Molecular and phylogenetic analysis of *Staphylococcus aureus* isolated from feline otitis externa

Lubna Abdul Kareem Abbas
Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

Amane Mohammed Rady
Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq, amani.m@covm.uobaghdad.edu.iq

Abstract

The study was conducted for the estimation of feline otitis externa caused by Staphylococcus aureus in Baghdad, a province of Iraq. One hundred and twenty ear swabs from cats with otitis externa were used for isolation of Staphylococcus aureus, after culturing on selective media Blood agar, Mannitol salt agar and Hi chrom agar, Gram staining and biochemical tests. The (n = 7) Staphylococcus aureus isolates were obtained from ear swabs of cats and confirmed by using polymerase reaction chain (PCR) for 23S rRNA and sequencing of this gene. Sequencing has been done in Korea by Macrogen and the evolutionary tree was drawn by using Molecular Evolutionary Genetic Analysis (MEGA). The results showed that the (7) isolates possessed the 23S rRNA gene (100%) by using specific primers at 350bp. The results of the phylogenetic tree revealed that the sequence of the (7) isolates revealed 99% compatibility with reference isolates at Gene Bank. It had been deposited under accession numbers (ID: ON908999.1, ID: ON909000.1, ID: ON909001.1, ID: ON909002.1, ID: ON909003.1, ID: ON909004.1, ID: ON909005.1)

Keywords: *Staphylococcus aureus, Feline otitis externa, 23srRNA, Sequencing*.

Introduction

Otitis externa is a condition that causes inflammation of the external ear canal, it is one of the most common and frustrating problems encountered in small animal practice. External otitis can be divided into two main types acute and chronic. Acute external otitis can be localized or diffuse (1). Otitis externa has a multifactorial etiology and bacteria play an important role in otic diseases and many studies have used samples collected from only one or both ears and considered them as different samples (2, 3). The causative factors for otitis externa have been grouped into predisposing, primary, and perpetuating factors (4). A variety of bacteria can be isolated from the ear canals of cats, including Staphylococcus, Pseudomonas, Escherichia, Proteus, Corynebacterium species and Enterococcus (5), lesions include ear pain, balance problems, itching, hemorrhagic lesions, and yellowish mucous discharge from the ear canal. In most cases, these clinical signs are significant and may be a sign of pathogenic infections (4). Staphylococcus spp. was the most prevalent among bacterial causes, accounting for 35.3% of all feline otitis externa cases and 75% of those caused by bacteria (6). Staphylococcus aureus is the second most prevalent pathogen in acute bacterial otitis externa, affecting up to 40%of Cases (7). Numerous reports reveal the zoonotic reveal transmission between companion animals and humans. The ability of

Staphylococcus aureus to cause disease is associated with a large number of virulence factors including (S. aureus enterotoxins, Panton-Valentine leucocidin, hemolysis Hl α and HI β), most of which participate in tissue invasion, colonization, and adhesion (8). Typically, these bacteria are resistant to the majority of common drug antimicrobial drugs, such as beta-lactams, aminoglycosides, and macrolides. The resistance of the antibiotics of these bacteria can result in therapeutic failure, increased medical expenses and mortality (9). There are several techniques were developed for the detection of S. aureus by using Polymerase chain reaction (PCR). The sequence analysis of the 23S rRNA gene provides a powerful mechanism for the accurate finding of pathogens particularly in cases with suspected bacterial disease (10, 11).

Material and Methods

Sample collection and identification

The study was conducted for the estimation of feline otitis externa caused by Staphylococcus aureus in Baghdad, The present study was approved by the ethical and research committee of the College of Veterinary Medicine, University of Baghdad, Ministry of High Education and Scientific Research. The 30 isolates were insured by the Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine in Baghdad after streaking the ear swabs of 120 cats with otitis externa on blood agar, Mannitol salt agar and Chrome agar. The isolated colonies were identified morphologically, culturally, and biochemically (12). Staphylococcus aureus isolates were confirmed by PCR amplification of the 23srRNA gene, and PCR assay was done by the following methods. In the first step, bacteria were exposed to DNA extraction using Bacteria Kit Geneaid. USA, second step, the purity and concentration of extracted DNA were measured using Nanodrop. One primer set was used in this study (Table 1) and the sequence of this primer gives an amplicon size reach of 350pb. The period of collection of these samples was from August 2021 to July 2022.

Table 1: Primer used to amplify 23srRNA gene of S. aureus

Primers Name		5`-3` (sequences)	Size	Reference
23srRNA	F	TCGGAATCTGGGAGGACCAT	350 bp	(13, 14)
	R	AATCGTAAGTCGGTTCGGTCC		

Sequencing

PCR products of the Staphylococcus aureus isolates were transferred to Macrogen Corporation in Korea for automated DNA sequencing by Sanger. The results were received by email then analysis was done using the Geneious software.

Results

Molecular study:

The present study demonstrated that (7) S. aureus positive which were detected by bacteriological isolates tested were positive for 23S rRNA gene by using the specific primer at amplicon size (350bp) (Figure 1).

Figure 1: 1 % agarose gel electrophoresis, DNA marker ladder (100 – 1000), control (lane 7) and lanes (1-6) were positive sample at 350 bp

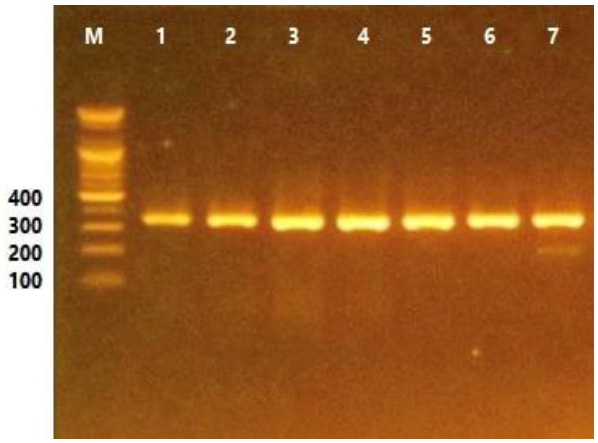
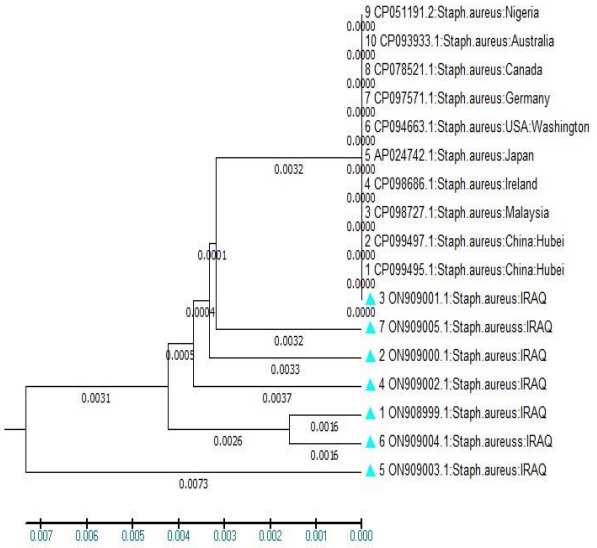


Figure 2: Neighbor-joining tree of the 23S rRNA gene of *S. aureus*



Sequence of 23S rRNA gene:

The results of the present study revealed that the sequence of the local isolates of S. aureus from ear swabs of cats was of different origins including: (Nigeria, Australia, Canada, Germany, USA: Washington, Japan, Ireland, Malaysia and China-Hubei) by 99% (Figure 2).

Seven isolates of Staphylococcus aureus were taken for 23S rRNA gene analysis, and the result was seven samples that match the global results by 99% (Table 2).

Table 2: Type of polymorphism in the S. aureus

No.	Type of substitution	Location	Nucleotide	Sequence ID with Submissions	Identities
1	Transvertion	108551	C\G		
	Transition	108552	G\A	ID: ON908999.1	99%
	Transvertion	108553	A\C		
2	Transition	108552	G\A	ID: ON909000.1	99%
	Transvertion	108553	A\C		
3	---------	--------	-------	ID: ON909001.1	100%
4	Transition	108297	C\T	ID: ON909002.1	99%
	Transvertion	108372	C\A		
5	Transition	108297	C\T		
	Transition	108324	C\T	ID: ON909003.1	99%
	Transition	108427	A\G		
	Transvertion	108468	T\G		
6	Transvertion	108551	C\G	ID: ON909004.1	99%
	Transition	108552	G\A		
7	Transition	108552	G\A	ID: ON909005.1	99%
	Transition	108554	C\T		

Discussion

In the present study, the PCR assay was performed to detect S. aureus based on the amplification of the 23S rRNA and gene. The present results were supported by Cremonesi 2013 in Italy, who reported that the (9) isolates of S. aureus encoded with 16S-23S rRNA goats (15). The present results were similar to previous studies done in Iraq that identified S. aureus isolates in dogs (13) and ewes milk samples that were carrying the 23S rRNA gene (14). Sequencing has been done in Korea by Macrogen and the evolutionary tree was drawn by using Molecular Evolutionary Genetic Analysis (MEGA) (16). It appeared when compared with the world that there were seven samples that matched the results of the mentioned countries by 99%. The results of the present study agree with Ahmed 2022 in Iraq, who recorded variable origins of the S. aureus isolates from milk samples of ewes including China, the United States, Japan, Taiwan, Germany, India, Nigeria and Argentina (14). The present results of the phylogenetic analysis showed that the local strains of S. aureus were compatible (99%) with other rescuers, strains in china with ID: CP099495.1 found that the methicillin-resistant S. aureus isolates were isolated from nasal swabs of pig farms and Farmer workers with different clonal lineages (17). In Nigeria, S. aureus isolates with ID: (CP 051191.2) were isolated from (urine blood, semen, endo-cervix, vaginal, wound and aspirates) of patients were differed primarily in their methicillin resistance gene carriage (18).

Conclusion

The result of the current study showed that all the isolates of Staphylococcus aureus from cases of feline otitis externa were positive for the 23S rRNA gene.

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