MOLECULAR ANALYSIS OF EFFLUX PUMPS AND QUORUM SENSING GENES IN MDR ACINETOBACTER BAUMANNII

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ABSTRACT: Acinetobacter baumannii (MDR-Ab) are common among clinical isolates worldwide and are a major therapeutic challenge. Identification of Acinetobacter species is complicated due to lack of standard identification techniques, the isolates were confirmed as Acinetobacter by PCR which was performed by housekeeping gene 16sRNA gene and confirmed as A. baumannii by bla_{Ova 51} genes. All of clinical isolates indicated multidrug resistant (MDR). The antibiogram typing represent the importance indicator to distinguish and differentiate between bacterial isolates according to origin of infection beside multidrug resistance pattern. A. baumannii isolates were typed genotypically by using antibiotic resistance genes, screen showed all 30 (100%) isolates were positive for $bla_{CTX and} bla_{Oxav51}$, 9(30%) were positive for bla_{IMP-1} and bla_{TEM} , 5 isolates (16.6%) were positive for bla_{VIM} , 12(40%) isolated were positive for bla_{SHV} and bla_{ParC} and only one isolate (3.3%) were positive for bla_{OXa23} and bla_{Oxa24} , while no isolate positive for bla_{gyr} . Whereas, Efflux pump genes screen showed all (100%) isolates were positive for AdeM, 12 (40%) isolates were positive for AdeL, 10(33.3%) isolates were positive for AdeH, 8(826.6%) isolates were positive for AdeG and AdeF and only one isolates were positive for Bap gene (biofilm associated gene). While, Ouorum sensing genes screen showed 13 isolates (43.3%) isolates were positive for RhlI gene, 12 (40%) isolates were positive for LasI gene, 10(33.3%) isolates were positive for Las R while 8 isolates were positive for RhlR gene. Five(16.6%) isolates were positive for Las R and RhlI genes and only one isolate isolates were Carrying for Las R and RhlI, Las I, RhlR genes. The virulence of the A. baumannii does not require, carrying full set of QS genes, AdeM predominance than other MATE Efflux Pumps genes, indicated have important role of resistance against many antibiotics in Iraqi A. baumanii.

Key words: Acinetobacter baumannii, quorum sensing genes, efflux pump genes, antibiogram typing.

INTRODUCTION

Acinetobacter baumanniiis an opportunistic pathogen that is responsible for a variable nosocomial infections, such as bacteremia, UTI, meningitis and pneumonia, especially in patients in the ICU (Antunes et al, 2014). Acinetobacter infections is broad and includes infection associated with tropical environments, wars and natural disasters and hospital outbreaks in humid climates, it naturally inhabits soil and water and other possible reservoirs include arthropods and animals food (Leung et al, 2006). In humans, can colonize on skin, burn, the respiratory and gastrointestinal tracts (Kanafani and Kanj, 2013). Tay and Yew (2013) reported the regulation of virulence behavior such asbiofilm formation and antibiotics resistance accrue due to AHL signallis of Q.S that regulatory mechanism among Gram-negative bacteria, and plays important role inexpression of virulence genes, antibiotic resistance in A.baumannii. Rahmati et al (2002) showed A. baumannii protecting themselves from

toxic effects of organic chemicals by efflux pump. Efflux-based mechanisms are responsible for resistance against many different antibiotics classes by ridding or pumping the drugs for reduction accumulation endogenous antibiotics. In the same manner He et al (2015) noticed correlation between quorum sensing and efflux pump. The major objectives of this study were there is Diagnostic technique to characterize A.baumannii for test the antimicrobial susceptibility and for study the relationship between Quorum sensing and Efflux pump genes dendrogram analysis.

MATERIALS AND METHODS

Specific primers were used and prepared according to the manufacture company information by adding deionized distilled water to obtain stock solution equal to 100pmol/îM. From the previous stock, 10ìl was diluted by adding 90 ìl distilled water to get a secondary stock concentration equal to 10pmol. PCR mixture for this gene composed from 12.5ìl of GoTaq®Green Master Mix,