Advances in Environmental Biology, 10(8) August 2016, Pages: 87-93

AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/

Comparison of three diagnostic methods for *Acinetobacterbaumannii* Isolatedfrom Baghdad Hospitals

Rajwa Hasen Esaa, Ema N N.Najii, Hussam Sami Awayid, Israa M.S. AL_KADMY

Department of Biology, College of Science, University of Mustansiriya, Baghdad, Iraq.

Address For Correspondence:
Hussam Sami Awayid, PhD..Department of Biology, College of Science, University of Mustansiriya, Baghdad, Iraq.
Tel: 00964 7807766568; E-mail: husamof@yahoo.com

Received 22 June 2016; Accepted 28 August 2016; Available online 31 August 2016

ABSTRACT

Background and Objectives: Precise identification of *Acinetobacterbaumannii* in clinical diagnostic laboratories is difficult since the commonly used commercial identification systems based on phenotypic tests may not cover the diversity of the genus and because several species are difficult to differentiate phenotypically,rapidly identify infected with*A. baumannii*may improve the efficiency ofinfection control practices.The aim of this study was to compare three diagnostic methods for*A. baumannii*isolates to reduce the time and cost of diagnosis method andshorten the time ofdelivery of antibiotictherapyfor patients and reduce risk. **Materials and Methods:**From February 2016 to August 2016, 55 gram negative bacteria isolates were collected from three main hospitalsin Baghdad. *A. Baumannii*isolates were diagnosed by three different methods, includingChromagarAcinetobacter, andChromagarOrientation,Vitek 2 system, and*16s RNA*, *RecA*,*gltA* detection by PCR. **Results:**Out of 55 gram negative bacteriaisolates,ChromagarAcinetobacter withChromagarOrintation, Vitek 2, PCRidentified47Acinetobacter species,(45*A. baumannii* with 2 *Acinetobacterradioresistans*) ,45*A. baumannii* isolates, respectively. The sensitivity and time for Chromogenicmedia,Vitek 2 and PCR, was 100% (24hr.) ,81.8% (8hr.), 100%(3hr.) respectively. **Conclusion:**PCR robustmethod for diagnosis *A.baumannii* in clinical laboratory where a Vitek 2 system and Chromogenic media need more time and routine test.

KEYWORDS: *Acinetobacterbaumannii*, Chromogenic media, Vitek 2 system, PCR.

INTRODUCTION

Most infections with *A. baumannii* involve organ systems that contain high levels of fluids. Such systems include among others the urinary and respiratory tract, peritoneal cavity, and are linked to indwelling devices. The difference between the infection and colonization with *A. baumannii* is difficult to differentiate. It is believed that the retrieval of *A. baumannii*in the hospitalized patient is a sign of severe illness, with a related mortality of about 30%[1].

Automated systems can identify this organism correctly to the species level, most participants reported using one of the following methods: Vitek1, Vitek2, MicroScan, BD Phoenix, API- 20E, API- 20NE, with and without classical tests [2].

Nevertheless, fourspecie *A. calcoaceticus*, *A. baumannii*, *Acinetobacter*genomic species 3 and *Acinetobacter*genomicspecies 13TU – must be distinguished using molecular techniques [3].

Chromogenic media that can rapidlyidentify patients colonized or infected with*A. baumannii*may improve the efficiency ofinfection control practices, shorten the time todelivery of appropriate antibiotic therapyfor infected patients and reduce mortality [4,5]. *A. baumannii*clinical isolates are commonly resistant to multiple

antimicrobial drug classes and have the ability to survive in the environment for prolonged periods of time, which facilitates their persistence in hospitals [6,7]. has become an increasingly prevalent cause of nosocomial infections especially immune-compromised and in Intensive Care Units (ICUs) patients in the last few years (8,9).Inthis study, we aimed to compare three diagnostic methods for*A. baumannii*isolates to reduce time and cost of diagnosis method andshorten the time todelivery of antibiotic therapy for patients and reduce risk.

MATERIALS AND METHODS

Isolation and diagnosis A. baumannii:

From February 2016 to August 2016,specimens were collected from infected patients with infections (UTIs), wounds, and sputum, Each swab taken carefully from the site of infection and placed in tubes containing readymade media to maintain the swab wet during transferring to laboratory. Each specimen was immediately inoculated on theCHROMagarAcinetobacter™ was prepared without and with supplementationwereDispersed slowly 32.8 g of powder base (B) in 1Liter of purified water then added 4.0 ml of the liquid supplement AC092(S) into slurry and one more time with MDR supplementCR102 1vial in 1Liter according to the manufacturer's instructions then Inoculated media were incubated at 37°C in aerobic conditions and evaluated after 18-24hr.Also CHROM orientation™ were dispersed slowly 33g of powder base in 1Liter of purified water and Stir until agar is well thickened.

These media were examined for presumptive colonies in accordance with the manufacturer's recommendations.

Vitek 2 system:

All bacterial suspensionwas taken from primary isolation plateswere adjusted toa McFarland standard of 0.5 in 2.5 ml of a 0.45% sodium chloride solution witha VITEK 2 Densi-Chek™ instrument (bioMe´rieux). The time between preparationof the inoculum and the filling of the card was always less than 30 min. Theformat of the GN plastic cardcontains 47 tests.The GN is a fully closed system to which no reagents have to be added. Thecard was put on the cassette designed for VITEK 2, placed in the instrument,automatically filled in a vacuum chamber, sealed, incubated at 35.5°C, andautomatically subjected to a colorimetric measurement by use of a new opticalreading head every 15 min for a maximum incubation period of 10 hr. Data wereanalyzed using VITEK 2 database version 4.01, which allows for organism identificationin the kineticmode after 6-8 hr. of incubation, and using quality controlled standard strains(*Acinetobacterbaumanii ATCC BAA-747*)obtained from American Type Culture Collection.

Genotype diagnosis ofA. baumannii by PCR.:

16s rRNA ,*gltA*and*recA*gene (a house keeping gene) was used for genotypic diagnosis. Specific primers listed in table 1 were employed and the amplified size was 240bp, 722bp and425bp respectively .Template DNA was prepared by boiling method byRuppé*etal*.[10]. Briefly, some isolated colonies of overnight growth bacteria were suspended thoroughly in 1 mL distilled water and boiled in a water bath for 6-10 min. After centrifugation, supernatant was used as templateDNA.PCR mixture of composed from 12.5 of GoTaq®Green Master Mix (2x) USA (promega), 5 µl template DNA, 1.5 µl primers (for each) final concentration (0.6pmol/µl),and nuclease free water up to 25 µl (4.5 µl).

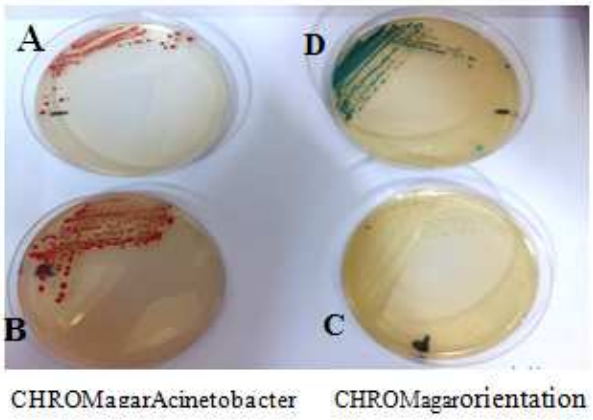
Table 1: The oligonucleotide PCR primers used in this study.

Gene	Primer	Oligonucleotide sequence (5'-3')	Annealing temp.°C time	Product size bp	Reference
16srRNA	16srRNA-F	5'-TTTAAGCGAGGAGGAGG-3'	58ºC for 30 sec.	240	(11)
	16srRNA-R	5'-ATTCTACCATCCTCTCCC-3'			
gltA	gltA-F	5'-AATTTACAGTGGCACATTAGGTCCC-3'	55°C for 1 min.	722	(12)
	gltA-R	5'-GCAGAGATACCAGCAGAGATACACG-3'			
RecA	RecA-F	5'-CACGCCGTAAGAGTGCATTA-3'	54 °C for 30 sec.	425	(24)
	RecA-R	5'-AACGGAGCTTGTCAGGGTTA-3'			

Results:

On CHROMagarAcinetobacter, 47Acinetobacter species appeared as bright salmon-redcolonies at 24 hours. Colony morphology of MDR and non-MDR-Acinetobacter wasindistinguishable on CHROMagarAcinetobacter. CHROMagarAcinetobacter also allowedgrowth of other red colonies. These red colonies were identified as

other gram-negativebacteria other than Acinetobacter and were distinguishable from Acinetobacter colonies byvariation of their red color, colony morphology and oxidase reaction. Eight isolates appearedas shiny red colonies, tested oxidase-positive and were identified as five *Pseudomonaspseudoalcaligenes* and three isolatesMoraxella group and onCHROMagarorientationAcinetobacter spp.Appeared asCreamy color, whileOther gram-negative bacteria showed blue color all summarized in the Figure1 and Table 2.



A-Acinetobacter spp. On CHROMagarAcinetobacter bright salmon-red colonies.
B-Other gram-negative bacteria On CHROMagarAcinetobacter shiny- red colonies.
C-Acinetobacter spp. OnCHROMagar orientation Creamy color.
D- Other gram-negative bacteria On CHROMagar orientation blue color.

Fig. 1: Cultured bacteria view inCHROMagarAcinetobacter and CHROMagarorientation after 24hr

Table 2: Comparison of CHROMagarAcinetobacterandCHROMagarorientationfor selection*A. Baumannii*

Isolate	No.	CH. Acinetobacter		Orientation CH.		CH. Acinetobacter Color/colony	CH.Orientation color/colony
		18 hr.	24 hr.	18 hr.	24 hr.		
Acinetobacter spp.	47	*47	47	* 47	47	bright salmon - Red/convex	Creamy/convex
Othergram negative Bacteria	8	*8	8	*8	8	shiny -Red/convex	blue/convex
Total	55	55	55	55	55		

*Small colonies, CH**.**Acinetobacter:CHROMagarAcinetobacterCH.Orientation:CHROM orientation

In Vitek-2compact systemidentification to species level. Out of the 55 strains,43 strains were isolated from sputum,5 from wound infections, and 7 from urine,The VITEK-2 compact system identified all the strains with a level of 95-99% probability (Table3). 45 strains of *Acinetobacterbaumannii*complex were identified with level 99%,5 strains *Pseudomonaspseudoalcaligenes*with level 99% ,3Moraxella group strains of probability 95%, and 2 strains*Acinetobacterradioresistans*with level 99%.all Summarized in the Table. 3

In PCR our result showed all genes were found to be present in all 45 (100%) *A. baumannii*clinical studied isolates and take time 3:00 hr. to completed for each gene. The results of the presence of16srRNA, gltA and RecAgenes are exemplified by the isolates shown in(Figure 3,4,5).

Table 3: results of identification all isolates by Vitek 2 compact system.

Isolates	No.	Probability of Id %	Time of Id inhr.
Acinetobacterbaumanii	45	99%	6:00 hr.
Acinetobacterradioresistans	2 2	99%	8:00 hr.
Moraxella group *Moraxella lacunata* *Moraxella nonliquefaciens* *Moraxella osloensis*	3	95%	10:00hr.
Pseudomonas pseudoalcaligenes	5	99%	10:00 hr.
Total isolates	55		

Id = Identification, No. =Number of isolates

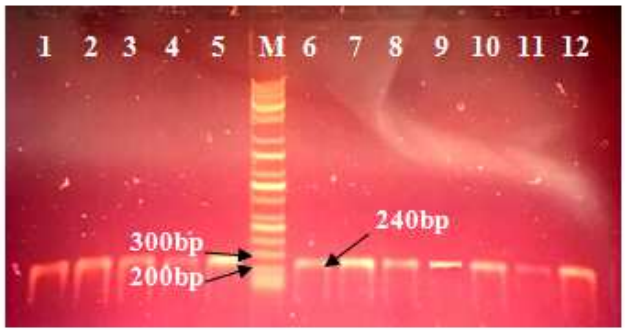


Fig. 3: Gel electrophoresis (1% agarose, 100 V/cm for 1hr. stained with ethidium bromide) for detect 16srRNA gene for some *A. baumannii*isolates. Line M 100bp DNA ladder, lines (1-12) positive results with 240bp amplicon.

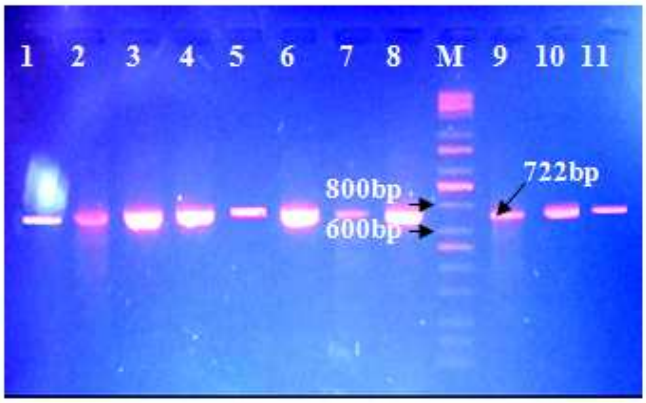


Fig. 4: Gel electrophoresis (1% agarose, 100 V/cm for 1hr., stained with ethidium bromide) for detect gltA gene for some *A. baumannii* isolates. Line **M** 100bp DNA ladder, lines (1,2,3,4,5,6,7,8,9,10, 11) positive results with 722bp amplicon.

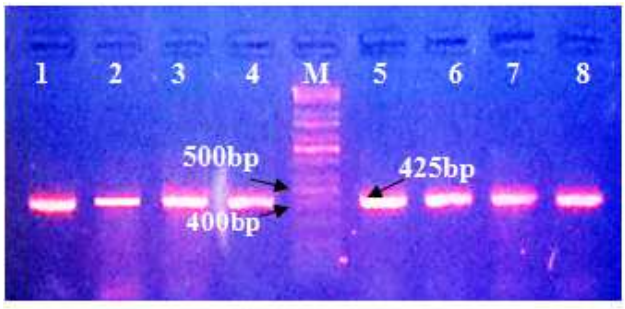


Fig. 5: Gel electrophoresis (1% agarose, 100 V/cm for 1hr., stained with ethidium bromide) for detect RecA gene for some *A. baumannii*isolateLine **M** 100bp DNA ladder, lines (1,2,3,4,5,6,7, 8) positive results with 425bp amplicon.

Discussion:

Our study results showed that CHROMagarAcinetobacter was 100% sensitive for bothMDR and non-MDR-Acinetobacter when compared to PCR but their study reported that CHROMagarAcinetobacter was both sensitive (91.7%) and specific (89.7%) for MDR-*A. baumannii*when compared to PCR also was an investigational culture media different from the one evaluated in our study, Their CHROMagarAcinetobacter contained agent that inhibits the growth of most gram-positive bacteria as well as carbapenem susceptible gram-negative bacteria while allowing Acinetobacter to appear as aqua blue colonies instead of the red colonies in our study [14]. While other study showed the cost of CHROMagar*Acinetobacter*was less thanthe cost of the molecular method, it had a comparableoutcome, detecting all MDR isolates (*Acinetobacterspp*, and others) with no missed cases[15]. When compared to CHROMagar orientation,Acinetobacter species appeared as bright salmon red colonies on CHROMagarAcinetobacter facilitating easier identification and isolation. However, CHROMagarAcinetobacter did notdifferentiate MDR from non-MDR-Acinetobacter and further susceptibility testing is neededto confirm multidrug resistance status. In addition, CHROMagarAcinetobacter allowsgrowth of red colonies that were identified as other gram-negative bacteria besidesAcinetobacter; however, these other bacteria could be distinguished from Acinetobacter byvariation of their red color, colony morphology and oxidase reaction[16]. Another study by[17] evaluated CHROMagarAcinetobacter for the ability to detect various other organisms such as *P.aeruginosa*, grew on CHROMagarAcinetobacter with the same redor similar red-orange color as *A. baumannii*. Their study also reported that CHROMagarAcinetobacter was 75% sensitive and 100% specific for *A. baumannii*.

Based on their studyresult advised against the use of CHROMagarAcinetobacter in the absence ofconfirmatory testing.In addition, some isolates of gram negative bacteria formed colonies of the same color asA. *baumannii*, suggesting the possibility of misdiagnosis[18]. While Acinetobacter species appear as creamy nontransparent convex colonies on CHROMagarorientation[19]. That was comparable with our study result. CHROMagarAcinetobacter and CHROMagarorientation may save time when the absence of a red color and creamy color respectively indicates theabsence of Acinetobacter species in a patient sample. However, a comprehensive time andcost-benefit analysis of using CHROMagarAcinetobacter and CHROMagarorientation versus other agar for isolating Acinetobacterthat Compatible withstudy conducted by[16]. The VITEK-2 compact system combines several advantages that may be of clinical interest for routine testing of gram negative rods isolated from clinical specimens like rapid identification, a simple methodology, a high level of automation and taxonomically updated databases.In other studies by[20] have proved an efficiency of VITEK-2 System with 95-99% probability of accurate identification of all strainsThis corresponds with our results, butin earlierstudy conductedby[21] results showed that the vast majority ofisolates belong to*A. calcoaceticus A. baumannii complex* (93.8%), but in our study we found majority of isolate was *A. baumannii complex* (81.8%), This may be due to the difference in increasing levels of capacity and automation[22]. The earlydiagnosis of *Abaumannii* with prompt applicationof appropriate therapy may prevent systemic involvement.The time required for the identification of*Abaumannii* based on culture methods is at least 18 hr. Table4.were recognized by the VITEK-2 framework as A. baumannii this outcome ought to be seen as an impediment of the phenotypic test, as opposed to the instability of VITEK-2 distinguishing proof results,since the phenotypic test can't separate microorganisms having a place with the ACB complex. The majority of the VITEK - 2 hardware utilized as a part of healing centers is really set up in a manner that when an animal types having a place with the ACB complex is recognized, it is naturally assigned as *A. baumannii*. [25].

The stamped contrast of the precision rates of the VITEK - 2 framework in distinguishing Acinetobacterwas found by test bunch. The exactness rates of the VITEK - 2 framework in distinguishing Acinetobacter clinical disconnects were 76.6% in the test bunch in study reported by [25] while in our study was 81.8% that near our consequence of the test bunch Table 4.

The main differences of these groups are the sample sizeThe proportion of*A. baumannii*may explain the difference of the accuracy rates because the performance of VITEK- 2 system for *A. baumannii* is good but not good for other strains. Since bacteria belonging to the non-ACB complex are rare, obtaining a sufficient number of isolates to study the characteristic features of each species is difficult. For further study of the clinical characteristics, pattern of resistance to antibiotics, and distribution of genes of each bacterial species, ansample number of strains of each species must be collected and identified by molecular biological methods.

PCR is a method that combines rapid nucleic acid extraction,amplification, and detection.The time for microbiological identification using PCR can be as shortas 3- 4 hrs, which greatly improves the turnaroundtimethe application of PCR was limited by the lowpositive predictive value, which might be because of thefollowing possibilities. First, negative culture results couldhave been obscured by prior antimicrobial therapy, whichwas applied when patient was first suspected.As such,the dead bacterial remnants would fail to grow on cultureplates but could be detected by PCR. Second, the moresensitive nature of PCR detection might lead to thedetection of a small number of *Abaumannii*, which mightnot be detected in culture or be outgrown by the otherbacteria present in the specimens. Third, the more sensitive PCR method might detecta small number of *A baumannii*, that is, however, not theresponsible agent for the infection.[23]. From the Table 4 it is clear

from the results indicated in there is a consensus expressed as agrrement % in the diagnostic accuracy by using PCR and Chromagar techniques.

Table 4: Compression between methods of diagnosis of *Acinetobacterbaumannii*

Method of diagnosis	Time	Cost	Sensitive	No. of isolates	Colony of bacteria
Chromagar	18-24 hr.	Highly	100%	47	Live bacteria
Vitek 2 system	6-8 hr.	Moderate	81.8%	45	Live bacteria
PCR	3-4 hr.	Cheap	100%	45	Dead bacteria
Agreement %	ChromagarvsVitek 2 system 86%		Chromagarvs PCR 100%		Vitek 2 systemvs PCR 86%

ACKNOWLEDGEMENT

The authors wish to express their gratitude toMustansiriya University of Sciences, baghdad, for financial and spiritualsupport specially doctor EmanNatiq, Rajwa Hassan andIssraSafi.

REFERENCES

[1] JUNG, Jaejoon; PARK, 2015. WoojunAcinetobacter species as model microorganisms in environmental microbiology: current state and perspectives .*Applied microbiology and biotechnology*, 99(6): 2533-2548.
[2] BASSETTI, Matteo, et al. 2008. Drug treatment for multidrug-resistant *Acinetobacterbaumannii* infections.
[3] PELEG, Anton Y., SEIFERT, Harald, PATERSON, David L. 2008. *Acinetobacterbaumannii*: emergence of a successful pathogen. *Clinical microbiology reviews*, 21(3): 538-582.
[4] KOLLEF, Katherine E., et al. 2008. Predictors of 30-day mortality and hospital costs in patients with ventilator-associated pneumonia attributed to potentially antibiotic-resistant gram-negative bacteria. *CHEST Journal*, 134(2): 281-287.
[5] AJAO, A.O., et al., 2011. Comparison of culture media for detection of *Acinetobacterbaumannii*in surveillance cultures of critically-ill patients. *European journal of clinical microbiology & infectious diseases*, 30(11): 1425-1430.
[6] NEONAKIS, Ioannis K., A. SPANDIDOS, Demetrios, PETINAKI, 2011. Efthimia. Confronting multidrug-resistant *Acinetobacterbaumannii*: a review. *International journal of antimicrobial agents*, 37(2): 102-109.
[7] LIAKOPOULOS, A., et al., 2012. Identification of OXA-23-producing *Acinetobacterbaumannii*in Greece, 2010 to 2011. *Euro Surveill*, 17(11): 20117.
[8] AYDEMIR, Hande, et al. 2012. Mortality attributable to carbapenem-resistant nosocomial *Acinetobacterbaumannii*infections in a Turkish university hospital.*Japanese journal of infectious diseases*, 65(1): 66-71.
[9] KABBAJ, Hakima, et al., 2012. Prevalence of Metallo-β-Lactamases Producing Acinetobacterbaumannii in a moroccan Hospital. *ISRN Infectious Diseases*.
[10] RUPPÉ, Etienne, et al., 2009. CTX-M beta-lactamases in Escherichia coli from community-acquired urinary tract infections, Cambodia. *Emerg Infect Dis.,* 15(5): 741-8.
[11] ROCHA, 2010. Carlos André Narciso da. Persistence and dispersion of Acinetobacter spp. in the urban water cycle.
[12] BARTUAL, Sergio G., et al. 2007. Development of a Multilocus Sequence Typing Scheme for Characterization of Clinical Isolates of *Acinetobacterbaumannii*.*Journal of clinical microbiology*, 45(6): 2101.
[13] CHEN, Te-Li, et al., 2014. Rapid identification of *Acinetobacterbaumannii*, Acinetobacternosocomialis and Acinetobacterpittii with a multiplex PCR assay. *Journal of medical microbiology*, 63(9): 1154-1159.
[14] GORDON, N.C., D.W. WAREHAM, 2009. Evaluation of CHROMagarAcinetobacter for detection of enteric carriage of multidrug-resistant *Acinetobacterbaumannii*in samples from critically ill patients. *Journal of clinical microbiology*, 47(7): 2249-2251.
[15] GHAITH,Doaa Mohammad; HASSAN, ReemMostafa; HASANINB, 2015. Ahmed Mohamed. Rapid identification of nosocomial *Acinetobacterbaumannii*isolated from a surgical intensive care unit in Egypt. *Annals of Saudi medicine*, 35(6): 440.
[16] AJAO, A.O., et al., 2011. Comparison of culture media for detection of *Acinetobacterbaumannii*in surveillance cultures of critically-ill patients. *European journal of clinical microbiology & infectious diseases*, 30(11): 1425-1430.
[17] AKERS, Kevin S., et al., 2010. HROMagarAcinetobacter is not selective for carbapenem-resistant *Acinetobacterbaumannii–calcoaceticus* complex .*Diagnostic microbiology and infectious disease*, 67(2): 209-211.

[18] PANAGEA,Theofano, et al., 2011. Evaluation of CHROMagar™ KPC for the detection of carbapenemase-producing Enterobacteriaceae in rectal surveillance cultures. *International journal of antimicrobial agents*, 37(2): 124-128.
[19] OHKUSU, Kiyofumi, 2000. Cost-effective and rapid presumptive identification of gram-negative bacilli in routine urine, pus, and stool cultures: evaluation of the use of CHROMagar orientation medium in conjunction with simple biochemical tests. *Journal of clinical microbiology*, 38(12): 4586-4592.
[20] SIMGAMSETTY, Susmitha, et al., 2016. Ease with VITEK 2 systems, biomerieux in identification of non-lactose fermenting bacteria including their antibiotic drug susceptibility: our experience. *International Journal of Research in Medical Sciences*, 4(3): 813-817.
[21] AL-HAMADANI, A., A. AL-MOHANA, A. AL-KHAZAALI, 2014. Emergence of plasmid mediated aac (6)-Ib-cr Gene in Flouroquinolon-resistant Acinetobacter spp. *Qadisiya Medicine Journal*, 10: 96-107.
[22] PINCUS, David H., 2006. Microbial identification using the bioMérieuxVitek 2 system.*Encyclopedia of Rapid Microbiological Methods. Bethesda, MD: Parenteral Drug Association*.
[23] CHIANG, Mei-Chun, et al., 2011. Polymerase chain reaction assay for the detection of *Acinetobacterbaumannii*in endotracheal aspirates from patients in the intensive care unit. *Journal of Microbiology, Immunology and Infection*, 44(2): 106-110.
[24] Sawsan, S. AL_Jubori, Israa M.S. AL_kadmy, ZuharI. JassimAL_Ani, 2016. Emergence of Multidrug resistance (MDR)*Acinetobacterbaumannii* Isolated from Iraqi hospitals.Advances in Environmental Biology, 10(5): 265-275.
[25] LEE, Min Jung, et al., 2014. Comparison of rpoB gene sequencing, 16S rRNA gene sequencing, gyrB multiplex PCR, and the VITEK2 system for identification of Acinetobacter clinical isolates. *Diagnostic microbiology and infectious disease*, 78(1): 29-34.