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Research article

Phylogenetic analysis of zoonotic *Acinetobacter* spp. isolated from Geoffroy's bat *(Myotis emarginatus)*, Northern Iraq

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Abstract

Bacteria that belong to the genus; *Acinetobacter*, especially *A. baumannii*, are considered as major multidrug-resistant nosocomial bacteria in hospital settings and intensive care units. This bacterium can be isolated from different animal species, and because its highly pathogenicity, control measures should be taken to lessen the animal sources and decrease the spread of this pathogen to public. The aim of the present study was to isolate and evaluate the phylogenetic status of the zoonotic *Acinetobacter* spp. from the Geoffroy's bat (*Myotis emarginatus*). The sample collection included 35 bats captured in some caves in Northern Iraq. Intestine parts and swabs were taken from each bat. The specimens were subjected to bacterial cultivation processes and *16S rRNA* gene and *beta lactamase* (*bla* $_{TEM-1}$) dependent polymerase chain reaction (PCR) methods to detect the genus level and the virulence activity, respectively. The phylogenetic tree was built utilizing the *16S rRNA* gene. The findings of the bacterial cultivation revealed the presence of the bacterium in 23 (65.7%) of the collected bats; however, the *16S rRNA*-PCR showed that only 10 (28.57%) of the bats demonstrated the incidence of this microorganism in their intestines. The *bla* $_{TEM-1}$-PCR reported that 4 (40%) isolates of the *16S rRNA*-PCR positive bats carried the β-lactamase gene in their genetic materials. The phylogenetic tree showed that the genetic similarity of the current study isolates was closely related to those from Egypt and China. The present data show that *Acinetobacter* spp. is present in the intestine of the Geoffroy's bat (*Myotis emarginatus*) located in some caves from Northern Iraq, and some isolates have virulence potential represented by the composition of the beta lactamase gene.

Keywords: *Acinetobacter*, Antibacterial resistance, Bats, *Myotis emarginatus*

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INTRODUCTION

The genus *Acinetobacter* is very diversified, including both positive and -negative oxidase activity, with no pigment, Gram-negative coccobacilli. There are over 50 different species of *Acinetobacter*, even though most of them are harmless environmental bacteria. Infectious strains of *Acinetobacter* are most often associated with *A. baumannii*, *A. lwoffii*, and *A. calcoaceticus*. In rare cases, other species of *Acinetobacter* have also been recorded as pathogens. These include *A. haemolyticus, A. johnsonii, A. junii, A. pittii, A. schindleri, A. nosocomialis,* and *A. ursingii*. Animal model investigations and multidimensional analyses of clinical evidence have shown that *A. baumannii* is the highest dangerous of all the species studied (Chiu et al., 2015; Wong et al., 2017).

The major issue is confirmed by the rapidity in which resistance genes may disseminate over the globe. The World Health Organization (WHO) acknowledged the alarming rise in the prevalence of multi-drug-resistant pathogens as a serious danger to global health (Bengtsson-Palme et al., 2018; Urban-Chmiel et al., 2022).

The members of the genus *Acinetobacter* spp. are widely known as environmental bacteria with some important exceptions of serious pathogens that could play potential role in human infections, especially those transmitted to patients in the ICUs. This effect is due to the fact that the environmental members of this genus can be transformed into highly resistant and virulent bacteria via their components of resistance gene, particularly beta lactam resistance (Jeon et al., 2015; Vázquez-López et al., 2020).

Acinetobacter spp. are prevalent in water, whether it be soil or sludge, a wetland, a pond, a sewage processing facility, a fish farm, or even the ocean. These environmental isolates may be significant environmental repositories for resistance components that change into clinically significant bacteria because they often include antibiotic resistance systems such as carbapenemases and extended-spectrum-lactamases (ESBLs) (Endo et al., 2012; Wongsrichai et al., 2021). Vegetables, meat, dairy product lines, and human skin have all been shown to have bacteria of medical interest, including the species *A. calcoaceticus, A. lwoffii, P. pittii*, and *N. nosocomialis*, Antibiotic-resistant gene elements have been found in such bacteria. Several environmental pathways have been suggested for the spread of *A. baumannii* strains with high levels of antibiotic resistance from animals to humans. These strains have been found on commercially available meat, vegetables, and a variety of animals. Monitoring investigations of skin colonization, especially in healthy persons, have shown that non-baumannii *Acinetobacter* spp. was predominant, while *A. baumannii* is seldom found as a colonizing organism of the skin among healthy subjects (Visca et al., 2011; Atrouni et al., 2016; Salzer et al., 2016).

In one study, 17 percent of healthy Texas military members had strains of the *A. baumannii-A. calcoaceticus* complex. However, these strains were genetically different from the bacteria discovered in affected troops returning from Iraq and Afghanistan, suggesting that they were not the cause of the infections. Because of this, it seems that healthy people seldom carry more dangerous species and strains (Griffith et al., 2006).

Nonetheless, *Acinetobacter* illnesses have increased in incidence and seriousness in recent decades as a result of the widespread and intensive use of mechanical ventilation, central venous and urinary catheterization, and antimicrobial treatment. These days, *Acinetobacter* infections have quickly expanded across healthcare facilities anywhere in the world. Infection rates are significantly greater in Intensive Care Units (ICUs). According to monitoring data from the 2009–2010 academic year of the United States National Healthcare Safety Network (NHSN), 1.8% of all healthcare-associated illnesses were caused by *Acinetobacter* spp (Sievert et al., 2013).

Geoffroy's bat (*Myotis emarginatus*) is thermophilic species, which lives in different parts of Europe and some countries in Asia, such as in Iraq and Iran. The bat uses caves and cracks of building walls as nesting houses. Metagenomic studies have found that this bat can include *Acinetobacter* spp. as one of the abundant fecal microbiota (Vengust et al., 2018). The presence of this bacteria in the gastrointestinal tract of this bat can alarm questions about its importance in spreading this bacterium to human beings. Thus, the current study was intended to isolate and evaluate the phylogenetic status of the zoonotic *Acinetobacter* spp. from the Geoffroy's bat (*Myotis emarginatus*).

MATERIALS AND METHODS

Ethical statement

All animal care and use in the current study were followed using institutional, national, and international ethically-approved protocols.

Collection of bats and specimens

The sample collection included 35 bats (*Myotis emarginatus*) captured in some mountain caves in Northern Iraq, during December to March, 2019. Euthanasia was used to sacrifice the bats. Intestine parts and swabs were taken from each bat. The specimens were subjected to bacterial cultivation processes and *16S rRNA* gene and *beta lactamase* (bla_{TEM-1}) dependent PCR methods to detect the genus level and the virulence activity, respectively. The work was performed in the Laboratory of Microbiology, College of Veterinary Medicine, University of Al -Qadisiyah, Al-Diwaniyah City, Iraq.

Bacterial isolation

The swabs from the intestine parts were cultivated on blood agar and aerobically incubated overnight at 37˚C for 24–48hrs. The morphological characteristics of colonies, Gram stain, and biochemical features were followed for the identification of the bacterial genus using methods described from (Fernando et al., 2016).

Molecular methods

DNA extraction

The DNA was extracted using the Genomic DNA Mini Kit (Geneaid) and following its protocol after *Acinetobacter* spp was cultivated in nutrient broth. The DNA was evaluated using a NanoDrop to determine the product quality and quantity. The DNA was deep-freeze-stored.

Polymerase chain reaction

The PCR reaction for each tube was prepared according to the kit manufacturer. The reaction included the use of the *16S rRNA* gene (for sequencing purposes) primers; F: CTGCCTATTAGTGGGGGACA and R: GCACCTCAGCGTCAGTGTTA (Khosravi et al., 2015), and the *bla* $_{TEM-1}$ gene (as an indication for ESBL-based virulence) primers; F: CGC CGC ATA CAC TAT TCT CAG AAT GA and R: ACG CTC ACC GGC TCC AGA TTT AT (Khalilzadegan et al., 2016). The PCR thermocycler employed the following conditions: for the *16S rRNA* gene, initial one-cycle of denaturation (4mins-94°C), 30 cycles of the following: denaturation (1min-94°C), annealing (40s-60°C), and extension (70s-72°C), and one-cycle of final extension (10mins-72°C). For the *bla* $_{TEM-1}$ gene, initial denaturation (4mins-94°C), 30 cycles of (denaturation (1min-94°C), annealing (40s-62.8°C), and extension (70s-72°C)), and final extension (10mins-72°C). The electrophoresis of the PCR products was run using a 1% agarose gel. The gel was visualized later utilizing a UV-equipped system.

Phylogenetic analysis

Only the *16S rRNA* gene PCR product was sent to Macrogen Company, Korea, for partial sequencing of the gene by using the Sanger sequencing method and employing the primers; F: CTGCCTATTAGTGGGGGACA and R: GCACCTCAGCGTCAGTGTTA. The phylogenetic tree was generated using NCBI websites and MEGA X software. In a brief, the clustalW was used, and the phylogenetic tree was constructed by evolutionary analysis by Maximum Likelihood method. Finally, the evolutionary analyses were conducted in MEGA X.

RESULTS

The findings of the bacterial cultivation revealed the presence of the bacterium in 23 (65.7%) of the collected bats; however, the *16S rRNA*-PCR showed that only 10 (28.57%) of the bats demonstrated the incidence of this microorganism in their intestines (Figure 1).

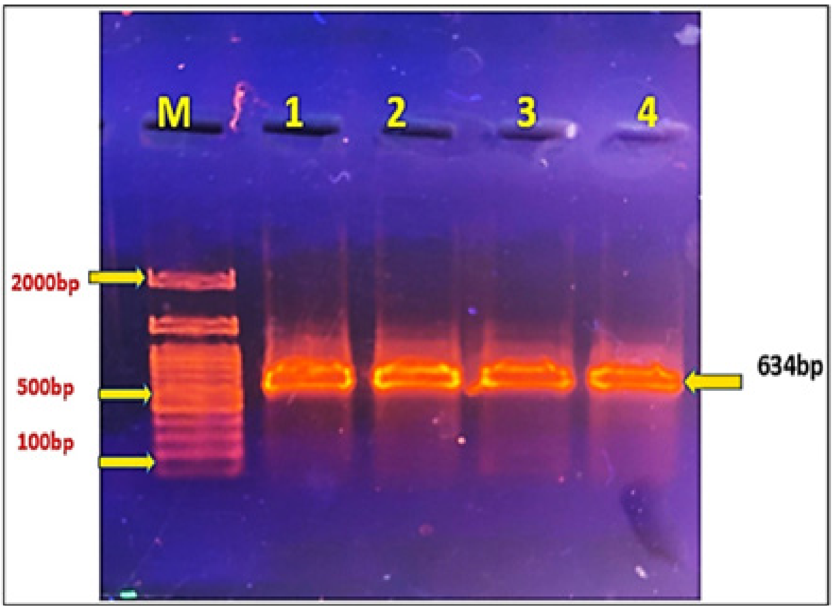

Figure 1 Electrophoresis image of the PCR products for the 16S rRNA gene-1% agarose gel of Acinetobacter spp from intestine samples of Bats. M lane: Ladder (100-2000), Lanes (1 to 4): positive PCR product samples at 634bp.

The *bla* $_{TEM-1}$-PCR reported that 4 (40%) isolates of the *16S rRNA*-PCR positive bats carried the β-lactamase gene in their genetic materials (Figure 2).

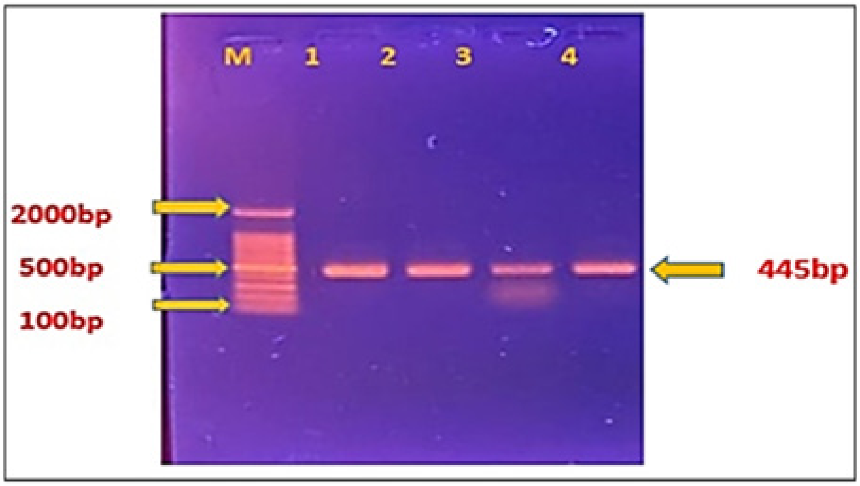

Figure 2 Electrophoresis image of the PCR products for the bla TEM-1 gene-1% agarose gel of Acinetobacter spp from intestine samples of Bats. M lane: Ladder (100-2000), Lanes (1 to 4): positive PCR product samples at 445bp.

The phylogenetic tree showed that the genetic similarity of the current study isolate (Acc. No.: MH997815.1) was closely related to those from China (Acc. No.: MT367764.1) and (Acc. No.: MN315450.1) (Figure 3) and as presented in Table 1.

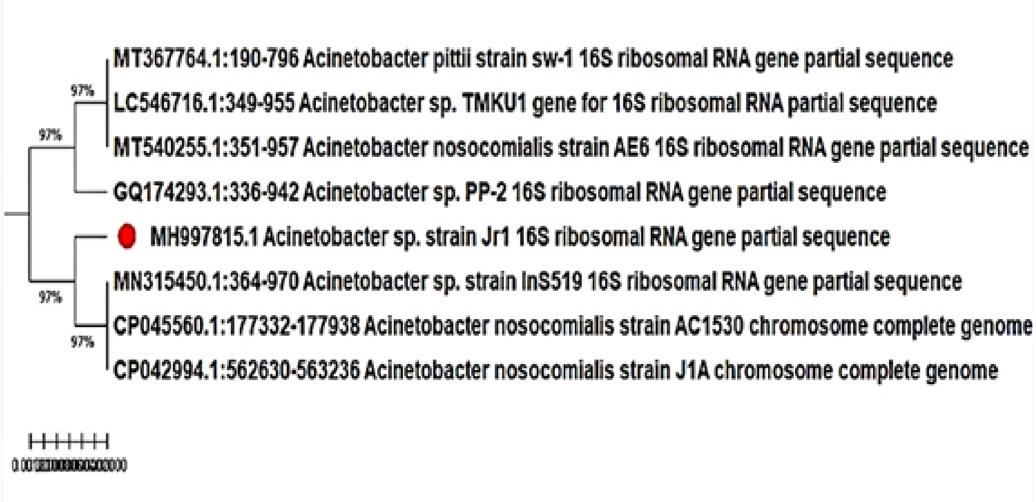

Figure 3 Phylogenetic tree for the 16S rRNA gene partial sequencing of Acinetobacter spp from intestine samples of bats.

Table 1 Identity of *16S rRNA* gene partial sequencing of *Acinetobacter* spp from intestine samples of Bats.

Accession number	Identity	Country	Host
MN315450.1	100%	China	soil InS05 from Antarctic
MT367764.1	99.84%	Egypt	*Aedes aegypti* adults
GQ174293.1	100%	China	Chunliu sewage
CP045560.1	99.3%		Homo-sapiens
CP042994.1	99%		Homo-sapiens

DISCUSSION

Numerous thousands of fatalities annually may be attributed to bacterial widespread resistance to antibacterial agents. The rise of antibiotic-resistant microorganisms, even to final option medications, is the biggest crisis humankind faces today (Thuan et al., 2022). The alarming growth of the problem that threatens population health on a worldwide scale and calls for international collaboration (Bengtsson-Palme et al., 2018; Urban-Chmiel et al., 2022).

One example of the potential pathogenic members of this group is *A. baumannii*, which is an opportunistic microorganism, often seen in people who are immune-compromised, especially those who have been in the hospital for a prolonged period of time (> 90 days). One, it is often found in water environments, and second, it has been demonstrated to colonize the skin and to be recovered in large quantities from the respiratory and oropharyngeal discharges of sick persons. Because of its broad antibiotic resistance spectrum, it has recently been labeled a "red alert" human pathogen, causing concern among medical professionals.

Some research indicated the presence of these bacteria, *Acinetobacter* members, in bats, as it is reported by Avena et al (Avena et al., 2016), who found that bat skin was dominated by two genera; *Pseudomonas* and *Acinetobacter*. Moreover, Korean research by Choy et al. (Cho et al., 2009) found that of 75 *Acinetobacter* isolated bacteria from two hospitals, and 61 members had genes for resistance to aminoglycosides.

The current work found that the *bla* $_{TEM-1}$ gene was detected in some of the recovered bacteria. This is an indication that these were ESBL-based isolates. It is important to mention that this gene needs only a limited number of specific single nucleotide polymorphisms (SNPs) to emerge bacterial isolates with high pathogenicity that could cause dangerous infection to public. Poirel et al (Poirel et al., 2003) found that *A. baumannii* isolated from patients in a hospital in France contained a clavulanic acid-inhibited ESBL, which comes with an agreement with current study results that showed the presence of these resistance genes in the bacterium isolated from bat. Khalilzadegan et al., (2016) reported that the bla_{TEM} gene was detected in 3.2% in their *A. baumannii* positive samples (22.3% 588) collected from the intense care units at hospitals in Tehran, Iran.

Our results indicated that the current sequenced isolate was nucleotide-similar to sequences from China recovered from oceanic and soil environments. The bacterium; *A. baumannii*, represents a major threat to hospital care units due to its resistance to a wide range of antimicrobial drugs. There could be some reasons that can be attributed for the similarity between the current study isolate and the Chinese ones. One reason is that bats feed on mosquitos that may carry infected blood with bacterial isolates imported by some means from China, such as human travel and imported animals. In addition, some bats decide to blood-feed directly on animals, including cows, which might be infected with the bacterium. The other reason is that some animal creatures, such as long-distance travelling birds, adapted to live in caves with bats. This co-presence may be considered as an ideal way for the transmission of the novel bacterium or its resistance-genes via plasmids to cave bats.

CONCLUSION

The present data show that *Acinetobacter* spp. is present in the intestine of the Geoffroy's bat (*Myotis emarginatus*) located in some mountain caves from Northern Iraq, and some isolates have virulence potential represented by the composition of the beta lactamase gene.

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CONFLICT OF INTEREST

No conflict of interest is declared.

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