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Original article

# Determination of phylogenetic relationships among methicillin-resistant *Staphylococcus aureus* recovered from infected humans and Companion Animals

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ABSTRACT

Companion animals carry different microorganism of severely public health hazard for human; the kindness relation and contact between humans and companion animals may the route in the transmission of most zoonotic bacteria, including Methicillin-Resistant *Staphylococcus aureus* (MRSA). Therefore, the current study investigate the companion animals mainly dogs and cat as a reservoir for MRSA and the genetic similarity between the recovered strains of MRSA from such companion animals and their owners. One hundred swabs were collected under aseptic condition from companion animals and seventy swabs were collected from nasal and soft tissue of the infected owners in contact. All samples were examined with standard microbiological techniques, antimicrobial sensitivity, molecular typing and genetic finger printing using RAPD-PCR to determine the genetic finger printing of the recovered strains from humans and companion animals. The prevalence of the MRSA was higher in dog's swabs than human swabs. Dog swabs showed a rate of (44.4%), cat's revealed (27.3%), while the owner swabs could detect (42.8%). The antibiotics profiles were 69.2% and all MRSA strains were positive for *mecA* gene (100%), while only 25 strains (38.5%) were positive for Panton Valentine Leukocidin (*PVL* gene). Phylogenetic tree revealed 4 clusters with complete genetic relatedness and higher identity between the strains recovered from humans and companion animals. Our results revealed that there is great similarity between the recovered strains, indicating that pets play an important role in colonization and transmitting MRSA to humans, and vice versa.



## 1. Introduction

Animals are the natural reservoirs of microorganism's complex populations. *Staphylococcus* is one of the most opportunistic bacteria frequently isolated genera from animals and human beings (Vitale et al., 2006; Aklilu et al., 2012). *Staphylococcus aureus* is a colonizing organism for skin and upper respiratory tract of an innocuous component of the commensal flora and causes invasive infection in both human and animals (Benito et al., 2016). It presented exclusively in humans, and also found in apparent healthy and diseases companion animals (dogs and cats) that considered family members (Aires-de-Sousa 2017; Bierowiec et al., 2019). The kindness relation and contact between humans and companion animals may the route in the transmission of most zoonotic bacteria, including MRSA. Companion animals are potential sources of public health concern of MRSA for human. The incidence of MRSA transmission phenomenon is unclear between people and companion animals (Bergstrom et al., 2012; Bierowiec et al., 2016).

Many authors suggested that humans are responsible for the initial colonization MRSA (Oehler et al., 2009; Smith and Pearson 2011) as they attract the infections with MRSA by Several exposures to hospitals and healthcare settings (Baptiste et al., 2005; Oehler et al., 2009). At the same times the isolates recovered from companion animals were often the same that recovered from infected humans (Weese et al., 2006; Faires et al., 2009). Indicating that there are recurrent infections and transmission of MRSA from infected outpatient's clinics (owners) to companion animals, and colonization of such strains occurs in such animal which become carriers and infect human cohabitants.

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Now, the population prevalence of MRSA transmission to others is relatively high. Therefore, studying of MRSA infection in cats and dogs is important to understanding public hazard of MRSA. Several studies showed that MRSA clones circulating in cats and dogs are similar to the ones identified in humans and belong mostly to house-contact clones (HC-MRSA). This is clinically important since HC-MRSA isolates usually carry more virulence genes, and also more resistance genes than MRSA originating from companion animals (Cuny et al., 2015; Haenni et al., 2015; Loeffler et al., 2010).

The current study investigate the companion animals mainly dogs and cat as a reservoir for MRSA and their owners or persons in contact with them and to investigate the genetic similarity between the MRSA recovered from the companion animals like and their owners using RAPD-PCR.

## 2. Materials and methods

### 2.1. Samples

One hundred swabs were collected from companion animals clinically suffered from otitis media and upper respiratory diseases; cats (55 nasal and ear swabs) and dogs (45 nasal and ear swabs). All animals lived in households in close contact with their owners. Seventy swabs from nostril, pharynx, and infected tissues were collected from the owners suffering from upper respiratory tract and pyogenic infections. All samples were taken under the acceptance of the owners.

### 2.2. Isolation and identification of Staphylococcus species from samples

All swabs were subcultured in Mannitol Salt Agar plates (BBL, United Kingdom) then incubated for 24 h. The pure colony was identified by Gram staining morphology, and traditionally method biotyping (catalase, coagulase tube test and sugars fermentation test) (Mutters et al., 2016).

### 2.3. Susceptibility testing

All *S. aureus* detected at culture and identification step of samples were subjected to antimicrobial susceptibility test using the disk diffusion method (Price et al., 2012). Eleven antibiotics of veterinary and/or human interest were examined: penicillin G, cefoxitin, kanamycin, gentamicin, tetracycline, erythromycin, spiramycin, lincomycin, chloramphenicol, enrofloxacin and vancomycin (Oxoid, United Kingdom). The result was interpretated according to CLSI (CLSI 2015).

### 2.4. Molecular typing

The multi-drug resistance isolates (MRD) were examined using multiplex PCR for simultaneous detection of 16S rRNA of *Staphylococcus aureus*, *mecA* and Panton–Valentine leukocidin (PVL) genes reported by Moussa et al. (2012).

Using three primer pairs, one of them specific to 16S rRNA of *S. aureus* "Staph756F & Staph750R primers" which amplify 756 base pair fragments specific of *S. aureus*, the second one amplify 433 bp fragments and specific for PVL gene (Luk-PV-1 and Luk-PV-2 primers) reported by McClure et al. (2006a, 2006b). The third pair amplify 1399 base pair fragments specific for *mecA* gene (mecA F and mecA R primers) reported by Weller (1999). The reaction mixtures and the PCR condition were carried out according to Moussa et al. (2012). Finger printing of MRSA by RAPD- PCR had been carried out according to the methods reported by (Mehndiratta and Bhalla 2012) using short size primers and the PCR product have been separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH). The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

## 3. Results

Positive microbiological data for *S. aureus* reveals Gram positive cocci arranged in clusters, golden yellow pin point colony, no effervescence of gas at catalase test, clotting rabbit plasma with coagulase test and ferment glucose.

Dog swabs show a rate of 44.4% (20/45), while, cats samples reveal 27.3% (15/55). The owner swabs detect 30 out of 70 with incidence 42.8%. All 65 *S. aureus* were identified from nasal swabs except two isolated from ear swabs from cases of otitis media. All *S. aureus* were subjected to antimicrobial susceptibility test using the disk diffusion method against 11 antibiotics. Forty five *S. aureus* were MRD with ratio 69.2%. The MRD profiles were: 10 were resistant for (penicillin G, cefoxitin, kanamycin, and gentamicin); 15 were resistant for (erythromycin, spiramycin, lincomycin, chloramphenicol, enrofloxacin); 18 were resistance for (penicillin G, erythromycin, spiramycin, kanamycin, vancomycin); two *S. aureus* isolated from cases of otitis media were resistant to all used antibiotic.

All MDR *S. aureus* were examined using multiplex PCR for simultaneous detection of 16S rRNA of *Staphylococcus aureus*, *mecA* and Panton–Valentine leukocidin (PVL) genes. The multiplex PCR could detect all the bacteriologically positive methicillin resistant *S. aureus* and all resistant strains for methicillin and oxacillin in few hours with 100% sensitivity and 100% specificity as shown in Fig. 1.

PVL genes were observed with only 25 strains (38.5%) as shown in Fig. 1.

RAPD – PCR of the recovered strains from human and companion using EP007, EP015, EP017, MN45 and KAY1 primers revealed characteristic RAPD profiles fingerprinting patterns based on the presence, size and the intensity of the amplified fragments. Moreover, it is noticed that the amplification reactions generated number of bands ranging from 3 to 10 bands with a molecular weight ranging from 180 up to 990 bp fragments as shown in Fig. 2. The majority of the examined MRSA had shared bands; but differs in their intensity. It's clear from the obtained data (Fig. 2) that the genetic profile of the strains recovered from humans and companions showed great similarity indicating the transmission of such strains between humans and companion animals.

The phylogenetic tree of the recovered MRSA from different species based on the similarity index between the strains, revealed 4 clusters. Most of the strains recovered from humans and companion were grouped in two clusters (3 and 4) and closely related

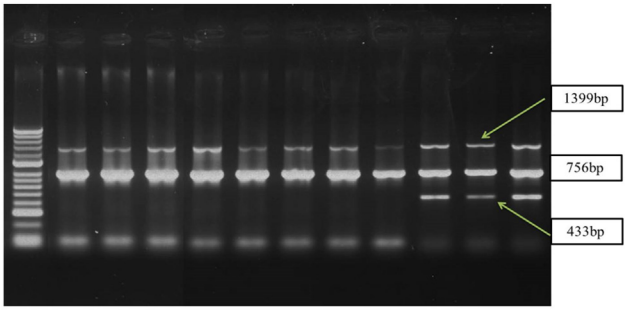


**Fig. 1.** Agarose gel electrophoresis showing multiplex PCR for simultaneous detection of 16S rRNA of *Staphylococcus aureus* (756 bp), *mecA* gene (1399 bp) and Panton–Valentine leukocidin (PVL) gene (733 bp).

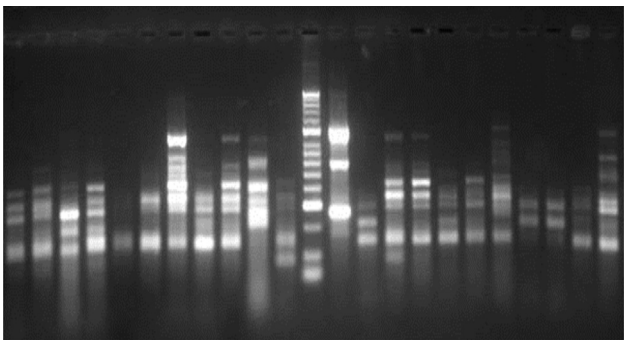

**Fig. 2.** Agarose gel electrophoresis showing RAPD-PCR of MRSA recovered from Humans, dogs and cats using EP015, EP017, EP007, MN 45 and KAY1 short size primers.

to each other with high similarity (similarity index varied from 0.0800 up to 0.0900 between the strains). While clusters number one and number 2 contain only one strain as shown in Fig. 3.

## 4. Discussion

*Staphylococcus aureus* is a colonizing organism for skin and upper respiratory tract of an innocuous component of the commensal flora and causes invasive infection in both human and animals (Benito et al., 2016). The present work analyzed the relation of companion animals mainly dogs and cats in transmission of MRD *S. aureus* to their owners. The incidence of positive *S. aureus* were high in dogs then human and later in cats in comparison with the number of samples collected from each. The same results were documented by many authors (Vitale et al., 2006; Bierowiec et al., 2016; Aires-de-Sousa 2017). MRD means that *S. aureus* were resistant for at least 3 antibiotics, the collected data from the present research revealed 62.2% of *S. aureus* were MDR with different antibiotic profiles as expressed by Bierowiec et al. (2019).

The virulence of the CA-MRSA especially in severs soft tissues and skin infections and necrotizing infections is attributed to the PVL gene (Vitale et al., 2006; Bierowiec et al., 2016; Aires-de-Sousa 2017). *mecA* gene for MRD in *S. aureus* has been considered as a house keeping gene (Bierowiec et al., 2019; Benito et al., 2016). Therefore, multiplex PCR for simultaneous detection of 16S rRNA of *Staphylococcus aureus*, *mecA* and *Panton–Valentine leukocidin* (*PVL*) genes had been carried out.

The multiplex PCR could detect all the bacteriologically positive methicillin resistant *S. aureus* in few hours with 100% sensitivity and 100% specificity which confirm the conclusion of Moussa and Shibl (2009) and Moussa et al. (2012). RAPD – PCR of the recovered strains from human and companion animals using EP007, EP015, EP017, MN45 and KAY1 primers revealed characteristic RAPD profiles fingerprinting patterns Fig. 2, Moreover, it is noticed that the amplification reactions generated number of bands similar in its molecular weight (shared bands) in all strains of humans and companion animals indicating the great similarity between The strains recovered from humans and pets (Loeffler et al., 2010; van Duijkeren, et al., 2011) however, these sharing bands differed in their intensity.

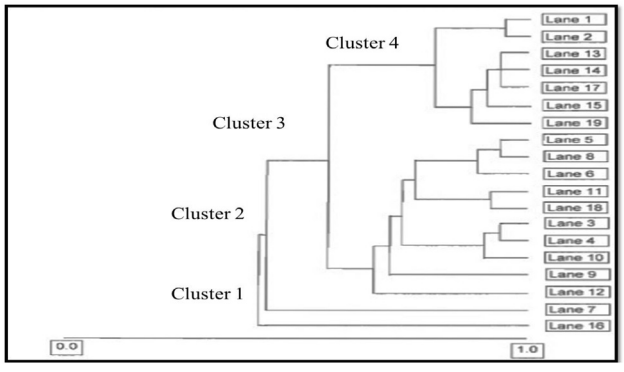


**Fig. 3.** Phylogenetic tree showing the genetic relatedness among MRSA recovered form humans and companion animals using EP015, EP017, EP007, MN 45 and KAY1 short size primers.

Most of the strains recovered from humans and companion were grouped in two clusters (3 and 4) and closely related to each other with high similarity (similarity index varied from 0.0800 up to 0.0900 between the strains) indicating the great similarity between The strains recovered from humans and pets (van Duijkeren et al., 2008; Frank et al., 2009). While, clusters number one and number 2 contain only one strain recovered from humans as shown in Fig. 3.

## 5. Conclusion

The previously revealed data confirmed that companion animals like dogs and cats are reservoirs for *S. aureus* and source of public health hazard especially with MRD *S. aureus* in owners.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Further Reading

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