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Comparison of CHROMagar™ *acinetobacter* and conventional methods for isolation *Acinetobacter baumannii* from tracheal aspirates patients with ventilator-associated pneumonia (VAP)

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ABSTRACT

Acinetobacter baumannii is the most common and increasingly important nosocomial pathogen associated with VAP. The aim of our study was to compare the capacity and performance of sheep blood agar, MacConkey agar and CHROMagar Acinetobacter for isolation and identification of A baumannii in tracheal aspirates specimens obtained from patients with Ventilator-associated pneumonia. (VAP) .Specimens was plated onto three media including: 5% sheep blood agar (SBA), MacConkey agar, and CHROMagar Acinetobacter. SBA was the gold standard to which all media was compared. There were 100 specimens collected during the study period from August 2012 through November 2012. SBA and CHROMagar detected 39 of 39 (100%) Acinetobacter isolates. CHROMagar Acinerobacter had 100% sensitivity and specificity. Nearly all isolates of Acinetobacter baumannii were multiple Drug resistant (MDR). Colisitin was the sole effective antibiotic against MDR isolates of A.baumannii.

Key words: *Acinetobacter baumannii*, CHROMagar Acinetobacter, Drug Resistance.

INTRODUCTION

Ventilator-associated pneumonia (VAP) is defined as pneumonia occurring more than 48 hours after patients have been intubated and received mechanical ventilation. [1].The incidence of nosocomial pneumonia in ventilated patients particularly in intensive care units (ICUs) is very high and ranging from 7% to more 40%. Such nosocomial infections prolong hospital stay and increases patients' mortality rate. There is still not well accepted gold standards methods for diagnosis of VAP, however rather there are some diagnosis methods with different sensitivity and specificity [2,3]. Despite remarkable progresses in diagnosis and treatment of VAP over the recent years, controversies persist over the optimal methods for diagnosis of VAP. Applying conventional laboratory methods is critical for identifying specific etiologic agents, for establishing appropriate treatment protocols [4, 5]. The bacteriologic diagnosis of VAP is still a controversial issue .This challenges in microbiology laboratories due to differentiation of between organisms responsible for the infection and colonizing normal flora. Recent studies raveled that

Acinetobacter baumannii is the most common and increasingly important pathogen associated with VAP in patients especially at ICUs wards [5, 6, , 7].

Acinetobacter baumannii is a ubiquitous pathogen that has emerged as a major cause of health care associated infections. *Acinetobacter baumannii* usually causes respiratory tract, urinary tract, blood stream and surgical site infections. They are of increasing importance because of its ability to rapidly develop resistance to the major groups of antibiotics [7-13]. There are few data available on the prevalence and drug resistance pattern of *A.baummannii* in our country. Knowledge about *A. baumannii* is much less developed than knowledge about other opportunistic pathogens such as *Pseudomonas aeru*ginosa. Difficulty for identifying this organism has led to the publication of data that are of questionable value [14]. In a previous study, we evaluated the performance of microbiology laboratories in Tehran and its districts in an External Quality Assurance Scheme (EQAS) for detection of *A. baumannii* as unknown microorganism. Of 487 laboratories involved in our survey, only 29.8% of laboratories correctly identified this organism [15]. The aime of this study was to compare the performance of CHROMagar Acinetobacter with conventional culture media for isolation and identification of *A.baumannii* in tracheal aspirates specimens. In addition we identified incidence and drug resistance pattern of all isolates *A,baummani* strains.

MATERIALS AND METHODS

This study was carried out in Microbiology Laboratory of Milad Hospital in Tehran. Milad hospital is a nonteaching and a 1000-bed tertiary care hospital. During August 2012 through November 2012,all tracheal aspirates specimens obtained from VAP suspected patients in ICUs of hospital were submitted to microbiology Laboratory. These samples were collected as part of routine infection control surveillance programs .We used a formulation of CHROMagar Acinetobacter that was introduced for isolation and detection of *Acinetobacter baumannii* .Acinetobacter CHROMagar was prepared from dehydrated powder and liquid by manufacture. supplement according the manufacture (CHROMagar ™ France, Paris). Other routine culture media such as MacConkey agar and blood agar were prepared in house according manufactures guidelines.

Tracheal aspirates specimens were inoculated directly to MacConkey agar, SBA and CHROMagar Acinetobacter. All plates were inoculated at 37ºC for 24 hours. All Specimens processed by the same laboratory technician. CHROMagar Acinetobacter, MacConkey agar compared to sheep blood agar for isolation of A.baumannii Oxidase negative colonies morphologically resembling to *A. baumannii* were identified using conventionally bacteriological methods[16, 17,18]. Susceptibility testing and interpretation of results was performed by disk diffusion method as recommended by CLSI guidelines. (19)

RESULTS

A total of 100 patients were subject of our study. Of them, 60 patients (60%) were male and 40 (40%) female. The median age was 57 years (range, 1 to 100 years). Fifty seven (57%) of patients were from emergency ward, and 43(43%) from ICUs. .All patients were mechanically ventilated. The source of the all positive clinical culture was tracheal aspirates. Of 100 patients, 80 patients had positive culture Thirty nine patients (39%) were considered to have infection or colonized by *A. baumannii*. .In addition *Acinetobacter baumannii* ,we isolated other organisms such as *S.aureus* ,*E.coli.K.pneumoniae,Enterobacter cloacae,S.malophilia* and *Candida albicans*. The frequency of isolated organism have been showed in Diagram-1. In our study we used SBA as a gold standard medium for growth and isolation of etiology agents of VAP including *Acinetobacter baumannii*. The results of SBA plate compared to CHROMagar Acinetobacter and MacConkey agar. SBA and CHROMagar Acinetobacter recovered all 39 (100%).CHROMagar Acinetobacter had 100% sensitivity and specificity for isolation of Acinetobacter. On CHROMagar .isolates of Acinetobacter *baumannii* appeared as bright salmon-red colonies at 24 hour as shown in Diagram-2 .The antibiotic resistance pattern of *A.baumanii* has been showed in Diagram -3. All isolates were resistant to third generation of cfephalosporins including ceftazidim, ceftriaxone, cefotaxim and ceftizoxime. More than 90% of isolates

were resistant to other antibiotics such as .imipenem gentamicin tobramycin and ciprofloxacin. All isolates of *A.baumanni* were susceptible to colistin.

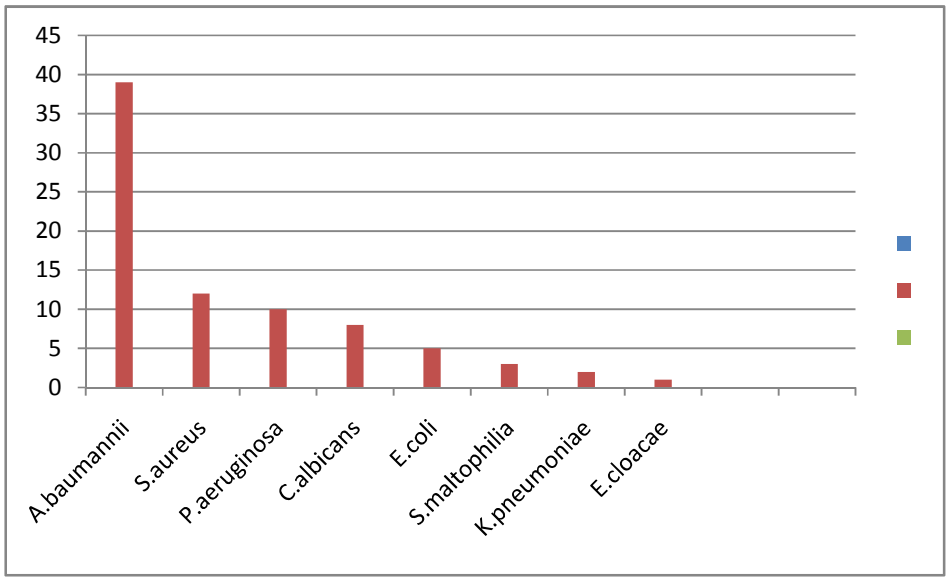
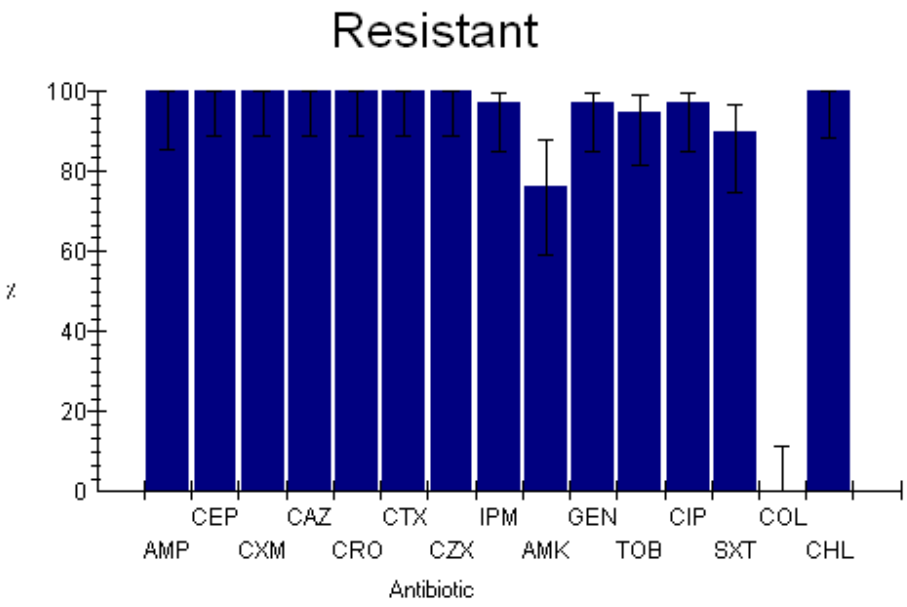


Diagram 1- Frequency of microorganisms isolated from VAP patients

Fig2-Colonies of *Acinetobacter baumannii* onCHOMagar Acinetobacter



AMP=Ampicillin,CEP=Cefpime,CXM=Cefuroxime,CAZ=Ceftazidime,CRO=Ceftriaxone, CTX=Cefotaxime.CZX=Cefazoline,IPM=Imipenem,AMK=Amikacin,GEN=Gentamicin,TOB=Tobramycine,CIP=Ciprofloxacin,SXT=Trimethoprim-Sulfamethoxazole, Col=Chloramphenicol,CHL=Colisitin.
Diagram2-Frequency of antibiotic resistance among A.baumannii isolates from tracheal aspirates in VAP patients

DISCUSSION

A. baumannii is a major hospital acquired pathogenenic microorganism in recent years. This organism usually affecting patients who are immunocompromised and those patients hospitalized in intensive care units [20, 21]. The economic impact of *A. baumannii* colonization and infection in hospitals is also substantial. Infections caused by *A.baumannii* often occur in outbreaks during which the bacteria are spread through contact with clinical personnel harboring the bacteria and as a normal flora[(22]. Recently, a wide verity of chromogenic media has been introduced that are designed to detect target pathogens with high sensitivity and specificity. These media contain enzyme substrates that release colored dyes upon hydrolysis, and resulting in pathogens forming colored colonies by pathogens which can easily be distinguished from commensal normal flora and environmental contamination. Nova days there are many chromomeric media for detection pathogens such as urinary tract infection agents, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Salmonella* spp. , *Candida* spp and antibiotic resistant organisms such as vancomycin resistant entrococci , ESBLs producing microorganisms, KPC and other Multiple drug resistant pathogens [23,,24,25,26].

CHROMagar *Acinetobacter* (CHROMagar, Paris, France) is a recently developed selective agar for the rapid isolation and identification of Acinetobacter .This medium contains components that inhibit the growth of most gram-positive and gram-negative bacilli. It incorporates substrates enabling color-based preliminary identification of colonies recovered within 18 to 24 h of inoculation. In this study, we evaluated the performance and ability of CHROMagar *Acinetobacter* for isolation and identification of *A.baumannii* in comparison to conventional method.

Previous studies have compared CHROMagar Acinetobacter to other methods for isolation and detection of Acinetobacter .In a study by Gorden et all they compared CHROMagar Acinetobacter to a molecular methods for detection MDR-Acinetobacter. .In their study CHROMagar Acinetobacter had 91.7% sensitivity and 89.7% specificity respectively [27]. Another study carried out by Akers et al reported that CHROMagar Acinetobacter was 75 sensitive and 100% specific for detection of Acinetobacter. CHROMagar Acinetobacter has been recently designed for selection of MDR_*Acinetobacter*[28] .A study by Wareham et al revealed that the addition of supplement to CHROMagar Acinetobacter inhibits

the growth of carbapenem sensitive strains of *A.baumannii*. However using of this kind of CHROMagar Acinetobacter needs further studies.

In addition to cost of equipment and labor the cost of CHROMgar Acinetobacter is higher than the cost of SBA, MacConbkey Agar and routine biochemical test for isolation and identification of *A.baumannii*, However usage of CHROMagar Acinetobacter is cost benefit, because many laboratories do not have capacity for primary isolation and identification of *Acinetobacter* by using routine bacteriological methods. In addition using of CHROMagar Acinetobacter may save time by detecting colonies in patient's samples. Early detection of Acinetobacter promotes treatment protocol, It will be useful if further studies carry out to compare CHROMgar Acinetobacter to other conventional culture media on their cost saving and time.[30]

CONCLUSION

In conclusion CHROMagar Acinetobacter permits the growth of *A.* baumannii in tracheal aspirates with high sensitivity and specificity. Our study revealed that Acinetobacter CHROMagar has a high sensitivity and specificity for detection of *A baumannii.*

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