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DETECTION OF BACTERIOCIN GENE FROM *KLEBSIELLA PNEUMONIAE*

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ABSTRACT

Bacteriocins are antimicrobial peptides produced ribosomally by *Klebsiella* species. They kill other members of the same or closely related species. The aim of this study was to detect bacteriocins gene in *Klebsiella pneumoniae* isolates. Thirty one isolates were identified as *K. pneumoniae* by biochemical and microbiological methods and confirmed by API 20E system and VITEK-2 system. The resistance rate of amikacin, ampicillin, augmentin, azetreonam and tetracycline was recorded (32.26%, 64.52%, 51.61%, 48.39% and 41.94% respectively). Klebicin gene cluster was detected in 15(48.39%) of *K. pneumoniae* isolates.

KEYWORDS: Bacteriocin, Klebicin, *Klebsiella pneumoniae.*

INTRODUCTION

Klebsiella pneumoniae is facultative anaerobic, nonmotile, Gram-negative, rod-shaped bacterium. It is encapsulated and lactose-fermenting.[1] *K. pneumoniae* is an important opportunistic pathogen that is responsible for causing both community and hospital acquired infections.[2] It can be carried asymptomatically in the intestinal tract, skin, nose, and throat of healthy individuals, but it can also cause a range of infections, most commonly pneumonia, wound, soft tissue, or urinary tract infections.[3] Because they survive in the environment and their competition with other microorganisms for resources, bacteria produce antimicrobial compounds to inhibit or kill other competing strains. All bacteria produce some types of antimicrobial peptides. These peptides are divided into two groups, the bacteriocins and the antibiotic peptides.[4]

Bacteriocins (klebocins or klebicins) are ribosomally synthesized natural antimicrobial peptides. These peptides are secreted by many varieties of bacteria for the purpose of killing other bacteria.[5] They differ from traditional antibiotics in having a relatively narrow spectrum of action and being lethal only for bacteria which are closely related to the producing strains. Andre Gratia was discovered the first bacteriocin in *Escherichia coli* since 1925. Bacteriocins of *Klebsiella* have been first described by Hamon, and Peron in 1963.[6]

Klebocins were proteins encoded by Klebocinogenic plasmid and also chromosomally encoded. They are produced by *Klebsiella* species carrying a Klebocinogenic plasmid that bears the genetic determinants for Klebocin synthesis, immunity and release.[7] Klebocins are organized into three domains, each one involved in a different step of the process of killing sensitive bacteria. Klebocins could exert their action by first binding to specific receptors, which are outer membrane proteins used for the entry of specific nutrients then translocated through the outer membrane and transit through the periplasm by either the Tol or TonB systems.[8] Klebocins would reach their target and act either by forming a voltage-dependent channel into the inner membrane or by using their endonuclease activity on DNA, rRNA or tRNA.[9]

The present study aimed to detect bacteriocin gene among *K. pneumoniae* isolates in Baghdad city.

MATERIALS AND METHODS

Samples Collection

Thirty one isolates of *K. pneumoniae* were isolated from sputum samples collected from patients suffered from pneumonia admitted at Baghdad Medical City (Baghdad Teaching Hospital and Gazi Al-Hariri Hospital) during the period from January/ 2015 to May/ 2015.

Identification of Isolates

The identification of all *K. pneumoniae* isolates were done by inoculation on blood agar and MacConkey agar for primary identification. They were incubated aerobically at 37˚C for overnight. All *K. pneumoniae* isolates were identified to genus and species level based on the standard biochemical and microbiological methods.[10] API-20 E system was used to confirm the identification.[11] The confirmative tests have been crowned by VITEK-2 System.

Antimicrobial Susceptibility Test

Antimicrobial susceptibility test was carried against five antibiotics (Amikacin, Ampicillin, Augmentin, Azetreonam and Tetracycline) by using the disk diffusion method (Kirby-Bauer Method) as recommended by the Clinical Laboratory Standards Institute (CLSI). The results were interpreted in based on the CLSI, 2014.[12]

Amplification of klebicin gene cluster

DNA extraction was carried out for 50 *K. pneumoniae* isolates by using ZR Fungal/Bacterial DNA MiniPrep™ kit. The klebicin gene cluster was amplified from extracted DNA. A 2300bp klebicin gene cluster was amplified using the primer forward (5'-GCTCTGTAACC TTCAAGTTCTC-3') and reverse (5'-CAAGCAAGATTACGGTCTACTC-3').[9] The PCR reaction mixture was composed of 12.5µl of green master mix, 3µl of template DNA, 2µl of each forward and reverse primer (10µM), then the volume completed to 25µl by free nuclease water. Amplification was carried out with the following thermal cycling profile: initial denaturation temperature at 94^0C for 5min and 32 cycles consisting of (denaturation temperature at 94^0C for 1min, annealing temperature at 54^0C for 40Sec and extension temperature at 72^0C for 2min) and 5min at 72˚C for the final extension. Amplified DNA products were resolved by electrophoresis on 1% agarose gels containing RedSafe™ Nucleic acid staining.

RESULTS AND DSCUSSION

K. pneumoniae is considered one of the most common Gram negative bacteria.[13] It is also an important pathogen in nosocomial infections.[14] The incidence of microbial infections has been increasing in the past few decades. This has led to the continuous and uncontrolled use of antimicrobial drugs for prevention and treatment in several parts of the world. This, in turn, led to the emergence of specific drug and multidrug resistance among various strains of microorganisms including *K. pneumonia.*[15]

Antimicrobial susceptibility testing has shown different degree of resistance against several antibiotics as shown in Table (1). There is an evidence for increasing the antibiotic resistance. The higher percentage of resistance was recorded against ampicillin (64.52%), followed by augmentin came in second-step with (51.61%). On the other hand, the percentages of resistance against azetreonam and tetracycline were convergent (48.39% and 41.94% respectively), while the percentage was 32.26% for amikacin resistance. *K. pneumoniae*

resistance to multiple antibiotics as discussed, this is thought to be in relation with Kaye *et al*.[16]

Table (1): Antibiotic susceptibility profile of *Klebsiella pneumoniae* isolates by disk diffusion method (n=31).

Antibiotics	Resistant *K. pneumoniae* Isolates	
	No.	%
Amikacin	10	32.26%
Ampicillin	20	64.52%
Augmentin	16	51.61%
Azetreonam	15	48.39%
Tetracycline	13	41.94%

Amikacin generally works through inhibition of bacterial protein synthesis by binding to 30s ribosome leading to misreading of mRNA.[17] The study findings showed that ten *K. pneumoniae* isolates were resistant to amikacin with percentage (32.26%) which is close to the result (28.12%) that reported by Aljanaby and Alhasani, (2016) in Kufa city.[18] This result is lower than other studies documented by Bratu *et al*, 2005 (45%) and Castanheira *et al*, 2008 (53.3%).[19,20] Ampicillin is the first "broad spectrum" penicillin group of beta-lactam antibiotics and is part of the aminopenicillin family. It acts as cell wall inhibitor for bacteria by inhibition of the transpeptidase enzyme.[21] In present study, most isolates showed high degree of resistance to ampicillin (64.52%). These findings were in agreement with those reported by Alain *et al*[22], and Kevin *et al.*[23] The study showed that 16(51.61%) of *K. pneumoniae* isolates were resistant to Amoxicillin/Clavulanic acid. Other result was reported in a study carried out by Aljanaby and Alhasani (2016) in Iraq, on the *K. pneumoniae* isolates showed resistance (93.75%) to this antibiotic.[18] *K. pneumoniae* isolates were showed high resistance to azetreonam. Fifteen *K. pneumoniae* isolates (48.39%) were resistant to azetreonam. This prevalence rate of azetreonam resistance was higher than these reported from 1998 to 2010 by Sanchez *et al*.[24] The study revealed that the tetracycline resistance was 41.94% which was higher than result reported by Aljanaby and Alhasani (2016), who found that the tetracycline resistant *K. pneumoniae* was 34.37%.[18]

Klebicins are classified into two groups, A and B, based on cross resistance[25], their mode of action on the target cell, result in pore formation in the outer membrane of the target cell making ionic channels in it. The other endonucleases klebicins hydrolyze the nucleic acid of the target cell in the cytoplasm.[26] Only two bacteriocins from *Klebsiella* have been sequenced, klebicins B[26] and CCL (NCBI accession AF190857). The klebicin B operon is a

chimera, composed of short regions each with a different evolutionary origin.[26] Klebicin gene cluster among 31 of *E. coli* isolates were conducted using specific primers. Figure (1) shows the result of PCR amplification. PCR products corresponding to Klebicin gene cluster (2300bps) were appeared in 15 isolates (48.39%).

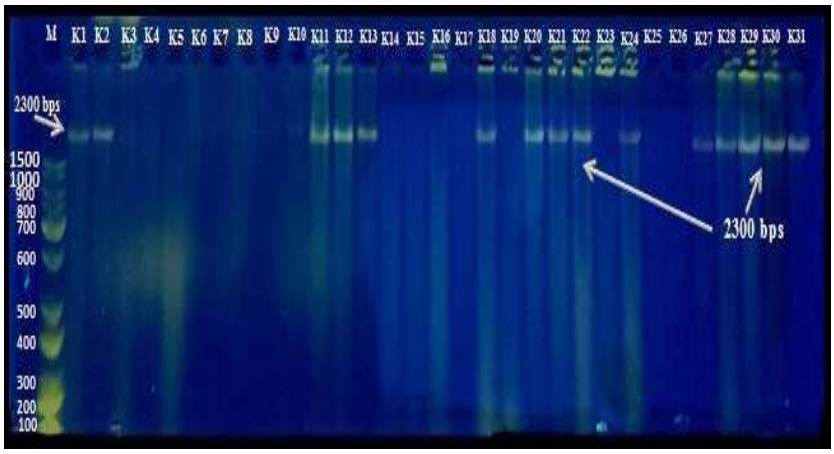

Figure (1): Analysis of *Klebicin gene cluster* of *Klebsiella pneomoniae* isolates. Lane(M): 100bp DNA Marker. Lane(K1,K2,K11,K12,K13,K18,K20,K21,K22, K24,K27,K28,K29,K30,K31): *Klebsiella pneomoniae* isolates positive for *Klebicin* gene cluster. All isolates are positive for *Klebicin* gene cluster and they are generated (2300bp) PCR product.

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