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Study of ecpA prevalence with biofilm formation ability in *Klebsiella pneumoniae* isolated from Respiratory Tract of Cattle in Certain area of Iraq

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Abstract: **In order to determine the percentage of Klebsiella pneumoniae which isolated from the upper and lower bovine respiratory tract, to determine a biofilm formation ability of these isolates, in addition to detection the presence of ecpA gene in these strains using conventional PCR assay. One hundred nasal swabs and same number of lung tissue samples were taken randomly from apparently healthy and diseased cattle that expressed respiratory clinical signs of different ages and gender and they were processed in laboratory of Veterinary Medicine College. The K. pneumoniae biofilm formation ability was detected by spectrophotometric method. Results of bacterial isolation showed that unhealthy animals exposed 7 (3.18%) of K. pneumoniae in lung samples and 4(1.8%) from nasal passages while apparently healthy animals revealed 1 (0.45%) and 3 (1.4%) K. pneumoniae isolates from lung and nasal passages respectively. The biofilm formation result showed that 10 (66.7%) were positive formers while negative formers composed 5(33.3%). Results of PCR assay revealed that 14(93.3%) of K. pneumoniae isolates had ecpA gene while 1(6.7%) of these isolates were lacked this gene.**

Keywords: **Respiratory tract, Klebsiella pneumoniae, biofilm formation, PCR, ecpA gene.**

I. INTRODUCTION

Bovine respiratory disease took into consideration as main economical disease resulted in comprehensive losses in the dairy and beef industries [1]. [2] mentioned that the infection has diversified etiology such as biological, nutritional and environmental factors that resulted in important economic problems among livestock leading to significant morbidity and mortality. There were microbiota situated as the bovine upper respiratory tract ubiquitous flora but they are turn into opportunistic pathogens when immune system impairment such Staphylococcus, Streptococcus spp., K. pneumoniae and etc. which can reach lung by inhalation then colonized in lung tissue and encouraged pulmonary tract infections such as bronchopneumonia [3]. However, K. pneumoniae was widespread opportunistic microbiota of human and animal that can infect large and small ruminant, horse, wild and companion animals [4].

[5] and [6] illustrated that the K. pneumoniae belongs to Enterobacteriaceae family and hypermucoid strains of this species induced invasive infections in human and animals species mainly mastitis in dairy cattle and bovine respiratory diseases. K. pneumoniae was important respiratory tract microbiota of all domestic animal species as well as cow milk and dry udder secretions and it was highly significant in dairy cattle due to remediation difficulties [7]. [8] reported that there were a wide spread of multidrug resistant K. pneumoniae among animals and human including third generation Cephalosporin (3GCs) and antibiotic resistance transmission between human and animal resulted in the important public health and economic troubles. [5] explained that K. pneumoniae virulence and resistance factors explicit by capsular polysaccharides, adhesins, siderophores and antimicrobial determinants which responsible for K. pneumoniae infections.

Moreover, host immune response resisted by a biofilm via restraint phagocytosis, opsonization process and mechanical host barrier such as organism removal by mucocillary system [9].

In Iraq, there is little study about the features and percentage of K. pneumoniae, therefore, the aims of current study were to isolate and identify K. pneumoniae and determining their owning of ecpA gene with biofilm formation ability.

II. MATERIALS AND METHODS

1. Sampling:

Total of (200) Slaughtered cattle's lung tissue and nasal swabs specimens were collected from pneumonic and apparently healthy cattle. Each sample was placed in separate sterile containers and tubes which were fully labeled with the date, tissue and animal identification.

These containers and swabs tubes were transported under cooled condition as soon as possible to laboratory for bacteriological examination.

2. Isolation and identification of K. pneumoniae:

The isolation of K. pneumoniae was accomplished according to [7] and the purified colonies were identify according to [13];[10], while [11] was confirmed diagnosis of purified K. pneumoniae colonies by detection presence of ecpA gene using conventional PCR assay.

3. Biofilm assay:

Quantitative biofilm formation assay (Spectrophotometric method) was performed according to [12] which include: Briefly, a colony from each isolated K. pneumoniae was grown in BHIB at 37ºC for 18hrs. 20µl of a bacterial suspension from broth culture of 0.5 McFarland standard (1.5×10 CFU/ml) was used to inoculate 96-well polystyrene plates containing 180 µl of BHIB, then the plates were incubated at 37 ºC for 24 h. Thereafter, the wells were evacuated from the medium, then were rinsed thrice with 1X PBS and fixed with 2% formalin at 4 ºC for 1 hr. Instantly, the wells were stained with 200 µl of 1% crystal violet for 20 min. Excess dye was eliminated, and 200 µl of absolute methanol was added to the wells. Determined color intensity using a Multiskan or Elisa reader at a wavelength of 620 nm. The ranking of biofilm-producing strains was established based on the absorbance readings as following: non biofilm producer, low, medium and high biofilm formers.

4. PCR assay:

The PCR assay was accomplished according to [14].

III. RESULTS

1) Bacterial isolates:

A total percentage of K. pneumoniae from lung tissue of diseased animals were 7(3.18%) while from nasal swabs were 4(1.8%) and total percentage of K. pneumoniae from lung tissue of healthy animals were 1(0.45%) while from nasal swabs were 3(1.4%).

2) Bacterial identification:

The fifteen bacterial isolates are Gram negative rods, straight, arranged singly or in pairs, their colonies on the MacConkey agar are pink, lactose fermenter and mucoid. They are non-motile and gave negative reaction for oxidase and a positive result for catalase. These features may indicate that the isolates are K. pneumoniae.

3) Biofilm assay:

Biofilm forming isolates categorization depending on absorbance readings obtained from ratio of blank (0.069) and positive control (0.744). If absorbance readings of isolates (<0.138) were considered non - biofilm producers; low biofilm -formers values ranked between (0.139 -0.276); absorbance reading value between (0.277-0.414) were deemed as medium biofilm-producer isolates, whereas isolates were classified as high biofilm formers with absorbance greater than (>0.415). Our analysis showed that 10/15 (66.7 %) of K. pneumoniae strains were able to form biofilm. Furthermore, 1/10(10%) of

strains were high biofilm formers, 5/10(50%) were medium biofilm formers and 4/10 (40%) were low biofilm former whereas 5/ 15(33.3%) were non biofilm formers.

4) Prevalence of ecpA gene:

The result of PCR assay revealed that 14 (93.3 %) of K. pneumoniae isolates were owned ecpA gene while 1(6.7%) of these isolates were lacked this gene as mentioned in the appendix.

IV. DISCUSSION

K. pneumoniae is the main opportunistic pathogen responsible for worldwide nosocomial and acquired community infections as meningitis, pneumonia and urinary tract infection [15] with tremendous range of reservoir sources as organic and inorganic bedding materials, soil, water, plants, dairy herds and their utensils and bovine skin, mucosae and feces[16]. K. pneumoniae infection controlling was depended on infected cases diagnosis and treatment, in current study, endeavors to isolate and identify K. pneumoniae from upper and lower respiratory tract of apparently healthy and unhealthy cattle were achieved.

The present findings disclosed that the unhealthy animals exposed high percentage of isolates as compared with the apparently healthy ones which may indicated that K. pneumoniae associated with respiratory tract infections of cattle, this result was in conformity with [7] in Egypt, who registered that high incidence of the Klebsiella spp. 62 (25.2 %) which isolated from (246) ailing animals from cattle predominantly with isolation percentage form (25.6 %) followed by sheep which consisted (24.2 %) and they detected that 44 (17.9 %) of inspected animals were revealed K. pneumoniae, also [17] found that K. pneumoniae occupied about 11 (64.7%) of 17 mastitic animals.

The actual finding elucidated that the isolated K. pneumoniae proportion from lung tissue 7 (3.18 %) was higher than nasal cavity isolates 4(1.8%) of animals expressed respiratory signs ,this result may indicated that animals may exposed to exertion factors that facilitate K. pneumoniae reaching, a normal flora to alveolar spaces ,this proof was concurrent with results of isolated bacteria from apparently healthy animals that exhibited high percentage of nasal cavity bacterial isolates 3(1.4%) as compared with proportion of lung isolates 1(0.45%).

The present result was in agreement with [7], who stated that (28.7 %) of the nasopharyngeal swabs from cattle were positive for K. pneumoniae isolates which followed the fecal samples isolates percentage, also [18] reported that the nasal and bronchial mucosa swabs of mammals and human suffered from respiratory sign such as severe pneumonia expressed positive K. pneumoniae isolates.

K. pneumoniae proportion isolated from lung tissue in present study was agreed with the finding of [19], who proved that this pathogen was isolated from (15.9 %) of sheep lung tissue suffering from pneumonia and pulmonary abscesses. isolated K. pneumoniae from nasal cavity of seemingly healthy animals may corroborate some authors statements which stipulated that K. pneumoniae is a nasopharyngeal as well as intestine normal flora of humans and animals [20]. However, environment may consider as important cattle infection source by K. pneumoniae in which this pathogen are present in soil, drinking water and plants.

Our result reported that 10 (66.7%) of K. pneumoniae isolates had biofilm creation ability which indicated that these local isolates were responsible for the cattle respiratory infection as long as K. pneumoniae can using biofilm in early infection stage [21].

The biofilm considered prime K. pneumoniae virulence factor responsible for infection, act as bacterial assistant to overcome antibiotic treatment along various biofilm formation stages (planktonic cells at mid-log and stationary phase, adherent monolayers and mature biofilms) and prevention C3b and IgG activity resulted in bacterial infection permanency [22]; [23].

The actual resule showed that among (15)bacterial isolates 10(66.7%)were biofilm producers, this result may referred to their survival ability in cattle flock environment and this was consent with [24], who mentioned that miscellaneous bacterial biofilm formers had survival ability in hospital circumference, surgical wound and medical implant, nevertheless the present result was inconsistent with [11], who proved that the K. pneumoniae was (100 %) biofilm inducers while our result correlated with [24], who reported that (76%)K. pneumoniae isolates were biofilm creators.

K. pneumoniae possessed certain genes such as the fimbrial ecpA gene that had biofilm production and drug resistant responsibilities, thus, our PCR assay results confirmed the result of biofilm formation screening and also supported idea of our isolates had variable virulence degree.

Our result found that 14 (93.3 %) among (15) K. pneumoniae isolates owned (ecpA) gene which corresponded with [14], who found that (66/69) clinical strains of K. pneumoniae had ecpA gene (96%) whilst [11], found that 50/ 50 (100%) of K. pneumoniae isolates possessed ecpA gene.

The changeful percentage of ecpA existence relied on nature of K. pneumoniae fimbriae that played a role in bacterial adherence to epithelial cells, biofilm formation on abiotic and biotic surfaces and considered as substantial virulent agent correlated with K. pneumoniae pathogenesis [25]. E. coli common pilus (ECP) is extracellular adhesive fiber disseminated in outer bacterial membrane, encoded by the chromosomal ecpRABCDE operon which substantiated firstly in association with E. coli strains, thereafter found in many other serious pathogenic Enterobacteria as Citrobacter rodentium, Shigella boydii, Enterobacter and K. pneumoniae which appear in (90 %) in the latter.

There was suggestion that (ECP) plays a dual role in early biofilm developmental phase and host cells discrimination [26]; [14]. It was recorded that (90 %) of K. pneumoniae strains isolated from hospital acquired infections produced ECP and biofilm formation that mediated chronic infections and resistant immune response [14].

V. CONCLUSIONS

The local K. pneumoniae isolates were biofilm producer strains as well as they expressed ecpA gene which responsible for biofilm formation and antibiotic resistance.

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