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Detection of Extended spectrum β -lactamases (ESBLs) produced by *Acinetobacter baumannii* isolated from burn patients in Al- Nasiriyah city Iraq.

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ABSTRACT

The aim of present study is to isolate and identify of Acinetobacter baumannii from burns infect as well as study their production for Extended spectrum β -lactamases (ESBLs).This study included 205 burn patients at Al-Hussien Teaching Hospital and other burn care units in Al-Nasiriyah city . The specimens were collected during period from August 2018 to February 2019. It were taken by burn swabs from pus of the burned area and then inoculated on blood agar and MacConkey agar. The isolates were diagnosed by microscopic examination, biochemical tests , API 20 E and Vitek- 2 system . It was found that 20 isolates of A.baumannii and 2 isolates of A. loffii . Where identified from this study . The study showed 18 (90%) out of 20 isolates of A. baumannii were appeared resistance to cefotaxime and ceftazidime(screening test of ESBLs) . Seven (35%) of isolates were producing ESBLs by confirmatory test(Modified double –disk synergy test (MDDST)) , and 13(65%) of isolates were negative results to production of the ESBLs.

Keywords— Acinetobacter baumannii , Extended spectrum β -lactamases ,ESBLs, burn patients

I. INTRODUCTION

Acinetobacterspp. are Gram negative coccobacilli, strictly aerobic, non-motile, nonfermentative, donot form spores and it is normal flora on skin and widely distributed in nature environment ,it considers opportunistic pathogen to human when appropriate conditions are available (Peleg et al., 2008 ; Doughari et al., 2011 ; Thomas et al ., 2018).

A.baumannii is distributed worldwide and is frequently emergence in a hospital and causes nosocomial infections such as meningitis, bacteremia, soft tissue infections, pneumonia and burns wound infections, increased infection of A.baumannii in burns patients due to the erosion of the protective layer of the skin , which make it easy to get the infected by the bacteria especially multi drug resistant (MDR) bacteria , including A. baumannii. These bacteria have the ability to resist drying and moist environment (Villegas and Hartstein, 2003 ; Jasem et al ., 2018). The common resistance mechanism of A. baumannii and most gram negative bacteria are production of enzymes, these coding by specific genes and transferred from cell to another via plasmids, or by DNA mutation in chromosome . The common enzymes were β-lactamase,include the penicillinases,cephalosporinases, oxacillinases, carbapenemases and extended spectrum β-lactamases , that hydrolyzes and resistance to cephalosporins, penicillins, and carbapenems (Bonomo and Szabo., 2006). The total number of the ESBLs now exceeds 200, the general principle of all the ESBL-detection methods are that the activity of the extended-spectrum of cephalosporins against the ESBL-producing organisms will be promote by presence the clavulanic acid (Shahid et al.,2009). Due to the increase in the spread of Acinetobacter baumannii resistant to β-lactams antibiotics especially the cephalosporins in the world and their emergence in our local hospitals, the this study was aimed to determine the ability of bacteria to produce Extended spectrum β-lactamase (ESBLs) by phenotypic methods.

II. MATERIALS AND METHODS

Specimens collections

Atotal of 205 burn patients of Al-Hussien Teaching Hospital and other burn care units in Al-Nasiriyah city were collected . The specimens were collected during the period from August / 2018 to February /2019. Swabs inoculated on blood agar and MacConkey agar. (Himedia , India) , isolates identification was performed by routine laboratory methods including API 20 E system(BioMerieux , France) . andVitek- 2 system (GN-ID card to Gram negative identification) (BioMerieux , France) .

Antibiotic susceptibility test

Antibiotic susceptibility testing was performed by the Kirby Bauer method on Mueller Hinton agar (BD&BBL ,USA) according to CLSI protocols(11) The antibiotics were ceftazidime ((CAZ)(30 µg)) ,
cefotaxime ((CTX)(30 µg))

antibiotics in front the augmentin (CLSI ,2006).

Detection of Extended spectrum β -lactamases (ESBLs) production

All isolates were appeared positive screening test were further tested to confirmatory test . It was done by modified double –disk synergy test ,it was used two plates of Muller Hinton agar , in one plate antibiotics including cefepime , aztreonam , ceftazidime , cefotaxime , and ceftriaxone were placing at distances 20 mm (center to center) from disk containing augmentin (10µgclavulanic acid +20µg amoxicillin) , in the other plate placed the same antibiotics without augmentin , then the plates a were incubated at 37°C for 18-24 hours .The positive result was decreased to antibiotic combined with a clear-cut enhancemet in the inhibition zone of

III. Results and Discussion

Twenty tow isolates were found to be related to *Acinetobacter*spp. , 20 (90.9%) of them were belonging to *A. baumanii* , and 2 (9.1%) isolates for *A. lwoffii* ,(Figure 1). Infection of *A.baumannii*are implicated across expend rang of anatomical area and in different severity as well as patients outcomes (Gordon and Warcham, 2010).

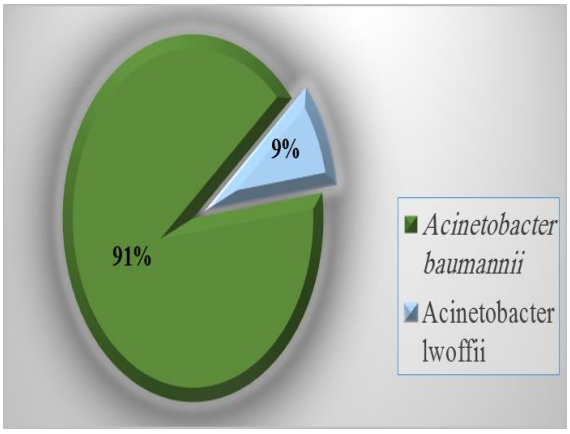


Figure (1): The isolation percentage of *Acinetobacter baumannii*

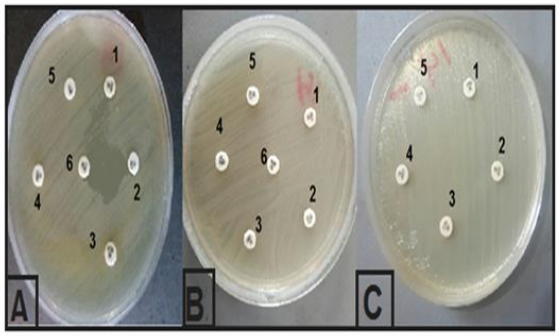
Acinetobacter baumannii became wide spread particularly in intensive care units and burn care units because its considered normal flora in human body and change to opportunistic pathogen which have resistance to antibiotics (Poirel et al., 2010).

Microbial colonization on open wounds and causes a local infection , it spread to deeper tissues lead to systemic infections, in most time the normal flora that opportunistic pathogens of burn injury (Ahmad et al., 2006).

The epidemiology of Acinetobacter baumannii was different from hospital to another, but in the most cases it became at the fifth rate of epidemiological bacteria in burns patients after Pseudomonas aeruginosa Staphylococcus aureus ,E. coli, Enterobacter spp. (Karah ,2011) .

Detection of ESBLs production

confirmatory test of ESBLs:The results of table (4-8) showed that 7 (35%) of isolates were producing ESBLs , and 13(65%) of isolates were negative results to production of the ESBLs,figure (2), and table (1)



1-Cefepime , 2-Ciftazidime ,3-Ceftriaxone ,4-Cefotaxime , 5-Aztreonam , 6-Augmentin(Amoxicillin –clavulanate) . Figure (2): Modified double –disk synergy test ((confirmatory test) (MDDST)) for detection ESBLs .A-positive result show enhancement of inhibition zon between augmentin and beta-lactam diskes ,B -negative result, C – results of beta-lactam antibiotics without augmentin.

Table (1): production of ESBLs by phenotypic tests .

Test	No. & (%) of ESBLs producers isolates	No. & (%) of ESBLs non producers isolates
Screening test	18 (90)	2(10)
Confirmatory test	7(35)	13(65)

The production of ESBLs is one of the important resistance mechanisms in the bacterial cell, the genes responsible for ESBLs travel through plasmids between bacteria within different groups .These enzymes have contributed to the development of resistance to antibiotics over the past years(Al-Marjani*et al.,* 2015) . The result of the present study agreed with the results of Mirnejad and Vafaei (2013) , they found that the ratio of ESBLs production was 20% , and these results were disagreement with Shyam*et al*. (2013) and Jabur (2014) they found that the ratio of ESBLs production was 99%.

Conclusion

The study shows that the *Acinetobacter baumanii* was produced ESBLs

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