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Phenotypic and genotypic characterization of *Klebsiella* species recovered from camels

A Thesis submitted

By

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For The Degree of Master In Veterinary Medical Sciences

(Microbiology)

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Abstract:

Klebsiella is one of the important bacteria that causes diseases in camels and human .This study included 44 *Klebsiella* strains were recoverd from 150 camels with an overall incidence 29.3% .The sample were collected from slaughtered house in El-qaliyobia and El-Monofia governorates. The biochemical identification reavealed that 12 isolates were *K.pneumoniae*, 26 *K.oxytoca*, 6 *K.rhinoscleromatis*. Antimicrobial susceptibility was tested by disk diffusion method indicated that the most effective antibiotic were Meropenem 95.4% followed by imipenen 81.8% and cefatizidime 54.5%. To test the virulence of the microbes many test were applied like Congo red binding assay.The mannose resistant haemagglutination pattern against red blood cells of cows, sheep, horse and human, the ability of the strain to produce enterotoxin and cytotoxicity of strains to Vero cells .12 strain of *K.pneumonia* and 12 strain of *Klebsiella oxytoca* ,6 strains *K.rhinoscleromatis* screened via PCR for four virulence genes encoding (*fim*H ,*tra*T,*iut*A ,*mag*A). All the strains were positive to *fim*H and *tra*T while one strain *K.oxytoca* not harbor *iut*A and all isolates don't possess *mag*A. Fifteen culturally positive *Klebsiella* strains (5 *K.pneumoniae* , 5 *K .oxytoca* and 5 *K.rhinoscleromatis*) were confirmed to be *Klebsiella* by PCR using genus specific primer *gyr*A , All 15 strains were *Klebsiella* isolates. To differentiate between the type of *Klebsiella* and the emphasis on the biochemical tests using the polymerase chain reaction test, the following genes were used *16S-23S ITS* , *peh*X and *pho*E for *K.pneumoniae* ,*K .oxytoca* and *K. rhinoscleromatis* respectively.

Keywords: *Klebsiella* species, dromedary Camels, Virulence genes, Antibiotic susceptibility

ACKNOWLEDGMENT

My thanks are submitted first and for most to Allah who gave me the strength and ability to complete this work.

I would like to express my deep thanks to my ***Professor Dr. Kamelia Mahmoud Osman,*** *professor of Microbiology Faculty of Vet. Med. Cairo University for her valuable smooth supervision, advices and continuous encouragement to complete this study and time she has devoted to the fulfillment of this work.*

My sincere gratitude and appreciation are also extended to ***Dr. Amany Ibrahim El bialy*** *chief Researcher at Animal Health Research Institute– Dokki, for her kind help, constant encouragement and generous advice during the performance of this study.*

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BHI	: Brain heart infusion
CAIs	: Community acquired infections.
CDC	: Centers for Disease Control and Prevention.
CPS	: Capsular polysaccharides
CR	: Congo red
DNA	: Deoxyribonucleic acid.
ESBL	: Extended spectrum beta lactamase.
ETC	: Extracellular toxic complex (ETC)
ETEC	: Enterotoxigenic E-Coli
***fim*H**	: Gene for detection of type 1 fimbriae
***gyr*A**	: DNA gyrase gene
Int	: Intermediate
(ITS)sequence	: internal transcribed spacer (ITS) gene sequence
***iut*A**	: A hydroxamate siderophore whose receptor
KPC	: *Klebsiella* producing carbapenemases
LPS	: Lipopolysaccharide
LT	: Heat-labile enterotoxin
***mag*A**	: Mucoviscosity-associated gene A
MDR	**:** Multidrug resistance.
MERS	: Middle East Respiratory Syndrome
MLST	**:** Multilocus sequence typing
MR	**:** Mannose resistant
MS	**:** Mannose sensitive
PCR	**:** Polymerase chain reaction.
***peh*X**	**:** Exopolygalacturonan Hydrolase X
***pho*E**	**:** phosphate porin gene
Res	**:** Resistant

***rmp*A**	: The plasmid gene confers a hypermucoviscous phenotype
Sen	**:** Sensitive
16S-23S ITS	: 16S ribosomal RNA internal transcribed spacer
***tra*T**	: Gene encodes an outer membrane protein
TSI	: Triple sugar iron agar.
UTIS	: Urinary tract infections.
VFs	: Virulence factors
XLD agar	: Xylose Lysine desoxycholate agar
YbtS	: yersiniabactin

1. Introduction

Klebsiella species are opportunistic pathogen that cause infection when the immune system is compromised. a number of factors contribute to virulence and pathogenicity in *K. pneumoniae* such as the capsular serotype, lipopolysaccharide, iron-scavenging systems and adhesions (**Wu, 2014**).

Camel is a uniquely morphological & physiological adapted animal in desert ecosystem with good potential to thrive well on meager resources under extreme climatic conditions. It has important utilities in human society such as drought, farming, milking and many other important farming purposes but sometime certain sudden environmental variations make this animal susceptible to various infections **(Kebede and Gelaye, 2010).**

K. pneumoniae capsular type 11 was isolated from the lungs of two adult camels in India affected by 'Khurak' a condition characterised by respiratory distress, pyrexia, prolonged cough, high morbidity but low mortality. *Klebsiella sp*. were iolated from lungs of slaughtered camels in Egypt (**Thabet, 1993**), Jordan (**Al-Tarazi, 2001**)**,** Mauritania (**Kane *et al*., 2005**), Pakistan (**Zubair *et al.*, 2004**) and Saudi Arabia (**Abdulsalam and Alhendi, 1999)**.

Infected camels can spread infections to human during meat processing and during migration (**Teshome *et al* .,2003**). Infection with pyogenic bacteria produces subsequent tissue injury in human and progresses to disease. For example, *Klebsiella pneumonia* causes severe respiratory tract infections in humans due to destructive changes, inflammation, hemorrhage, and necrosis that occur in pulmonary tissue **(Hackstein *et al* ., 2013)**. Pneumonia, liver abscess, and meningitis have

also been reported following infection with *Klebsiella* spp **(Shon *et al* ., 2013).**

The increase in resistance of Gram-negative bacteria is mainly due to mobile genes on plasmids that can readily spread through bacterial populations. Standardised plasmid typing methods are enhancing our understanding of the host ranges of these elements and their worldwide distribution (**Carattoli, 2009**).

The treatment of infections caused by *Klebsiella pneumoniae* is always problematic, because the bacterium is naturally resistant to benzylpenicillin (penicillin G) and aminopenicillins (ampicillin) (**Gould, 2016**).

Antibiotics may either kill or inhibit the growth of bacteria. Some strains of *Klebsiella pneumoniae* are resistant to a wider range of antibiotics. Resistance of *Klebsiella* to previously sensitive antibiotics is also increasing in the recent years due to overuse and misuse of antimicrobial agents and or natural causes (**Murray *et al.*, 2005**).

Only a few of the antimicrobial agents are effective for the treatment of severe nosocomial infections due to their natural resistance of ESBL producing *K.pneumoniae* towards ampicillin and amoxicillin. Infections caused by ESBL-producing pathogens are problematic because when co-resistance to other antimicrobial class is present, limited antibiotic options are available currently, imipenem or meropenem is regarded as the drug of choice for infections caused by ESBL-producing pathogens (**paterson and Bonomo ., 2005**).

Infections caused by multidrug-resistant bacteria are increasing in many countries.The selective pressure resulting from the use and overuse of antibiotics are important risk factors for the acquisition of drug-

resistant bacteria. Isolates carrying class 1 integrons were more likely to be resistant to cefotaxime, ceftriaxone, ceftazidime, amoxicillin-clavulanic acid, aztreonam, ciprofloxacin, tobramycin, tetracycline, trimethoprim-sulfamethoxazole, gentamicin, and cefepime than integron-negative isolates (**Peerayeh *et al* ., 2014**).

The fimbria has been described as a microbial surface component that mediates specific attachment to eukaryotic cell membrane. Fimbrial mediated adherence has been proposed as an important virulence factor in the development of urinary tract infection. Adherence of pyelonephritic *E.coli* has been correlated with their ability to cause a D-mannose resistant haemagglutination of human erythrocytes and their degree of virulence may be related to the mannose content of the capsular polysaccharide (**Duncan *et al* ., 2005)** .

Haemagglutination by Type 1 fimbriae termed as mannose sensitive (MS), inhibited by D-mannose, bind to mannose containing receptors**.** The second heterogeneous class of fimbriae, produce mannose resistant (MR) haemagglutination, is not inhibited by D-mannose and bind to a variety of receptors present. Both MR and MSHA fimbriae are produced during Urinary Tract Infections (UTIs) and cause bacteria to adhere to urinary tract epithelium, though MSHA fimbriae type I are non-virulent determinants, colonize better than non-fimbriate strains (**Grover *et al* .,2013)**.

The factor responsible for *K. pneumoniae* pathogenicity includes the production of heat -labile and heat-stable endotoxins.The lipopolysaccharide has been linked with the extensive tissue necrosis that complicates *Klebsiella* infections. The production of an extracellular toxic complex (ETC) that has been shown to be responsible in mice for lethality and extensive lung necrosis is composed of 63% capsular

polysaccharide, 30% lipopolysaccharide, and 7% protein .Mucoid strains of K1 or K2 serotype were more virulent to mice than non-mucoid strains of the same serotype (**YU *et al* ., 2007)**

The production of heat-labile enterotoxin (LT) by *Klebsiella* spp cause the main role in the pathogenesis of diarrhoea. The genes encoding enterotoxins are located mostly on the plasmids and hence they can be transferred among G (-) microorganisms, such as *Klebsiella pneumoniae* (**Mavziutov *et al* ., 2007).**

In *K. pneumoniae* the type 3 fimbriae mediate the biofilm formation on biotic and abiotic surfaces .Biofilm are complex three-dimensional structure formed by communities of inter or intra species microorganisms attached to a surface or interface enclosed in an exopolysaccharide matrix of microbial and host origin (**Murphy and Clegg , 2012) .**

Biofilm provides protection to antibiotic treatment, attack by phagocytosis and harmful molecules and facilitates bacterial communication leading to expression of virulence determinants. Biofilm formation proceeds in two stages a rapid attachment of the bacteria to the polymeric surface is followed by a more prolonged accumulation phase that involves cell proliferation and intercellular adhesion. The ability to adhere to materials and to form biofilm is an important feature in the pathogenesis of *Klebsiella* associated community acquired infections (CAIs) due to the colonization of the polymeric surface by forming multi-layered cell clusters, embebbed in extra cellular material (**Vuotto *et al* ., 2014**) .

Multiple *Klebsiella* components (e.g., fimbriae, siderophores, O antigens, and capsular antigens) have been considered to be potential virulence factors .Of these factors, capsular antigens are probably