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Phylogenetic Analysis of *Acinetobacter baumannii* Isolated from Veterinary Necessities

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Abstract

Acinetobacter baumannii can be isolated from different animal species, and because of its highly pathogenicity, control measures should be taken to lessen the animal sources and decrease the spread of this pathogen to the public. the present study aimed to evaluate the phylogenetic status of this pathogen isolated from different veterinary sectors using the 16s rrna gene so as to determine the extent of genetic relatedness of this bacterium between these sectors. eight isolates of a. baumannii were previously isolated from different veterinary necessities (slaughter houses, private veterinary clinics, and public veterinary centres). the isolates were confirmed at the species level through PCR amplification of 16s–23s ribosomal DNA using real-time PCR. the phylogenetic tree was constructed utilizing the sequences of the 16s rrna gene for these isolates and for a. baumannii from the gene bank. the data showed that all isolates were genetically similar, and the genetic similarity of the current study isolates was closely related to those from different countries including Saudi Arabia, Turkey, Brazil, Uk, Spain, Iran, and Iraq. The present data suggest that a. baumannii from this study could have come from those countries during the course of animal importation; in addition, there was a cross-transmission of these isolates between various veterinary sectors. this will recommend good hygiene practices to minimize the risk of bacterial transmission between different veterinary sectors.

Keywords: *Acinetobacter baumannii*, veterinary necessities, 16S rRNA, clonal relatedness.

Introduction

Acinetobacter species have been associated with serious infections, including bloodstream infections, meningitis, pneumonia, wounds, urinary tract infections, and infections of the skin and soft tissues [1]. Numerous sources of Acinetobacter baumannii have been identified and tested, including the raw meat of goats, camels, and sheep in Iran and the commercial raw meat of chicken, turkey, and pork in Switzerland [2]. Acinetobacter baumannii has been identified in veterinary settings and in a variety of animal species. Cattle were found to carry A. baumannii, particularly in their nostrils, and some of the isolates have also been connected to human disorders [3]. Dairy cows have higher prevalence rates of A. baumannii than do beef cattle and calves. Depending on the type of bird and the area it inhabits, this bacterium can also be discovered in the habitats of animals, birds, and wildlife [4].

A. baumannii isolate-infected animals are most likely nosocomial infections, based on trends of antibiotic resistance and genomic study. However, isolated isolates of A. baumannii have also been discovered from horses in different stables that were not suffering from any specific illness [3]. The presence of carbapenem-resistant A. baumannii in horses, commonly admitted to hospitals, poses a concerning public health risk. Companion animals like dogs, cats, and horses are significant in A. baumannii-related infection [2]. However, the natural reservoir of this bacterium remains unclear, and the role of Acinetobacter species in illnesses

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affecting hospitalized animals is poorly documented [3]. In addition, A. baumannii has been discovered to be present in a number of different species, including rats, rabbits, ferrets, snakes, and ducks. Companion, wildlife, and food-producing animals are all very different. Companion animal-to-human transmission is more likely since the A. baumannii isolates discovered in pets and humans are almost identical [5].

The 16S rRNA gene sequence analysis has proven useful in deciphering the makeup of the bacterial communities. Since the 16S rRNA gene is found in all bacteria, it is possible to infer the genetic links between all bacterial species by analyzing the 16S rRNA sequence, according to a number of publications [6].

The aim of this study was to use molecular analysis with 16S rRNA gene sequencing to establish phylogenetic tree relationships of A. baumannii collected from veterinary supplies. This is the first phylogenetic analysis of A. baumannii that has been reported in veterinary supplies, as far as we are aware

Material and Methods

Bacterial Isolates

A total of eight A. baumannii isolates were previously recovered from various veterinary sectors including slaughter houses, private veterinary clinics and from public veterinary centers [7]. The isolates were stored in brain heart infusion broth (Lab M, UK) with 30% glycerol at -20°C. All isolates were sub-cultured on to CHROMagar Acinetobacter (CHROMagar, France) and reconfirmed thorough PCR amplification of 16S-23S ribosomal DNA using the Realtime PCR.

DNA Extraction and Molecular Detection of A. baumannii Isolates

Garcia and his colleagues [8] state that the thermal separation method was used to separate the DNA specimens. 150 µl of the supernatant was then saved in order to be used as a "PCR DNA" template. A "nanodrop (Thermo Scientific, USA)" was employed to assess the purity and concentration of extracted DNA.

Real-time PCR was used to identify 16S-23S ribosomal DNA using the forward primer 5'CATTATCACGGTAATTAGTG and the reverse primer 5'AGAGCACTGTGCACTTAAG in order to detect A. baumannii. Twenty µl of Sybr green master mix (Add SYBR Master from Addbio/Korea) , 1 µl of each set of primers (10 pmol/µl for each primer) , 1 µl of sample DNA (50–100 ng/µl)، and 7 µl DNase/RNase-free water were used to create the PCR mixes. The PCR cycler was configured as follows in a qPCR Bio-Rad CFX96 (BIO RAD/Germany): After an initial denaturation period of 3 minutes at 95°C, there are 30 cycles of 45 seconds at 95°C, 45 seconds at 62°C, and 45 seconds at 72°C [9].

Gene sequence processing

Sequencing samples were chosen according to the sources of each sample. After the PCR product from several positive samples was chosen, it was sent to Macrogen, Korea, for sequencing analysis using both forward and reverse primers. The 16s rRNA gene was one of the genes examined in this work, and it was amplified using a set of broadly applicable primers [10]. Using BioEdit (V7.0.5.3) software, consensus sequences were created after the raw data from sequencing were aligned. These consensus sequences were then compared to nucleotide sequence data that was obtained from the GenBank database.

Phylogenetic analysis

Sequencing findings were compared using 16S rRNA gene sequence analysis using GenBank data and the NCBI's basic local alignment search tool program (http://www.ncbi.nlm.nih.gov). Molecular evolutionary genetic analysis software (MEGA 6.1) was used to create a phylogenetic tree using distance matrices and the Maximum Composite Likelihood substitution approach. A bootstrap study using 1000× data sets was used to determine how reproducible the node was for tree topology [11]. A total of 17 A. baumannii isolates (8 from this study and other 8 from NCBI and one *Staphylococcus aureus* strain from NCBI was used as outgroup, were used in this study to construct the phylogenetic tree analysis.

Results

The present study was conducted to analyze the phylogenetic tree of multi-drug *A. baumannii* isolated from veterinary necessities obtained from our previously published study [7]. Briefly the isolated bacteria were positive on Acinetobacter CHROMagar and Vitek2 system confirmed by real-time PCR using specific primerswas 4% in thigh and 2% in breast samples. The incidence of *S. Typhimurium* was 2% in thigh samples only (Table 7).

Every sequence that had been obtained during this investigation was added to the GenBank Database with accession codes (OP810392.1, OP810499.1, OP810400.1, OP810405.1, OP810409.1, OP810410.1, OP810413.1, and OP810414.1). The Duhok *A. baumannii* isolates were clustered into a single cluster (Fig.1) based on the examination of nucleotide sequences and phylogenetic tree. The isolates from this study,

on the other hand, were dispersed between different isolates from different countries and from slaughterhouses, while the reference strain from other countries, which was obtained from the NCBI database, was clustered to diverse isolates from different countries (with a similarity percentage of approximately 99–100%). One isolate from a veterinary clinic was clustered to a isolate from Saudi Arabia accession No. U85706.1 with identity (100%) and Tukey (98%) (Fig. 2).

Discussion

Phylogenetic analysis of A. baumannii isolated from veterinary necessities aims to contribute to our understanding of the epidemiology, evolution, and potential sources of this bacterial species [12]. In addition, comparing the phylogenetic relationships of isolates can be beneficial for the identification of potential outbreaks, track the spread of isolates, and develop strategies for infection control [13]. This can give some information which are valuable for public health authorities, veterinarians, and researchers working on infectious disease control [14]. According to the above-mentioned points, this study was aimed to assess the phylogenetic tree relationships of A. baumannii isolated from veterinary necessities with those reference isolates obtained from gene bank.

According to the phylogenetic relationship, all isolates from this study, were closely related, suggesting that there were a cross-transmission between different veterinary sectors (slaughterhouse, government veterinary hospital and private veterinary clinic).

Generally, there are several ways of bacteria transmission in different veterinary sectors include areas such as farms, animal clinics, research facilities, and slaughterhouse. Bacteria can transmit by direct animal contact and this can occur in settings such as farms where animals are kept in close proximity or animals moving between different veterinary sectors, such as being transported from a farm to a research facility or veterinary clinic [15]. Equipment, and instruments can become contaminated with bacteria, if these items are not properly cleaned and disinfected between uses or between different sectors, they can serve as a source of transmission [16].

Veterinary professionals, researchers, and farm workers can inadvertently transfer bacteria between different sectors. This can happen through contact with animals, equipment, or other contaminated surfaces [17]. Some bacteria can be transmitted through the air. Airborne transmission may occur when animals in one sector share the same airspace with animals in another sector and this most commonly seen within the slaughterhouse [18]. Insects and other vectors can carry bacteria from one place to another. For example, flies can pick up bacteria in a farm setting and then transfer them to animals in a different location [19].

Contaminated water sources can serve as a vehicle for bacterial transmission between different veterinary sectors. This can occur, for instance, in slaughterhouses [20].

Some bacteria including A. baumannii can be transmitted between animals and humans. If veterinary professionals or researchers come into contact with infected animals and do not follow proper biosecurity measures, they may inadvertently carry bacteria to other sectors and to human being [21]. Previous study has proven that A. baumannii emerged in the veterinary sector (the source for human infection was from the veterinary sector as indicated by their genetic relatedness [7]. All of the above-mentioned risky factors may support the results of this study that all isolated isolates were genetically related.

In this study, all A. baumannii isolates were showed clustering to different isolates from divers' countries, suggesting that these isolates from veterinary sector could come from those countries with the course of animal importation. In our region, there is no such biosecurity measures, including testing, quarantine, and surveillance protocols at border zones to test the carrier imported animals, this may lead to the potential spread of pathogens to local animal populations with a serious consequence for both animal and human health [22]. To prevent these negative outcomes, it is crucial for nations to implement and enforce effective biosecurity measures at their border zones. This includes rigorous testing, quarantine periods, and surveillance protocols to ensure that imported animals are free from harmful pathogens [23].

Conclusion

In summary, phylogenetic analysis of A. baumannii isolated from veterinary necessities is a multifaceted approach that addresses both basic scientific questions about the genetic diversity and evolution of the bacterium, as well as practical concerns related to public health, and infection control in veterinary settings. Effective biosecurity measures, such as quarantine protocols, proper cleaning and disinfection procedures, and education on hygiene practices, are essential to minimize the risk of bacterial transmission between different veterinary sectors. Developing and implementing these measures can help prevent the spread of diseases and maintain the health of humans and animals in various settings.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

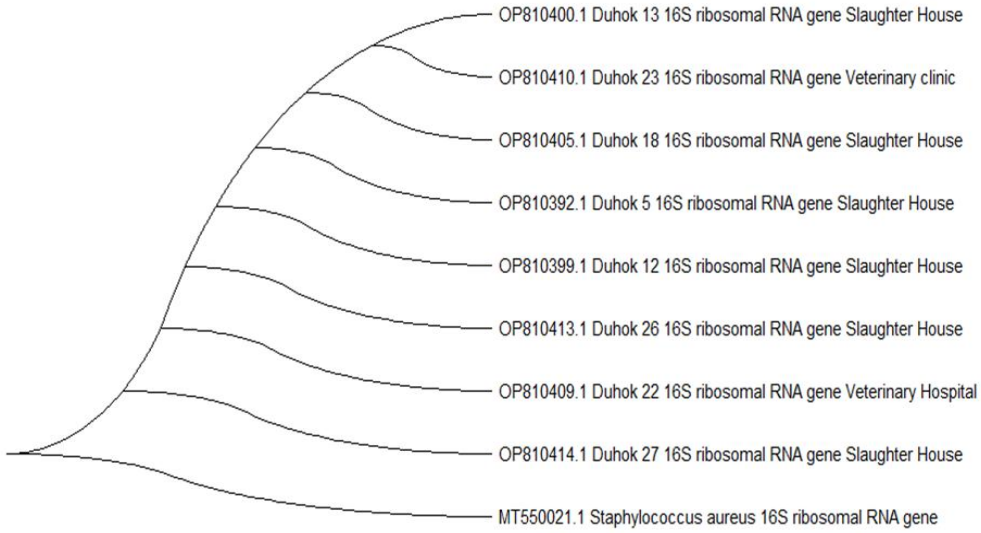

Fig. 1. Phylogenetic tree for samples used in this study. Staphylococcus aureus strain was utilized as out group species

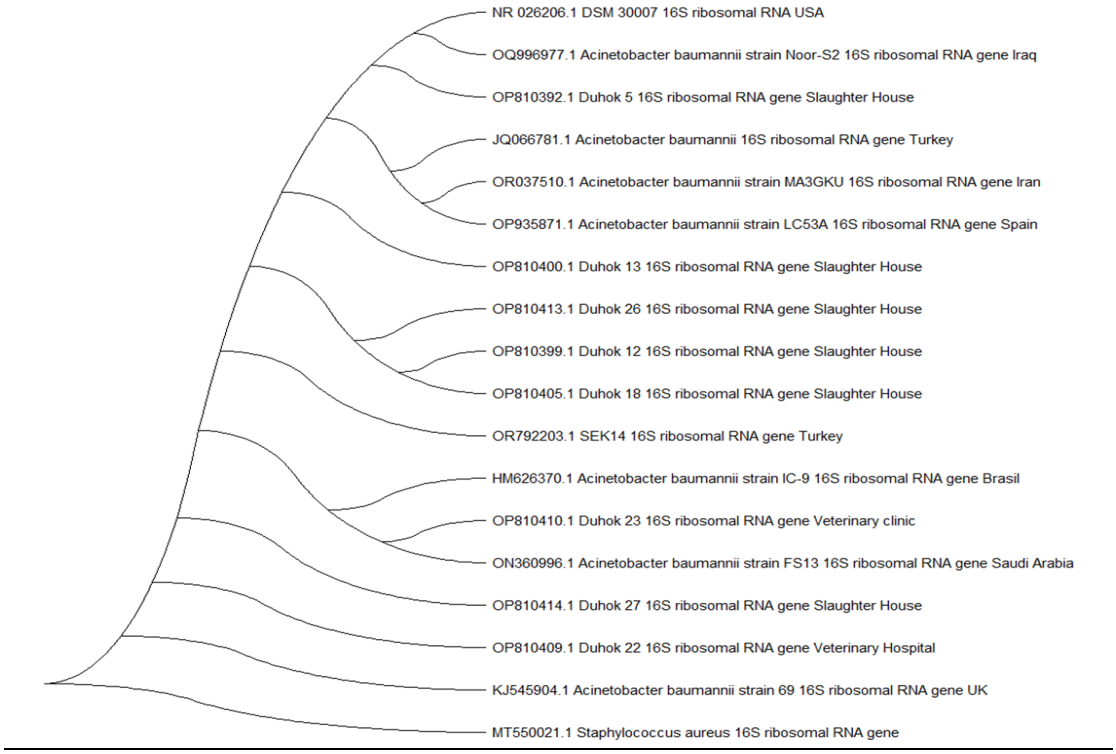

Fig. 2. Phylogenetic tree for samples used in this study and sequences from different countries retrieved from NCBI databases. Staphylococcus aureus strain was utilized as out group species.

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التحليل الوراثي لبكتيريا *Acinetobacter baumannii* المعزولة من المستلزمات البيطرية

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الملخص

يمكن عزل بكتيريا *Acinetobacter baumannii* من أنواع حيوانية مختلفة، ونظرًا لقدرتها العالية على الإمراض، يجب اتخاذ تدابير المكافحة لتقليل المصادر الحيوانية وتقليل انتشار هذا العامل الممرض بين الجمهور. هدفت الدراسة الحالية إلى تقييم الحالة التطورية لهذا الممرض المعزول من قطاعات بيطرية مختلفة باستخدام جين *16s rRNA* وذلك لتحديد مدى الارتباط الوراثي لهذه البكتيريا بين هذه القطاعات. تم عزل ثماني سلالات من فطر *A. baumannii* سابقاً من مختلف المستلزمات البيطرية (المجازر، العيادات البيطرية الخاصة، والمراكز البيطرية العامة). تم تأكيد العزلات على مستوى الأنواع من خلال تضخيم PCR للحمض النووي الريباسي 16S – 23S باستخدام PCR في الوقت الحقيقي. تم إنشاء شجرة النشوء والتطور باستخدام تسلسل جين الرنا الريباسي 16S لهذه السلالات ولـ *A. baumanni*i من بنك الجينات. أظهرت البيانات أن جميع العزلات كانت متشابهة وراثيا، وأن التشابه الوراثي لعزلات الدراسة الحالية كان وثيق الصلة بعزلات دول مختلفة بما في ذلك المملكة العربية السعودية وتركيا والبرازيل والمملكة المتحدة وأسبانيا وإيران والعراق. وتشير البيانات الحالية إلى أن *A. baumannii* من هذه الدراسة يمكن أن يكون قد جاء من تلك البلدان أثناء استيراد الحيوانات، بالإضافة إلى ذلك، كان هناك انتقال لهذه السلالات بين مختلف القطاعات البيطرية. وهذا سيوصي بممارسات النظافة الجيدة لتقليل مخاطر انتقال البكتيريا بين القطاعات البيطرية المختلفة.

الكلمات الدالة: *Acinetobacter baumannii*، الضروريات البيطرية، الرنا الريباسي 16S، الارتباط النسيلي.