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Molecular characterization of methicillin resistance *Staphylococcus aureus* (MRSA) from eye infections in domestic cats in Mosul city

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A B S T R A C T

The spread of antibiotic resistance has become a global threat in both human and veterinary sectors. In this study, Methicillin-resistant *Staphylococcus aureus* (MRSA) strains were previously isolated from cats with eye infections have characterised using PCR and DNA sequencing. The *mecA* resistance gene, in addition to *clfA, cfB and coa* virulence factor genes were used as molecular markers. The results indicated successful amplification of all these genes with PCR product size 147, 288, 203 and 674 bp, respectively. Additionally, the sequencing results of *mecA* gene confirm the PCR results, and 4 MRSA isolates were registered in the GenBank under accession no. PQ181561, PQ181562, PQ181563 and PQ181564. The phylogenetic analysis of the *mecA* gene indicates that the source of these bacteria is human. In conclusion, pet cats serve as reservoir for MRSA and could transmit such zoonotic pathogen to humans in contact with these animals.

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Introduction

The misuse of antibiotics in treating bacterial infections in both human and veterinary fields has accelerated the development of antibiotic resistance against different types of antibiotics [1-3]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered one of the most important hospital-acquired infections [4]. For many years, it was believed that MRSA infection was limited to humans and the transmission only occurred between susceptible individuals, such as elderly patients or patients hospitalised due to burns [3, 4]. However, the infection with MRSA was extended to cover different animal species including pets [5-7]. Many studies reported that human was considered the main reservoir host that transmits MRSA to animals [8-10]. Raising companion animals, especially imported cats, inside homes has become of great importance to many people. Nevertheless, direct contact with these pets or indirect contact with their waste has become a major source of risk for many pathogens, including MRSA [11, 12]. Many studies have reported the isolation and identification of MRSA in cats, and this might have an important role in the zoonosis and retransmission of MRSA to humans again [11, 13, 14], resistant to methicillin, is encoded by the *mecA* gene, which produces the penicillin-binding protein (PBP). This resistance is considered part of the resistance to β-lactams [15, 16]. Additionally, the identification of virulence strains of MRSA in pet cats is of great importance due to their ability to transmit to humans [17, 18]. Virulence factors such as clumping factor, which is encoded by two genes *clfA* and *clfB*, in addition to coagulase which is encoded by the *coa* gene, are considered the main virulence factors that enable *S. aureus* to survive and avoid host defence phagocytosis [19, 20]. Although many studies in Mosul city have reported the isolation and identification of MRSA from different animal sources such as milk, meat and broilers and fish [5, 8, 21, 22], no studies have reported the detection of MRSA in pet animals, especially cats. Accordingly, this study was designed to molecular characterize MRSA from domestic cats in Mosul city.

Materials and Methods

Bacterial isolates

A total of 19 *Staphylococcus aureus* glycerol stock isolates were included in this study. These isolates were obtained from a previous related study that attempted to isolate bacteria from cats suffering eye infections of different ages and sexes. These isolates were identified by standard culture method [12]. Also, further identification of *S. aureus* isolates at the species level was done previously using polymerase chain reaction targeting the *nuc* gene. Meanwhile, methicillin disc was used to confirm the presence of MRSA based on antibiotic results using the disc diffusion method.

Culture on HiChrome^{TM} MeReSa Agar Base

All *S. aureus* isolates were cultured on HiChrome^{TM} MeReSa Agar Base with selective supplement (Himedia, India). The culture media was prepared according to manufacturer instructions. This chromogen agar selectively identifies MRSA based on the color of the colonies obtained after incubation at 37 °C for 24 h.

Molecular diagnosis and characterization

DNA extraction

All 19 isolated of *S. aureus* were subjected to DNA extraction using AddPrep Bacterial Genomic purification kit (Addbio, Korea). Briefly, 2-3 pure colonies of *S. aureus* isolate were picked and mixed with lysosome buffer containing Lysozyme (50 mg/ml) and incubated 37 ℃ in the water bath (Memmert, Germany) for 1 hour followed by centrifugation at 13,000 rpm for 3 minutes. The supernatant was discarded and the sediment was preserved. A lysis solution containing Proteinase K Solution (20 mg/ml) was added and incubated at 56 ℃ for 15 minutes. The Binding of DNA was achieved by adding binding solution 200 µl with 200 µl of absolute ethanol and mixed well then centrifuging at 13,000 rpm for 4 minutes. The supernatant was transferred to the spin column with the collection tube and centrifuged at 13,000 rpm for 1 min. the column was washed twice with 500 µl of Washing 1 Solution and Washing 2 Solution, respectively and centrifuged each time at 13,000 rpm for 1 min. Finally, the spin column was dried and the DNA was eluted by adding 80 µl of Elution Solution and centrifugation at 13,000 rpm for 1 min. The extracted DNA was kept at -20 °C for further assays.

Polymerase chain reaction (PCR)

The primers used in this study were purchased from (Macrogen Co., Korea) (Table 1).

The PCR master mix was set in a 30 µl volume containing 15 µl of Add Taq DNA Master (2x) (Addbio, Korea) 1 µl of each forward and reverse primer, and 11 µl of PCR grade water, while 2 µl of DNA was added as a template. The PCR was performed using (T100 BioRad Thermocycler, USA). The conditions included Polymerase activation at 95 °C for 10 min, then 38 cycles of denaturing at 94 °C 45 seconds, annealing (as mentioned in Table 1 for each primer) for 45 seconds and extension at 72 °C for 1 min. Final Extension for one cycle at 72 °C for 5 min followed by holding step at 4 °C. After amplification, the PCR product were subjected to gel electrophoresis using 1.5% agarose stained with gel red dye (Addbio, Korea) and 8 µl of the DNA ladder (AddBio, Korea) was used as marker. After electrophoresis, the gel was documented, for the determination of expected bands.

Table 1. Primers used in PCR with respective annealing temperature and amplicon sizes

Gene	Primer	Sequence 5' – 3'	Tm °C	Size bp	Reference
mecA	mecA-F	GTGAAGATATACCAAGTGATT	52	147	[23]
	mecA-R	ATGCGCTATAGATTGAAAGGAT			
clfA	clfA-F	ATTGGCGTGGCTTCAGTGCT	60	288	[24]
	clfA-R	CGTTTCTTCCGTAGTTGCATTTG			
clfB	clfB-F	ACATCAGTAATAGTAGGGGCAAC	55	203	[24]
	clfB-R	TTCGCACTGTTTGTGTTTGCAC			
Coa	coa-F	ATAGAGATGCTGGTACAGG	55	674	[25]
	coa-R	GCTTCCGATTGTTCGATGC			

DNA sequencing and analysis

A total of 4 positive samples were sequenced according to the Sanger sequencing procedure. The 4 samples were also submitted to GenBank (NCBI) to obtain the accession numbers.

Results

The results of chromogen agar confirm the presence of bluish colonies indicating the presence of methicillin resistance property Figure 1. Additionally, the PCR results targeting the *mecA* gene showed clear bands of 147 bp indicating that these isolates have methicillin resistance gene Figure 2. Also, the PCR results confirm the presence of *clfA, clfB and coa* genes that are responsible for clumping factors A and B (Figures 3 and 4), and the coagulase activity of *S. aureus*, respectively Figure 5. Further confirmation for MRSA was obtained from sequencing and 4 isolates were successfully deposited in the GenBank under the accession no. PQ181561, PQ181562, PQ181563 and PQ181564. The alignment results using BLAST on the NCBI website and phylogenetic tree analysis showed 100% similarity to other isolates from China KX639007, USA CP163245 and CP160409, and Japan AP024289, AP024291, AP024293, AP024294, AP024296, AP024298 as showed in Figure 6.

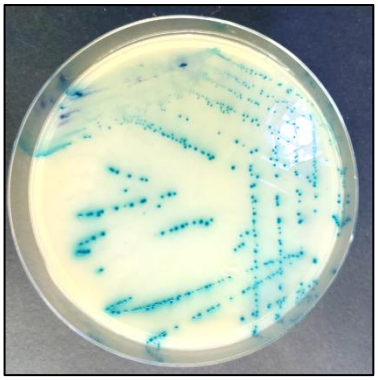

Figure 1. Growth of *Staphylococcus aureus* colonies on HiChromeTM MeReSa Agar Base for the diagnosis of methicillin-resistant *Staphylococcus aureus*.

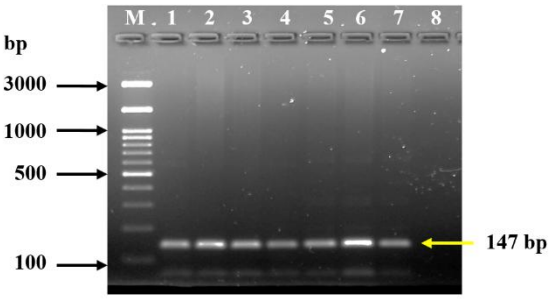

Figure 2. Agarose gel electrophoresis to detect the *mecA* gene of MRSA with a size of 147 bp. Lane M represents the DNA ladder, lanes 1-7 represent positive samples, lane 8 represents the negative control.

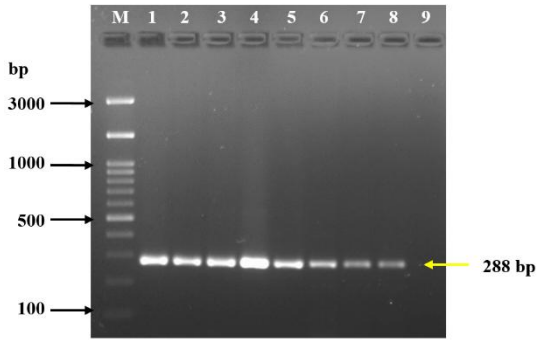

Figure 3. Agarose gel electrophoresis to detect the *clfA* gene of MRSA with a size of 288 bp. Lane M represents the DNA ladder, lanes 1-8 represent positive samples, lane 9 represents the negative control.

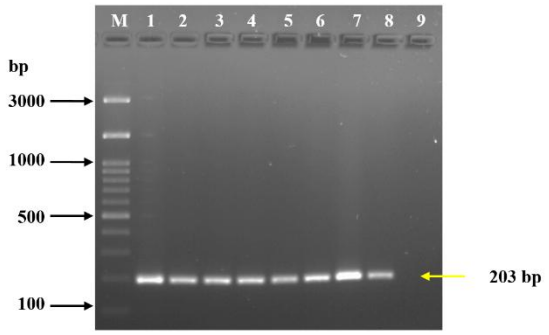

Figure 4. Agarose gel electrophoresis to detect the *clfB* gene of MRSA with a size of 203 bp. Lane M represents the DNA ladder, lanes 1-8 represent positive samples, lane 9 represents the negative control.

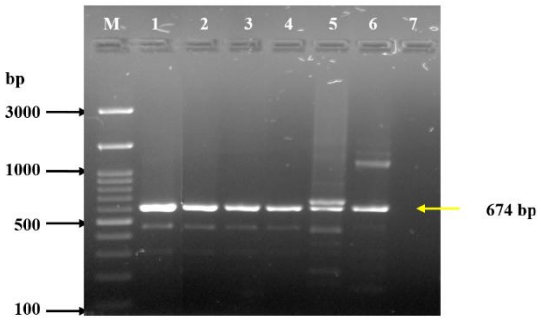

Figure 5. Agarose gel electrophoresis to detect the *coa* gene of MRSA with a size of 674 bp. Lane M represents the DNA ladder, lanes 1-6 represent positive samples, lane 7 represents the negative control.

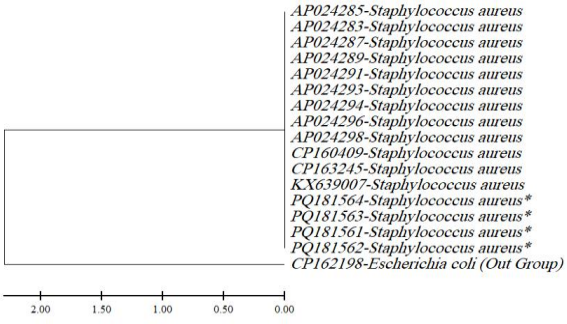

Figure 6. Phylogenetic tree analysis of *mecA* gene of MRSA local isolates * compared to other isolates in the GenBank.

Discussion

The emergence of MRSA in animals and the risk of its transmission to humans is of great public health importance [26, 27]. Nowadays in Mosul city, cats become the most favourable pet to keep at home. However, the risk of zoonosis still exists due to the absence of strict hygienic procedures adopted by their owners. In this study molecular characterization of MRSA isolated from eye infections in cats was performed. The results showed that the *mecA* gene was detected in MRSA isolates indicating the significance of such bacteria as a risk factor that might spread to the environment or other animals and could affect humans. Several studies have indicated the risk of these animals in transmitting MRSA, and high rates have been recorded among these animals and their owners, especially with the spread of imported cats that are not subject to comprehensive veterinary examinations. [9, 28, 29]. Accordingly, many studies have indicated the isolation of MRSA from cats as well as by their owners [28, 30]. Sing *et al.* (14), who indicated that isolation of MRSA from a cat in a person's house and at the same time, MRSA was isolated from his wife who was suffering from an abscess. The study indicated the isolation of MRSA from all members of the same family who did not suffer from any bacterial infections. In a study by Van Duijkeren [7] also indicated that 15/20 (75%) of the dogs and cats examined were positive for MRSA, while the percentage in their owners was 2/45 (4%), which indicates the existence of a close relationship between owners and companion animals [29, 31]. The analysis of the *mecA* gene results also gave 100% identity with the human isolates from China, the United States and Japan. This identicalness indicates that the source of origin of the isolates from cats is of human origin and that cats raised in homes are considered reservoir hosts for these germs [26, 32, 33]. The genes responsible for virulence were also detected, including *clfA*, *clfB,* and *coa*. Most of the isolates carried the virulence clumping factor in addition to the production of the coagulase enzyme. The results of the molecular diagnosis of the virulence factors are consistent with the biochemical tests that were previously performed on the isolates, and this is an indicator of the ability of these isolates to cause infections, especially when the appropriate conditions are available. The role of the clumping factor clfA was recorded as facilitating the adhesion of invading bacteria to the blood protein fibrinogen, while there are not enough studies on the role of *clfB* in pathogenesis. Also, the detection of the coagulation enzyme in MRSA isolates indicates the ability of these bacteria to overcome the body's defences by forming a protein barrier resulting from the conversion of fibrinogen to fibrin, which contributes to preventing the arrival of white blood cells to the site of infection, which facilitates the role of bacteria in colonization and multiplication [19, 20, 34].

Conclusion

Detection of MRSA from eye infections in cats in addition to virulence factors may constitute a risk factor for human public health and facilitate zoonosis, especially when these cats are kept at home.

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Competing Interests

The authors should declare that there are no competing interests.

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