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Research Article

Molecular Profile and Antibiogram of Methicillin Resistant *Staphylococcus aureus* Isolated from Domestic Dogs in Port Harcourt: A Public Health Concern

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Abstract

Objective: Methicillin resistance *Staphylococcus aureus* is one of the most common bacterial zoonotic infectious agent that is transmitted to humans by dog. This study was carried out to determine the potentials of domestic dogs to act as reservoirs for transmission of MRSA in Port Harcourt Rivers State, Nigeria and to possibly x-ray it's potential public health implication

Methods: 210 swab samples from the mouth, nose and skin of 70 dogs (from private residences, dog farms, and private veterinary clinics) were collected and cultured for the recovery of *Staphylococcus aureus* using standard microbiological procedures. PCR assay were used to detect the presence of *mecA* genes and confirmed the identity of *S. aureus* isolates. Disk diffusion technique was used to determine the antibiotic susceptibility against 8 antimicrobial agents.

Results: Overall, 202 *Staphylococcus spp*. were isolated from the sampling sites (Mouth, Nose and Skin) of 70 dogs, consisting of 177 (87.62%) *S. aureus* and 25(12.38%) coagulase negative *Staphylococcus pathogens*. Out of the 177 *S. aureus,* 42(23.73%) were MRSA while 135 (76.27%) were MSSA. However, the results of the Susceptibility pattern of MRSA isolates showed that levofloxacin was the most effective among all the antibiotics used in this study. 28 (66.67%) isolates out of the 42 MRSA positive isolates were sensitive to levofloxacin while 2(4.76%) were resistance to levofloxacin. Amoxicillin was the most resisted antibiotic, 37(88.10%) isolates out of the 42 MRSA positive isolates were resistance to amoxicillin while 5(11.90%) of the 42 MRSA positive isolates were sensitive to amoxicillin. All the 42 MRSA isolates had multidrug resistance index of > 0.2. Polymerase chain reaction (PCR) and Agarose gel-electrophoresis analysis revealed that the MRSA isolates carries *mecA* gene. Further analysis of the resistant determinants by BLAST revealed that all the resistant *Staphylococcus* strains were *S. aureus* strains.

Conclusion: This study revealed that dogs in study area harbors MRSA, which is of public health concern. Therefore, there is an urgent need for proper public health enlightenment advocacy on the risks associated with pet dog ownership and the need for proper hygiene while handling these pet dogs in our community.

Keywords: Molecular Profile; Antibiogram; Methicillin Resistant; *Staphylococcus aurous;* Domestic Dogs; Port Harcourt; Public Health; Risk

Introduction

Due to increasing urbanization with its attendant increase in anti-social behaviors - crimes, etc., many homes now keep dogs- both as security and as pets. This has also increased interaction between dogs and man with attendant risk of zoonotic transmission of infectious agents to man. Dogs are widely becoming a potent source of transmission of zoonotic infections. Zoonotic diseases can be transmitted to human by infected saliva, aerosols, contaminated urine or feces and direct contact with infected dog. Studies have shown that Methicillin resistance *Staphylococcus aureus* remains one of the most common bacterial zoonotic *i*nfection transmitted to humans by dogs [1,2].

Staphylococcus aureus, is a commensal that is often present asymptomatically on parts of the human body such as skin, skin glands, and mucous membranes, including noses and guts of healthy individuals [3,4].

The presence of *S. aureus* in human and animal bodies can lead to infection of the host. It is a main cause of infection acquired in the community and the hospital. It also has the ability to cause serious infections such as endocarditis and osteomylitis that affect the respiratory tract, bloodstream, skin and soft tissues [5].

Clinically, one of the utmost challenges with *S. aureus* is its ability to resist the effects of antibiotics of different classes [6]. The acquisition of resistance determinants by *Staphylococcus aureus* has been the greatest challenge to the treatment and control of staphylococcal infections since *S. aureus* has the highest pathogenic potential among the several species of staphylococci. Methicillin resistance is connected with possession of *mecA* gene which bestows resistance to all β-lactam antibiotics. Determinants of methicillin resistance *mecA* gene is situated on a large mobile genetic element, the staphylococcal cassette chromosome mec (SCCmec), enabling horizontal transmission between staphylococcal isolates [7]. Strains of MRSA have been reported in companion animals like dog and cat, free-living wild animals and aquatic species [8].

There is a continuing change in the prevalence and epidemiology of MRSA, with new strains being discovered universally. Hence, a nonstop awareness for MRSA via checking the host specificity, transmission routes and characteristics of novel strains in our environment is paramount. Nigeria lacks comprehensive studies focusing MRSA colonization in dogs with context to antibiotic susceptibilities of these strains. Therefore, the present study was designed to assess the prevalence of MRSA *(mecA* status), antibiotic susceptibility profile and potential risk factors associated with dogs in Port Harcourt.

Material and Methods

Description of the study area

This study was carried out on dogs from private veterinary clinics, dog farms, and from private homes within Port Harcourt Metropolis. It is located 4°451N6°5O1E/4.750°N6.833°E of the Niger Delta and is rich in rainforests and mangrove swamps. This study was conducted at the Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Rivers State.

Sample collection

A total of 210 samples were collected from the nostrils, mouths and skins of dogs sampled within Port Harcourt Metropolis from private residences, dog farms, and private veterinary clinics. Each dog had three samples taken; sterile swabs were pre-moistened in a sterile solution of phosphate buffered saline (PBS) and place parallel to the skin's surface and rubbed back and forth around the chest region of each dog to capture the skin microbiota. Nasal swabs were collected by inserting a sterile swab into the nostril of each dog to a distance of about 0.5–1.0 cm. While the third set of samples were collected from the side of the mouth of each dog. The swab sticks were placed in an ice pack, taken to the laboratory and cultured within eight hours of collection at a temperature of 37°C for 48 hours [9].

Isolation and identification of MRSA through cultural techniques

Samples collected were cultured on mannitol salt agar (Oxoid, UK) and incubated aerobically at 37 °C for 24–48 h. The cultures were then examined for the presence of *S. aureus* (yellow colonies) a microscopic appearance after Gram staining, and subculture unto chocolate agar. Collected samples were grown on mannitol salt agar (Oxoid, UK) and incubated aerobically at 37°C for 24-48 hours. Cultures were then checked for the presence of *S. aureus* (yellow colonies) and subcultured onto chocolate agar. The presumptive *S. aureus* isolates were further examined for pigments and coagulase production [10]. *S. aureus* confirmed isolates were

subjected to oxacillin disk diffusion test following guidelines of Clinical Laboratory Institute [11]. Well-isolated methicillin-resistant colonies were selected and inoculated onto nutrient agar slants and 10% v/v glycerol. Stock cultures were kept in the lower compartment of a refrigerator at a temperature (8-12°C) [12].

Molecular identification

DNA extraction (Boiling method)

Five milliliters of an overnight broth culture of the bacterial isolate in Luria Bertani (LB) was spun at 14000rpm for 3 minutes. The cells were re-suspended in 500ul of normal saline and heated at 95^0C for 20 minutes. The heated bacterial suspension was cooled on ice and spun for 3 minutes at 14000rpm. The supernatant containing the DNA was transferred to a 1.5ml microcentrifuge tube and stored at -20°C for other downstream reactions.

DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was launched by double clicking on the Nanodrop icon. The equipment was initialized with 2ul of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestal; the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the "measure" button.

16S rRNA amplification

The 16s rRNA region of the rRNA gene of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.5uM and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light transilluminator.

Sequencing

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25ul BigDye® terminator v1.1/v3.1, 2.25ul of 5 x BigDye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing condition were as follows 32 cycles of 96°C for 10seconds, 55°C for 5seconds and 60°C for 4minutes.

Phylogenetic analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLAST. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 [13]. The bootstrap consensus tree inferred from 500 replicates [14] is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method [15].

Antimicrobial susceptibility testing

The agar disk diffusion susceptibility test of 8 antimicrobials gentamicin (30 ug), chloramphenicol (30 ug), erythromycin (15 µg), ceftazidime (30 µg), ceftriaxone (30 ug), levofloxacin (5 ug), amoxicillin (10 ug) and ciprofloxacin (5 ug) was carried out by using the Clinical and Laboratory Standard Institute guidelines [11].

Determination of multiple antibiotic resistance (MAR) index

For the purposes of this investigation, multi-drug resistance was defined as the resistance of *S. aureus* isolates to three or more classes of antibiotics. The formula MAR = a/b, where a represents the number of antibiotics for which the test isolates showed resistance, and b represents the total number of antibiotics for which the test isolates were assessed sensitively, is used to calculate multi-antibiotic resistance (MAR) by index of individual isolates [16].

Data analysis method

Data analysis was done using SPSS (Statistical Package for Social Sciences,) version 23.0 with *P*-value set at 0.05 level of significance.

Statistical packages used included, Prevalence rates of different sample types, Descriptive statistics and Chi Square.

Results

Molecular identification of *Staphylococcus aureus*

Results of Agarose gel electrophoresis on Plate 1 showed the amplified 16S rRNA gene bands at 1500bp. Lane L represents the 100 bp molecular ladder. The obtained 16s rRNA sequence from the isolate (Figure 1) produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of isolates 1, 2, 3, 4 and 5 showed a 100% similarity to *S. aureus*. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolates within the *Staphylococcus* spp. and revealed a closely relatedness to *Staphylococcus aureus.*

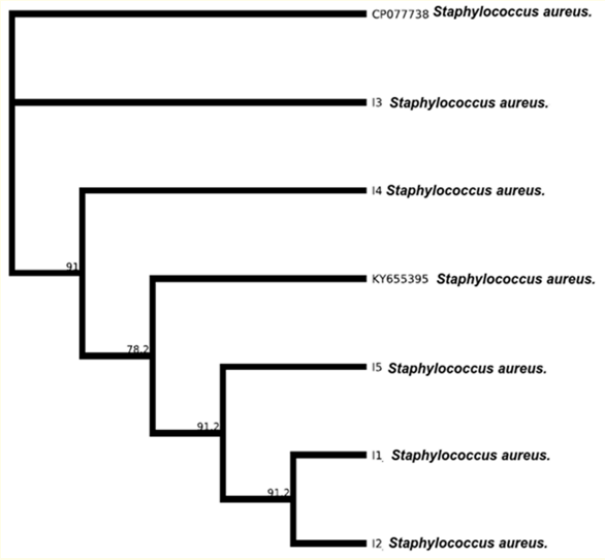

Figure 1: Phylogenetic tree showing evolutionary relatedness between isolates.

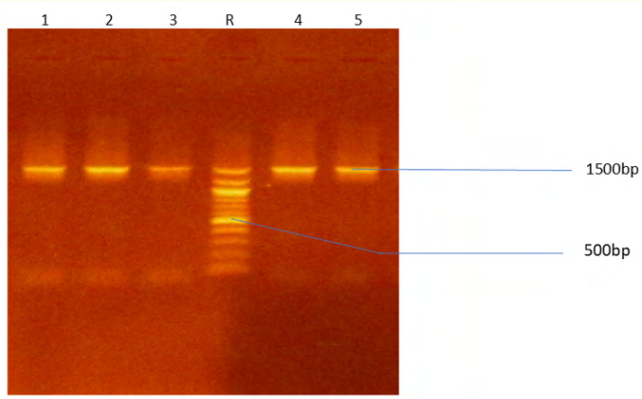

Plate 1: Agarose gel electrophoresis of MRSA mecA gene of some selected bacterial isolates.

Lane 1 – 5 represents 16SrRNA gene bands (1500bp) (lane 1 = Nostril, 2 = Mouth, 3 = Skin, 4 = Skin, and 5= Skin are positive samples from the various sampling sites on the dogs showing the 1500bp gene). Lane R represents the 500bp Molecular weight maker.

Prevalence rates of *Staphylococcus* spp., *S. aureus* and coagulase negative *staphylococcus* (CONS) from different sample type

This present study revealed that out of the 202 *Staphylococcus* spp. Isolates recovered from the sampling sites (Mouth, Nose and Skin) of 70 dogs, 87.62% were *S. aureus* and 12.38% were Coagulase Negative *Staphylococcus*, of these the nose has the 37.85%, the mouth has 31.68% and the skin has 25.99% (Table 1).

Identification of methicillin susceptible *S. aureus* (MSSA) and methicillin resistance *S. aureus* (MSSA)

The isolates that persisted in the presence of oxacillin with inhibition ≤10 mm were considered as Methicillin-Resistant. Out of a total 177 *S. aureus* isolates recovered from the Mouth, Nose and Skin of the 70 dogs (Table 2), 135(76.27%) were MSSA, of these, the mouth has the predominant 56(31.64%), the nose has 54 (30.51%) and the skin has the least occurrence 25(14.12%). While, 42(23.73%) were MRSA, of these, the skin had the predominant 21(11.86%), followed by the nose 13(7.34%) and mouth had the least 8(4.52%) (Table 2).

This study also revealed that out of the 70 dogs recruited for this study only 33% (23) have at least one of its sites colonized by MRSA. While 67% (47) were not colonized by MRSA (Figure 2).

Sample/ Swab Type	*Staphylococci spp.* n(%)	*S. aureus* n(%)	CONS n(%)
Mouth Swab	67(33.17)	64(31.68)	3(1.48)
Skin Swab	66(32.67)	46(25.99)	20(9.90)
Nose Swab	69(34.16)	67(37.85)	2(1.0)
Total	202 (100)	177(87.62)	25(12.38)

Table 1: Prevalence Rates of *Staphylococcus* spp., *S. aureus* and Coagulase Negative *Staphylococcus* (CONS) from different sample type.

Sample/ Swab Type	*S. aureus* n	MSSA n (%)	MRSA n (%)
Mouth Swab	64	56(31.64)	8(4.52)
Skin Swab	46	25(14.12)	21(11.86)
Nose Swab	67	54(30.51)	13(7.34)
Total	177	135(76.27)	42(23.73)

Table 2: Prevalence Rates of *S. aureus*, MSSA and MRSA from different sample type.

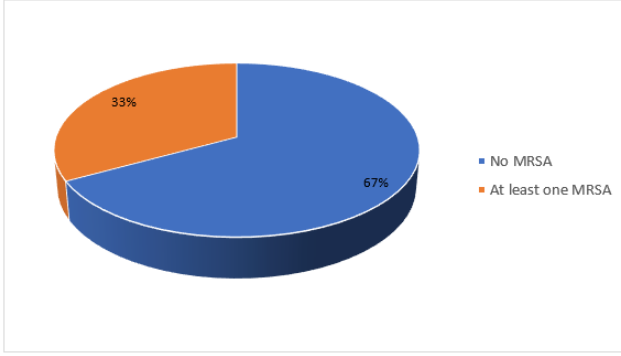

Figure 2: Prevalence of MRSA on recruited Dogs.

Antibiotic susceptibility pattern among methicillin resistant *Staphylococcus aureus* (MRSA) Isolated

Results of susceptibility pattern of MRSA isolates as presented in Table 3 revealed that the isolates showed the highest susceptibility to Levofloxacin (66.67%) followed by Ceftriaxone (54.76%) and Ciprofloxacin (52.38%). The resistance was high with Amoxicillin (88.10%) and Chloramphenicol (40.48%).

Multi-antibiotics resistant indices of the MRSA Isolates

All the 42 MRSA isolates had multidrug resistance index of > 0.2 while 13 isolates 30.95% had 0.2 as their Multidrug resistance index (Table 4).

Antibiotics	Concentration (µg)	Resistance n (%)	Intermediate n (%)	Susceptible n (%)
Amoxicillin	10	37(88.10)	0(0.00)	5(11.90)
Ceftazidime	30	15(35.71)	22(52.38)	5(11.90)
Ceftriaxone	30	6(14.29)	14(33.33)	23(54.76)
Ciprofloxacin	5	6(14.29)	14(33.33)	22(52.38)
Chloramphenicol	30	17(40.48)	13(30.95)	12(28.57)
Erythromycin	15	10(23.80)	21(50.00)	11(26.19)
Levofloxacin	5	2(4.76)	12(28.57)	28(66.67)
Oxacillin	1	42(100.00)	0(0.00)	0(0.00)

Table 3: Antibiotics susceptibility pattern for Methicillin-Resistant *S. aureus* isolates.

MAR index	No. of isolates (%)
0.2	13(30.95)
0.3	17(40.48)
0.4	0(0)
0.5	8(19.05)
0.6	3(7.14)
0.7	1(2.38)

Table 4: MAR index of Methicillin Resistant *Staphylococcus aureus* (N = 42).

Discussion

The emergence of MRSA in pets is of utmost concern when considering animal health, and also more importantly, the possibility for pets to act as a means by which human are colonized or infected by MRSA [17,18]. As a result of animal-to-human transmission of MRSA, there is likelihood that the infection will continue to spread from animals to human and afterward human to human until the animal is free of infection [19].

This study established the presence of MRSA in pet dogs in Port Harcourt. Other studies have also isolated MRSA from dog [20,21]. This study revealed that out of the 70 dogs recruited 33% (23) have at least one of its sites colonized by MRSA. In the study area those working as veterinary staff lack adequate knowledge on proper handling of dogs, veterinary staff and dog owners having non-protected contact with pets. And there is no effective policy in the study area to ensure that dog owners take proper care of their dogs and to check quackery. Thus, there could be possible transmission of MRSA from human to dog or dog. The prevalence of MRSA reported in the current study is in agreement with the findings of Shoaib., *et al*. [9] which reported a nasal carriage of 33.91% in Pakistan, but at variance with that reported by Habibullah., *et al*. [21] in dogs (42.62%). The variation in prevalence could probably be as result of unsanitary conditions in the study area, and geographical variation.

The prevalence of nasal carriage of MRSA in dogs in this study was 7.34% but it is not also in agreement with Yaser., *et al.* [2] which reported a 5.3% nasal carriage in Jordan. Comparison of MRSA carriage rates reported by different studies is challenging, since various sampling strategies and isolation methods can be used for assessing staphylococcal carriage. This might be also as a result of variation in locations, regulations and policies on vaccination of pets, and the influence of genetic and environmental factors, etc.

The obtained 16S rRNA sequence from the isolate produced an exact match during the megablast search for highly similar sequences. The 16S rRNA of the isolate showed a percentage similarity to other species at 100%. The evolutionary distances computed were in agreement with the phylogenetic placement of the 16S rRNA of the isolates within the *Staphylococcus* spp. and revealed a close relatedness to *Staphylococcus aureus.*

The Results of Susceptibility patterns of MRSA to antibiotics showed a decreasing trend of resistance in the order: Oxacillin (100%), Amoxicillin (88.10%), Chloramphenicol (40.48%) and Ceftazidime (35.71%). This may be as a result the fact that they are commonly used by veterinary personnel and dog owners. This study showed that commonly used antibiotics such as Amoxicillin is no longer reliable in treating staphylococcal infections in this region as clearly seen in the 88.10% resistance of the isolates to these antibiotics. Further, the high resistance to the beta-lactam antibiotics can be explained by the extensive and uncontrolled use of these antibiotics as well as affordability. This result agrees with the results of Egege., *et al*. [6], which showed high resistance (100%) to Amoxicillin. It is however in contrast with another study in Jordan, wherein 46% of MRSA isolates were resistant to amoxicillin [2].

A high percentage of the MRSA isolates were susceptible to Levofloxacin (66.67%), Ceftriaxone (54.76%) and Ciprofloxacin (52.38). Levofloxacin was the most sensitive among all the antibiotics used in this study. This high Susceptibility to Levofloxacin could be that Levofloxacin isn't among the commonly used antibiotics the veterinary personnel and dog owners. This was higher than the findings of Min., *et al.* [22] which Levofloxacin has a susceptibility of 34.7%. This difference could be as a result of the exposure and indiscriminate use of Levofloxacin in that environment, different methods for susceptibility testing, and different breakpoints for the evaluation of the results.

Considerable Multiple Antibiotic Resistance (MAR) indices were recorded in the present study. A MAR index greater than 0.2 was recorded for all isolates tested. This is suggestive of a high-risk source of contamination and an environment where antibiotic is often used. Previous studies from Osundiya., *et al*. [16], made similar observation.

Exposures to contaminated sources are carrier sources for spread of MRSA. MRSA persists because of its ability to resist antibiotics, formation of pathogenic protective biofilms, and evasion of immune system through specific molecular patterns. On the other hands, transfer of typical strains associated with community (CA-MRSA), livestock associated (LA-MRSA), and hospital acquired (HA-MRSA) to non-specific hosts is another clue for extended persistent and spread of MRSA strains.

Conclusion and Recommendation

Dogs are responsible for the transmission of several zoonotic diseases and MRSA to their owners because they are reservoir and vector to this diseases and infections. Thus, dog owners should be educated or sensitized on the zoonotic diseases and their ways of transmission to reduce these infections in human population. Dog owners are recommended to practice proper hygiene while handling these pet dogs in our community and vaccinate their dog(s) regularly.

Conflict of Interest

None was reported among authors.

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Bibliography

1. Ghasemzadeh I and Namazi S H. "Review of bacterial and viral zoonotic infections transmitted by dogs". *Journal of Medicine and Life* 8.4 (2015): 1-5.

2. Yaser H T., *et al*. "Molecular study on methicillin-resistant *Staphylococcus aureus* strains isolated from dogs and associated personnel in Jordan". *Asian Pacific Journal of Tropical Biomedicine* 5.11 (2015): 902-908.

3. Omeed S and Chandrashekhar G U. "Gram Positive Bacteria". *National Library of Medicine* (2022).

4. Devriese L A., *et al*. "*Staphylococcus pseudintermedius* sp., a coagulase-positive species from animals". *International Journal of Systematic and Evolutionary Microbiology* 55.4 (2005): 1569-1573.

5. Fazakerley J., *et al*. "Heterogeneity of *Staphylococcus pseudintermedius* isolates from atopic and healthy dogs". *Veterinary Dermatology* 21.6 (2010): 578-585.

6. Egege SR., *et al*. "Detection of Methicillin-Resistant *Staphylococcus aureus* in Ready-to-Eat Shellfish (Corbiculid heterodont) in Bayelsa State, Nigeria". *Microbiology Research Journal International* 30.3 (2020): 22-35.

7. Ito T., *et al*. "Staphylococcal Cassette Chromosome mec (SCCmec) analysis of MRSA". *Methods in Molecular Biology* 1085 (2014): 131-148.

8. Weese J S. "Methicillin-resistant *Staphylococcus aureus* in animals". *Institute for Laboratory Animal Research Journal* 51 (2010): 233-244.

9. Shoaib M., *et al*. "Diversified epidemiological pattern and antibiogram of mecA gene in *Staphylococcus aureus* isolates of pets, pet owners, and environment". *Pakistan Veterinary Journal* 40.3 (2020): 331.

10. Cheesbrough M. "District Laboratory practice in tropical countries". 2nd edition, Part Two Cambridge University Press (2006): 62-70.

11. Clinical Laboratory Standards Institute (CLSI). "Performance standards for antimicrobial disk and dilution susceptibility tests for Bacteria Isolated from Animals". Approved Standard M31-A3. CLSI, Wayne, PA (2020).

12. Alonso S. "Novel preservation techniques for microbial cultures". In Novel food fermentation technologies. Springer, Cham (2020): 7-33.

13. Saitou N and Nei M. "The neighbor-joining method: A new method for reconstructing phylogenetic trees". *Molecular Biology and Evolution* 4 (1987): 406-425.

14. Felsenstein J. "Confidence limits on phylogenies: An approach using the bootstrap". *Evolution* 39 (1985): 783-791.

15. Jukes TH and Cantor CR. "Evolution of protein molecules". In Munro HN, editor, Mammalian Protein Metabolism, Academic Press, New York (1969): 21-132.

16. Osundiya OO., *et al*. "Multiple Antibiotic Resistance (MAR) indices of pseudomonas and Klebsiella species isolates in Lagos University Teaching Hospital". *African Journal of Clinical and Experimental Microbiology* 14.3 (2013): 164-168.

17. Weese JS., *et al*. "Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the household". *Veterinary Microbiology* 115.1-3 (2006): 148-155.

18. Cuny C., *et al*. "Livestock associated MRSA (LA-MRSA) and its relevance for humans in Germany". *International Journal Medical Microbiology* 303 (2013): 331-337.

19. Leonard F C and Markey B K. "Methicillin resistant *Staphylococcus aureus* in animals. A review". *Veterinary Journal* 175 (2008): 27-36.

20. Wedley AL., *et al*. "Carriage of *Staphylococcus species* in the veterinary visiting dog population in mainland UK: molecular characterisation of resistance and virulence". *Veterinary Microbiology* 170 (2014): 81-88.

21. Habibullah A., *et al*. "Prevalence and molecular detection of methicillin-resistant *Staphylococcus aureus* from dogs and cats in Dhaka City". *Bangladesh Journal of Veterinary Medicine* 15 (2017): 51-57.

22. Min SH., *et al*. "*Staphylococcus pseudintermedius* infection associated with nodular skin lesions and systemic inflammatory response syndrome in a dog". *The Canadian Veterinary Journal* 55.5 (2014): 480.