Original article

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

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1. Introduction

Animals are the natural reservoirs of microorganisms' 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 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.

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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 investigates the companion animals mainly dogs and cats 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 interpreted according to CLSI (CLSI 2015).

2.4. Molecular typing

The multi-drug resistance isolates (MDR) 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 bp 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 fermentation 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.
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 Shihi (2009) and Moussa et al. (2012). RAPD – PCR of the recovered strains from human and companion animals using EP007, EP015, EP017, MN45 and KAY1 short size primers.

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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