* has multidrug-resistant phenotypes. Resistance to broad spectrum β -lactams, aminoglycosides, fluoroquinolones, and carbapenems are observed in this bacteria, which complicate the treatment of this pathogen

Objectives: The current study aimed to identify resistance isolate *Acinetobacter baumannii* from different wards of a teaching hospital in Georgia, Adjara and determine the susceptibility pattern of these bacteria.

Materials and Methods:- Susceptibility profile and identification of the infection *Acinetobacter baumannii* (n=14) isolates collected in different hospital services (2013-2015) were performed by disc diffusion methods according to the CLSI guidelines, and API 20E, respectively. Disk diffusion method was employed to evaluate antimicrobial susceptibility against *CTX-cefotaxime*, *PIP-Piperacilin*, *DOR-Doripenem*, *TIC-Ticarcilin*, *MRP-Meropenem*, *FEP-Cefepim*, *CAZ-Ceftazodome*, *IMI-Imipenem*, *TIC/ACC- Ticarcilin/Clavulonic*, *CIP-ciprofloxacin*, *STX-Sulfonamid+timetri*, *CN-Gentamicin*, *TE-Tetracilin*, *TOB-Tobramicin*, *PIP/TAZ-Piperacilintazobactam*, *NET-Netilmicin*, *CT-Colistin* and *AK-amikacin*. Genes of family's *blaOXA51*, *blaOXA40*, *blaOXA58* and *blaOXA23* group were investigated by PCR. Sequencing was performed using group-specific primers

Results:- Among the 14 isolated *A. baumannii*, Samples cultured from the Sputum (25%), Biological fluid (17%), Most of the isolates (60%) were obtained from intensive care unit (ICU). Isolated *A. baumannii* showed high resistance to the evaluated antibiotics except

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TE-Tetracyclin, which showed only 21.4 % resistance. Also, 78.57% and 100% of the isolates were identified as MDR.

Conclusions:- The result of the current study showed the growing number of nosocomial infections associated with XDR *A. baumannii* causing difficulties in antibiotic therapy. Resistant strains increasingly cause public health problems; therefore, their early detection is essential for healthcare centers.

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Introduction:-

Carbapenem resistance in *A. baumannii* is now an emerging issue worldwide [Krol...2009]. Hospital outbreaks have been described from various geographic areas [Landman...2002] and this organism has become endemic in some of them. Emergence and spread of multidrug-resistant (MDR) *Acinetobacter baumannii* in nosocomial settings has become a serious concern in clinical practice. The organism commonly targets critically ill patients in intensive care units (ICU) and burn wards [Peleg...2008]. There is a wide variety of clinical manifestations of *Acinetobacter baumannii* infections, including hospital-acquired pneumonia, bloodstream infection, urinary tract infection, meningitis and wound infections. Over the last decade, *Acinetobacter baumannii* has become a serious and emerging nosocomial pathogen worldwide [Peleg...2008].

Acinetobacter baumannii has multidrug-resistant phenotypes. Resistance to broad spectrum β -lactams, aminoglycosides, fluoroquinolones, and carbapenems are observed in this bacteria, which complicate the treatment of this pathogen [J Coelho...2004].

Control of multidrug-resistant and extensively drug-resistant *Acinetobacter spp.* infections is an important challenge for clinical microbiologists and physicians. Its ability to survive in hospital environment and its capability to persist for long periods of time on surfaces make it a common cause of healthcare-associated infections and multiple outbreaks [PE Fournier...2006]. The prevalence of *A. baumannii* in healthcare centers has increased around the world [Ahmed...2010; Cisneros...2005].

Nevertheless, the most widespread carbapenemases in *A. baumannii* are class D β -lactamases. Three main acquired carbapenem-hydrolysing class D oxacillinase (CHDL) gene clusters have been identified either in the chromosome or in plasmids of *A. baumannii* strains, represented by the blaOXA-23-, blaOXA-24/40-, and blaOXA-58-like genes [Poirel...2006]. The OXA-type carbapenemases comprise four broad groups: blaOXA-23-like, blaOXA-40-like, blaOXA-58-like and an intrinsic blaOXA-51-like [Tsakris...2006; Cicek...2014; Kusradze...2011].

Nosocomial outbreaks of imipenem (IPM)-resistant *A. baumannii* producing these OXA enzymes have been reported worldwide: OXA-24-like (OXA-24, OXA-25, OXA-26 and OXA-40) were found in Spain, Belgium, Portugal, Czech Republic, France and the USA; OXA-23-like (OXA-23, OXA-27 and OXA-49) were identified from Europe, Singapore, China, Brazil, Australia, USA, Algeria, Egypt, Libya, South Africa, Thailand, Tunisia, South Korea, Colombia, Iraq and French Polynesia; and OXA-58-like were identified in France, Spain, Belgium, Turkey, Italy, Austria, Greece, the UK, Argentina, Australia, the USA, Kuwait and Pakistan [Peleg...2008; Mugnier...2010]. blaOXA-51-like genes are endogenous and specific to *A. baumannii*. [Woodford...2006; Brown...2005]

Objectives:-

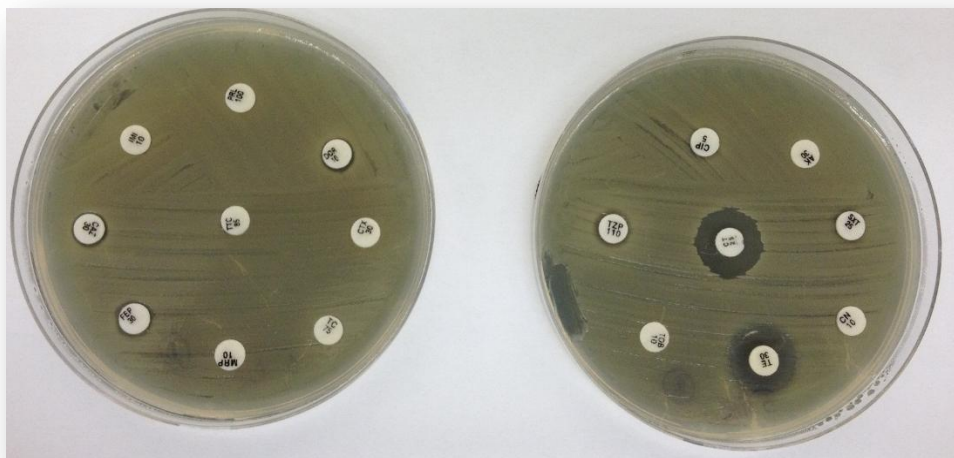
The current study aimed to identify *Acinetobacter baumannii* by molecular method and determine its separation among different wards in hospital and determine the antimicrobial patterns of these bacteria.

Materials and Methods:-

Antimicrobial susceptibility testing:-

Disk diffusion method was performed to test the susceptibility of *Acinetobacter baumannii* isolates to common antibiotics on Mueller-Hinton agar, with an inoculum equal to 0.5 McFarland turbidity according to CLSI [16]. The plates were incubated at 37°C for 18-24 hrs. And the inhibition zone diameters around the antibiotic discs were measured. There were samples of sputum and biological fluids. All isolates were examined for the antibiotic

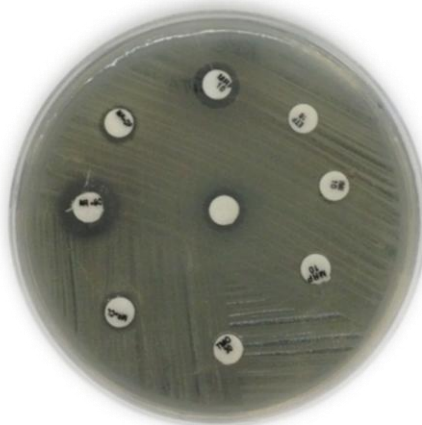
resistance of the following antibiotics: *CTX-cefotaxime*, *PIP-Piperacilin*, *DOR-Doripenem*, *TIC-Ticarcilin*, *MRP-Meropenem*, *FEP-Cefepim*, *CAZ-Ceftazodom*, *IMI-Imipenem*, *TIC/ACC- Ticarcilin/Clavulonic*, *CIP-ciprofloxacin*, *STX-Sulfonamid+timetri*, *CN-Gentamicin*, *TE-Tetracilin*, *TOB-Tobramicin*, *PIP/TAZ-Piperacilintazobactam*, *NET-Netilmicin*, *CT-Colistin* and *AK-amikacin*. were placed around an Ticarcilin-clavulanic acid disc (85 mg) at interdisc distances (centre to centre) of 20 mm on Muller-Hinton agar inoculated by bacterial suspension equal to 0.5 McFarland. (Picture 1)



Picture 1:-*Acinetobacterbaumanniii*-Antimicrobial susceptibility testing.

Imipenem-EDTA synergy test:-

EDTA(ethylene-diamine-tetraaceticacid) is a polyaminocarboxylicacid that binds metalions like zinc and can in activate the metallo-beta-lactamases. Therefore, it is used for the phenotypic detection of MBL production in clinical isolates (Pitout, 2007)Picture 2.



Picture 2:- EDTA synergy test-*Acinetobacterbaumanniii*..

PCR of Blaoxa-51-like Gene:-

PCR- Polymerase chain reaction technique has been used of *blaOXA51*, *blaOXA40*, *blaOXA58* and *blaOXA23* Genes to confirm the species of *A. baumannii*, genomic DNA of all *A. baumannii* isolates, PCR was conducted to identify *blaOXA51* genes, which was endogenous to *A. baumannii*. With specific forward and reverse primers. Table 1.

The PCR conditions were as follows: initial denaturation at 95°C for five minutes followed by 35 cycles of 94°C for 30 seconds, 52°C for 30 seconds, and 72°C for 45 seconds and then 72°C for 10 minutes. Reactions were performed with 2 µl DNA template.. PCR products were analyzed by electrophoresis on 1.2% agarose gel in a tris-borate-

EDTA buffer (TBE) buffer at 85 volts. PCR amplicon size was calculated by comparison to molecular weight size marker (1000 bp DNA ladder). Then the PCR products were visualized under UV light [Safar...2013].

Table 1:-Primers of *Acinetobacterbaumanniii*

Gene	Primers (5'- 3')	Condition PCR
<i>bla_{OXA}-23</i>	5'-ATGAATAAATATTTTACTTGC-3' 5'- TTAAATAATATTTCAGCTGTTT-3'	Initialdenaturationat94°C7min;denaturation at94°C-40s, annealingat57°C-40s elongationat72°C-1m, repeated for 30 cycles; Finalextensionat72°C-7 minutes
<i>bla_{OXA}-40</i>	5'- ATGAAAAAATTTTATACTTCC-3' 5'- GGTCTACAKCCMWTCCTCCA-3'	
<i>bla_{OXA}-51</i>	5'- ACAGAARTATTTAAGTGGG-3' 5'- GGTCTACAKCCMWTCCTCCA-3'	
<i>bla_{OXA}-58</i>	5'- ATGAAATTATTAATAAATATTGAGTTTAG-3' 5'- TTATAAATAATGAAAAACACCCAAC-3'	
	5'- AACCACACGATGTGGGTAGC-3'	

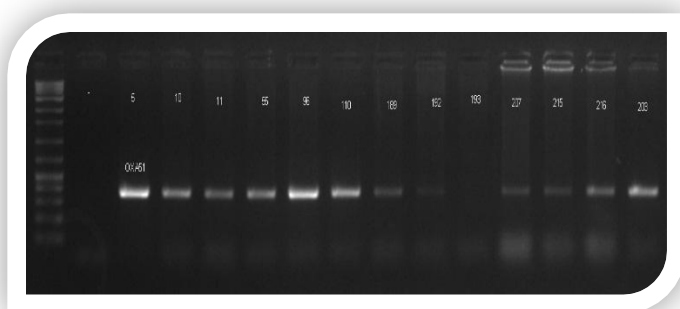


Figure 1. PCRamplification_-negativecontrol,+1,+5-positivecontrolof blaOXA51 geneof *Acinetobacter baumannii*isolates.

All the samples of *Acinetobacter baumannii* contains carbapenem bla_{oxa} 51 gene. These data are similar to those conducted worldwide research [Woodford ... 2006; Brown ... 2005], including studies conducted in Georgia [Kusradze ... 2011].The sample of a wounded soldier during the war in Iraq in 2007, containing bla_{oxa} 51 gene,as well as bla_{oxa} 23 gene, has been described in the article written by Kusradze. The sample of phlegm, taken by a wounded soldier being treated at the military hospital of Gori during the Georgian -Russian war in 2008, was studied in 2009. The sample was considered nosocomial, and it contained bla_{oxa}24 gene and as well as bla_{oxa}51 gene, but as the result of our study, conducted in some hospitals in Adjara in 2013-2015, the samples contained bla_{oxa}51 and bla_{oxa}40 genes. (Figure 1, Table 1)Like data of Kusradze, bla_{OXA}-58 was not detected in any isolates in this study.

Table 1. *Acinetobacterbaumannii* –Sample which contains bla_{oxa}51 and bla_{oxa}40

Sample	Oxa51	Oxa40
110	oxa51	
10	oxa51	oxa40
55	oxa51	oxa40
192	oxa51	oxa40
5	oxa51	oxa40
119		
191		
193		
96	oxa51	oxa40
189	oxa51	
11	oxa51	oxa40
207	oxa51	oxa40
215	oxa51	oxa40
216	oxa51	oxa40
203-H	oxa51	oxa40

Results:-

Fourteen *Acinetobacter baumannii* producing **blaOXA51**, **blaOXA40**, **blaOXA58** and **blaOXA23** gene isolates were detected in different biological samples, namely in sputum (n=10), blood (n=1) and abdominal fluid (n=3), collected in different hospital services. The infection isolates showed an extended resistance profile to aminoglycosides, fluoroquinolones and tetracycline. Isolates showed specific amplification for **blaOXA51**, **blaOXA40**, families. As the diagram shows all sample was resistance to the antibiotics **CTX-cefotaxime**, **PRL-Piperacilin**, **TIC-Ticarcilin**, **CAZ-Ceftazodome**, **CT-Colistin**, **TIC/ACC- Ticarcilin/Clavulonic**, **tazobactam**,,. Also quite high rate of resistance to antibiotics **STX-Sulfonamid+timetri**, **CIP-ciprofloxacin**, **CN-Gentamicin**,, **TOB-Tobramicin** **PIP/TAZ-Piperacilin**, **NET-Netilmicin**, and **AK-amikacin** **DOR-Doripenem**, **MRP-Meropenem**, **FEP-Cefepim**, **IMI-Imipenem**,. Relatively sensitive antibiotic was **TE-Tetracilin**.

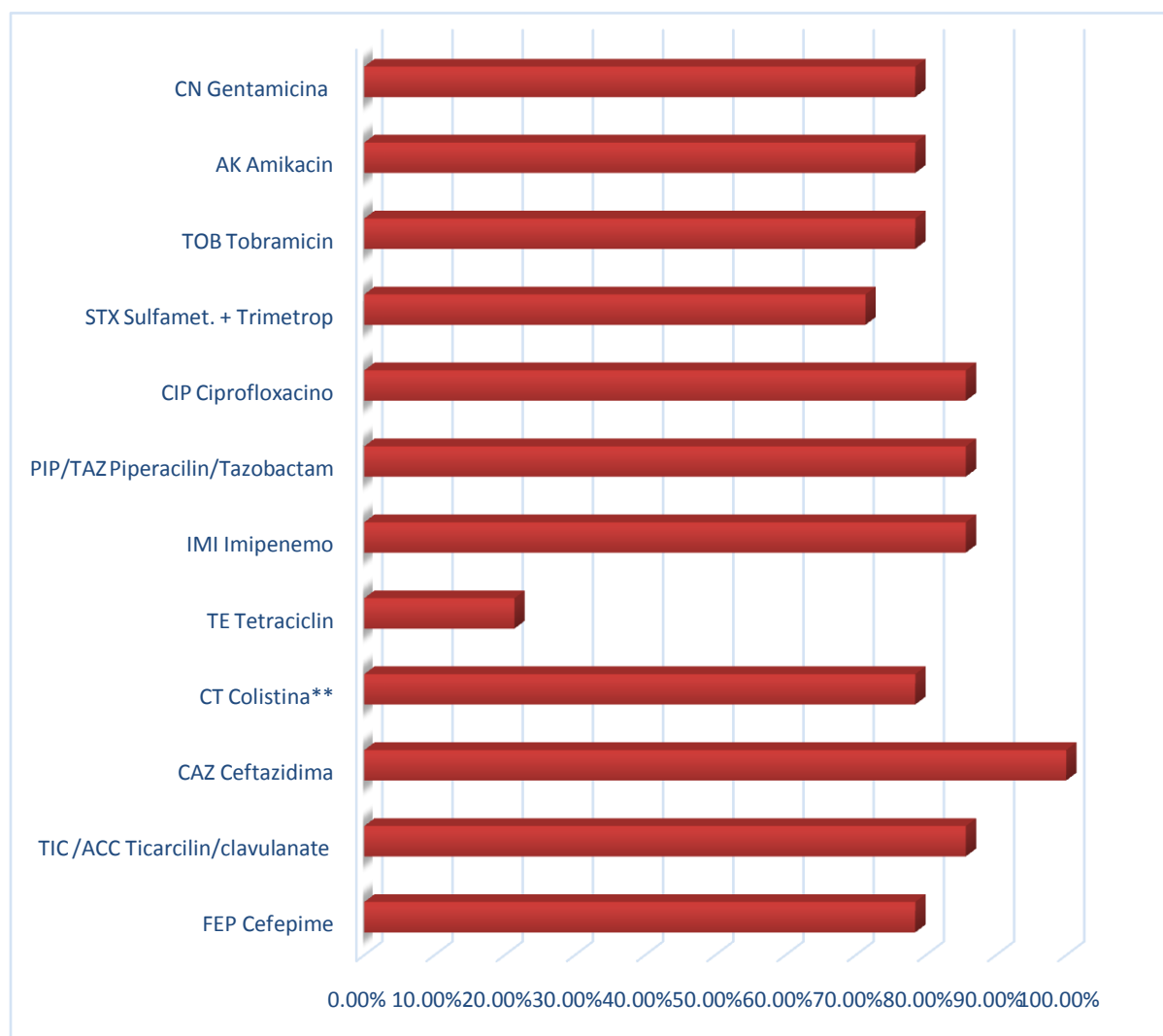


Figure 2 . Profile of Antibiotic Resistance *Acinetobacter Baumannii*

Acinetobacter baumannii showed a high resistance to almost all antibiotics. As the diagram shows the highest resistance was revealed towards **ceftazidim**, it was **100%**, towards **ciprofloxacin**, **piperacillin**, **tazobactam**, **imipenem** and **ticarcilin / clavulanic** -**90%**, towards **gentamicin**, **amikacin**, **tobramycin**, **colistin** and **cefepim** - **80%**. It should be noted that our study was different from the research conducted in Georgia. In particular, according to the article „**Molecular detection of OXA carbapenemase genes in multidrug-resistant *Acinetobacter baumannii* isolates from Iraq and Georgia**“ [Kusradze...2011] , all the samples were susceptible

to **colistin**, but as the result of our study, **80%** of the samples were resistant to **colistin**. In general, the resistance profile to broad spectrum antibiotics was similar. The conducted studies showed that the resistance to **sulfamet+trimetrop** was **70 %**, and a relatively low resistance to **tetracycline** was **20 %** (Figure 2).

Discussion:-

Acinetobacter spp. is the second most commonly isolated non-fermenter in human specimens (after *Pseudomonas aeruginosa*). *Acinetobacter spp.* appears to be an important cause of ICU infections. Multidrug-resistant *Acinetobacter spp.* is alert pathogens, mostly in ICUs and is related with outbreaks of infection. Almost similar results were observed in a study by Sana Islahi in India. Most of the strains were highly resistant to the antibiotics. Therefore, treatment of these infections are complicated. Evidence has accumulated that contaminated surfaces cause the epidemic and endemic transmission of many MDR and XDR bacteria. [Hossien...2014]. ~

Our results showed that this group of antibiotics had low-level resistance tetracyclin**20%**, highest resistance was revealed towards ceftazidim, it was **100%**, towards ciprofloxacin, piperacillin, tazobactam, imipenem and ticarcilin / clavulanic -**90%**, towards gentamicin, amikacin, tobramycin, colistin and cfepim - **80%**, The conducted studies showed that the resistance to sulfamet+trimetrop was **70 %**. **Also**, Isolates showed specific amplification for **blaOXA51**, **blaOXA40**, families.

In summary, our results demonstrate the need for effective surveillance of antimicrobial resistance in *A. baumannii* in Adjara Region and suggest that it is essential to use antibiotics with the most caution to prevent the emergence of drug-resistant strains. Furthermore, these findings indicate that the prevalence of antibiotic-resistant *A. baumannii* is high in Adjara Region, especially for the antibiotics of choice. This is an emerging concern to public health, particularly in the clinical management of persons with life-threatening *A. baumannii* infections. The results of this study confirm what some other studies have shown, that the length of hospital stay and antibiotic use prior to infection are significantly associated with increased risk of an antimicrobial resistant *A. baumannii* infection.

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Referenc:-

1. Krol V, Hamid NS, Cunha BA. Neurosurgically related nosocomial *Acinetobacter baumannii* meningitis: report of two cases and literature review. *J Hosp Infect.* 2009 Feb. 71(2):176-80. [Medline].
2. Landman D, Quale JM, Mayorga D, Adedeji A, Vangala K, Ravishankar J, et al. Citywide clonal outbreak of multiresistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in Brooklyn, NY. *Arch Intern Med.* 2002;162:1515–20
3. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev.* 2008;21(3):538–82. doi: 10.1128/CMR.00058-07. [PMC free article] [PubMed] [Cross Ref]
4. A.Y. Peleg, H. Seifert, D.L. Paterson ***Acinetobacter baumannii*: emergence of a successful pathogen** *Clin Microbiol Rev*, 21 (2008), pp. 538–582
5. . Poirel L, Nordmann P (2006) Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. *Clin Microbiol Infect* 12: 826-836.
6. IaKusradze^a, Seydina M. Diene^b, Marina Goderdzishvili^a, Jean-Marc Rolain^b Molecular detection of OXA carbapenemase genes in multidrug-resistant *Acinetobacter baumannii* isolates from Iraq and Georgia. *International Journal of Antimicrobial Agents*, Volume 38, Issue 2, August 2011, Pages 164–168
7. PE Fournier , H Richet . The epidemiology and control of *Acinetobacter baumannii* in health care facilities. *Clin Infect Dis.* 2006;42:692–9.
8. Ahmed Hamouda , Benjamin A. Evans , Kevin J.Towner , Sebastian G. B. Amyes . Characterization of Epidemiologically Unrelated *Acinetobacter baumannii* Isolates from Four Continents by Use of Multilocus Sequence Typing, Pulsed-Field Gel Electrophoresis, and Sequence-Based Typing of blaOXA-51-like Genes. *Journal of Clinical Microbiology.* 2010.
9. Cisneros , J. Rodriguez-Bano , F. Fernandez-Cuenca , A. Ribera , J. Vila , A. Pascual , et al. Risk-factors for the acquisition of imipenem-resistant *Acinetobacter baumannii* in Spain: a nationwide study. *Clin. Microbiol. Infect.* 2005;11:874–879

10. J Coelho , N Woodford , J Turton , D M Livermore . Multiresistant *Acinetobacter* in the UK: how big a threat? *Journal of Hospital Infections*. 2004;58:167–169
11. HossienFazeli 1 ; AzadehTaraghian 1,*; RazieKamali 1 ; FarkhondehPoursina 1 ; Bahram Nasr Esfahani 1 ; ShararehMoghim „Molecular Identification and Antimicrobial Resistance Profile of *Acinetobacterbaumannii* Isolated From Nosocomial Infections of a Teaching Hospital in Isfahan, Iran” *Avicenna J ClinMicrobInfect*. 2014 October; 1(3): e21489.
12. N Woodford , M. J. Ellington , J. M. Coelho , M. E. Ward , S.Brown , J. F. Turton., et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int.J. Antimicrob. Agents*. 2006;27::351–353.
14. C H  ritier , L Poirel , Fournier PE , JM Claverie , D Raoult , P Nordmann . Characterization of the naturally occurring oxacillinase of *Acinetobacterbaumannii*. *Antimicrob Agents Chemother*. 2005;49(10):4174–9.
13. S Brown , H. K. Young , S. G. B. Amyes . Characterisation of OXA- 51, a novel class D carbapenemase found in genetically unrelated clinical strains of *Acinetobacterbaumannii* from Argentina. *Clin. Microbiol Infect*. 2005;11:15–23
14. SafarFarajnia , FatemehAzhari , MohammadYousefAlikhani , MohammadKazemHosseini , AmirPeymani , NasrolahSohrabi . Prevalence of PER and VEB Type Extended Spectrum Betalactamases among Multidrug Resistant *Acinetobacterbaumannii* Isolates in North-West of Iran . *Iran J Basic Med Sci*. 2013;16:751–5.
15. P.D. Mugnier, L. Poirel, T. Naas, P. Nordmann **Worldwide dissemination of the *bla*_{OXA-23} carbapenemase gene of *Acinetobacterbaumannii*** *Emerg Infect Dis*, 16 (2010), pp. 35–40
16. A.Y. Peleg, H. Seifert, D.L. Paterson ***Acinetobacterbaumannii*: emergence of a successful pathogen** *ClinMicrobiol Rev*, 21 (2008), pp. 538–582
17. Cicek AC, Saral A, Iraz M, Ceylan A, Duzgun AO, Peleg AY, Sandalli C. *OXA- and GES-type beta-lactamases predominate in extensively drug-resistant Acinetobacterbaumannii isolates from a Turkish University Hospital. ClinMicrobiol Infect*. 2014;20(5):410–5. **View ArticlePubMedGoogle Scholar**
18. Tsakris A, Ikonomidis A, Pournaras S, Tzouvelekis LS, Sofianou D, Legakis NJ, Maniatis AN. *VIM-1 metallo-beta-lactamase in Acinetobacterbaumannii. Emerg Infect Dis*. 2006;12(6):981–3. **PubMed CentralViewArticlePubMedGoogle Scholar**