STUDY ON PREVALENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN POULTRY MEAT IN COIMBATORE DISTRICT

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Abstract

Methicillin-resistant Staphylococcus aureus (MRSA) are a major public health problem. Study reports from World Health Organization (WHO) indicate that 20% of the documented S. aureus infections across the globe are due to MRSA, and in some developing countries the rate has gone up to 80%. Routine screening for MRSA is one of the most important technique for the successful infection control. The present study was envisaged to evaluate the prevalence of MRSA in poultry meat samples collected from retail poultry meat outlets. A total of 60 samples were collected from different retail outlets across Kerala state. Out of 60 samples 17 were found positive for S. aureus (28.3%) and PCR analysis using mecA gene confirmed that 4 isolates were MRSA (6.6%). All MRSA isolates were subjected to antimicrobial susceptibility test. MRSA isolates were found sensitive to tetracycline and chloramphenicol and found resistant to gentamicin, co-trimoxoazole, ciprofloxacin, methicillin, ampicillin, amoxyclav, cefuroxime, vancomycin and erythromycin indicating all MRSA isolates are multidrug resistant. The results from this study clearly suggest the increasing resistance of S. aureus to β-lactam antibiotics is responsible for the emergence of MRSA.

Key words: MRSA, *Staphylococcus aureus*, PCR, *mec*A, Antibiogram

1. INTRODUCTION

Staphylococcus aureus is a pathogens of greatest concern to human because of its intrinsic virulence because of its ability to cause a diverse array of life-threatening infections and its capacity to adapt to different environmental conditions (Deurenberg *et al*., 2007). *S. aureus* colonizes the nasopharynx, skin, and gut mucosa of various host species such as humans and birds (Geenen *et al*., 2013). The prevalence MRSA isolates in livestock have gained great attention in recent years (Feßle et al., 2011). The identification of livestock-associated MRSA in food-producing animals has raised questions regarding the presence of MRSA in food of animal origin.

MRSA have acquired the *mec*A gene which encodes an alternative penicillin-binding protein 2α with reduced affinity for methicillin (Nworie *et al*., 2013). This gene complex also allows cross resistance to non-

beta lactam antibiotics such as clindamycin, ciprofloxacin, cotrimoxazole, erythromycin and gentamycin because of the presence of insertion sites for plasmids and transposoons. Recently, MRSA has been increasingly reported as a potential zoonotic pathogen (Assafi et al., 2020; Bakeet and Darwish, 2014) isolated from a number of animal species such as Dogs, Cats, Horses, Sheep, Pigs and Chickens worldwide (Becker *et al*., 2002).The presence of MRSA in poultry and poultry products is of great concern to the poultry industry because of the risk to human health. The major risk is associated with the production of staphylococcal enterotoxins by MRSA strains, which can cause staphylococcal foodborne illness (Lee et al., 2018; Sergelidis and Angelidis. 2017). Raw meat handling, cross contamination, and undercooked meat consumption may lead to MRSA infections (Kluytmans, 2010).

The objective of this study was to estimate the prevalence of MRSA in poultry meat to provide epidemiological data regarding the overall occurrence of this microorganism in the poultry meat outlets across Kerala state.

2. MATERIALS AND METHODS

2.1. Sample collection

Poultry meat samples were collected from retail outlets in sterile sample collection bags. one gram of meat was weighed and added 10 ml of and beaten down using a stomacher. 1 ml of sample mix was inocculated in to primary enrichment media (peptone water) and incubated at 37^0C for 24 hrs.

2.2. Isolation of Staphylococcus aureus

A standard protocol described by Lancette and Bennett (2001) was used for the isolation of *Staphylococcus aureus*. Swab tips were suspended in 10 ml of staphylococcal enrichment broth and incubated at 37°C for 24hr. A loopful of the inoculum from the enrichment broth was streaked onto Baird-Parker (BP) agar plates and incubated at 37°C for 24h. After incubation, the colonies having characteristic appearance on BP agar medium were selected and transferred onto nutrient agar slants and incubated at 37°C overnight.

2.3. Screening *S. aureus* on MRSA selective agar plates

The isolated *S. aureus* bacterial strains were streak plated on HiCrome™ MRSA Agar (HiMedia, India) plates. Plates were incubated at 28C for 24 hours in an incubator, MRSA colonies will be giving blue coloured colonies on selective agar plates.

2.4. *Preparation of DNA template*

DNA isolation from isolated sample of *S. aureus* were carried out using PureLink™ Microbiome DNA Purification Kit (ThermoScientific, USA) following the manufacturer's instructions.

2.5. PCR confirmation of MRSA using *mecA*

Identification of MRSA isolates to the species level was verified by PCR using primers *mec*A gene (Brakstad *et al.*, 1992). The presence of *mec*A gene was verified in *Staphylococcus aureus* isolates using the following primers. *mec*A gene, For- 5'-AAA ATC GAT GGT AAA GGT TGG C-3' and *Rev* 5'-AGT TCT GCA GTA CCG GAT TTG C-3' that are expected to yield a PCR product of 533 bp for *mec*A gene. PCR was performed in a 25µl reaction mixture with a PCR buffer containing 200 µm concentration of each deoxynucleoside triphosphate (dNTP), 10 mM Tris-Hcl (pH 8.3), 1.5 mM $MgCl_2$, 1 unit of Taq polymerase (Promega), 0.25 µM concentration of each primer and 2.5 µl of DNA template. DNA amplification was carried out for 34 cycles in 25 µl of reaction mixture as follows: denaturation at 94°C for 50 seconds, annealing at 57°C for 50 seconds and 58°C for 50 seconds for *nuc* and *mec*A gene respectively, extension at 72°C for 50 seconds with a final extension at 72°C for 5 minutes. The amplified products were analyzed by agarose gel electrophoresis in 1.5% gel.

2.6. Antimicrobial Susceptibility Test

Antimicrobial Susceptibility Test (AST) was carried out for the MRSA isolates using disc diffusion method according to Clinical and Laboratory Standards Institute methods. Briefly, each of the isolate was inoculated into nutrient broth (HiMedia) and incubated at 37°C for 24. Turbidity of the growing culture was adjusted to correspond with that of a barium sulphate (0.5 MacFarland) standard. About 0.1 ml of the nutrient broth culture was subsequently inoculated onto Mueller Hinton agar plates and spread over the surface with sterile L rods (Spreaders). Antimicrobial discs were then placed on the surface of each plate by means of antibiotic disc dispenser and incubated at 37°C for 24 hours. Diameters of inhibition zone were measured using a transparent ruler and the interpretative breakpoints for resistance were determined by comparing zone diameters as recommended by Clinical and Laboratory Standards Institute and readings were recorded. The methicillin-resistant *Staphylococcus aureus* isolates were tested against a panel of 11 antimicrobials namely; ampicillin (10µg), amoxyclav (amoxicillin and clavulanate) (30µg), cefuroxime (30µg), chloramphenicol (30µg), co-trimoxazole (25µg), ciprofloxacin (5µg), erythromycin

(15µg), gentamycin (10µg), methicillin (5µg) and tetracycline (30µg), vancomycin (10µg).

3. RESULT AND DISCUSSION

MRSA has been increasingly reported as emerging problem particularly in small animals and poultry (Broens, 2011). The presence of MRSA in poultry farms, slaughter houses, carcases, or food of poultry origin has been reported in recent years (Persoons et al., 2009). In the present study, 22 of the 60 poultry meat samples evaluated confirmed the presence of *S. aureus***.** They showed the characteristic un-crowded gray black to jet black coloured colonies on BP agar plates (**Fig.1a**), Only four out of the 60 samples tested showed characteristic groth on MRSA selective agar plates (**Fig.1b**).

Figure 1: (**a**) *S. aureus* with characteristic black colonies on Baird-Parker agar plates (**b**) Multi-drug resistant *S, aureus* with characteristic blue colonies on MRSA selective agar plates.

PCR analysis were carried out using *mec*A gene specific primers resulted with 533bp amplicon confirming that the 4 strains that were MRSA positive (**Fig. 2**).

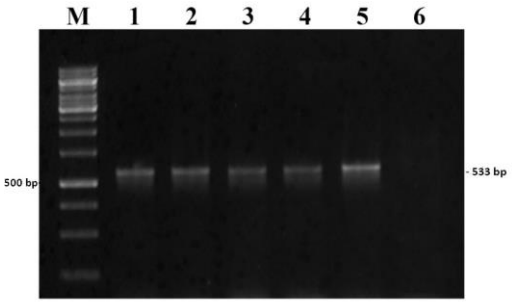

Figure 2: PCR amplification of *mec*A gene, lane**-M** molecular merker**;** Lane **1-4;** MRSA positive samples with 533bp amplicon of *mec*A gene; Lane**-5** Positive control; Lane**-6** Negative control**.**

Antibiogram study were carried out for all the MRSA isolates. These isolates were found sensitive to antibiotics tetracycline, chloramphenicol and found resistant to gentamicin, co-trimoxoazole, ciprofloxacin, methicillin, ampicillin, amoxyclav, cefuroxime, vancomycin and erythromycin indicating all MRSA isolates obtained in this study are multidrug resistant. Results clearly suggest the increasing resistance of *Staphylococcus aureus* to β-lactam antibiotics causing emergence of MRSA.

4. CONCLUSION

The present study revealed that 28.% of poultry meat samples tested were positive for the presence of *S. aureus* and of these 6.6% isolates were found to be MRSA carrying the *mec*A gene and all the MRSA isolates were multidrug resistant. Methicillin resistant *Staphylococcus aureus* is a condition

that needs to be given close surveillance due to its zoonotic importance.

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6. REFERENCES

1. Assafi, M. S., Hado, H. A., & Abdulrahman, I. S. (2020). Detection of methicillin-resistant Staphylococcus aureus in broiler and broilers farm workers in Duhok, Iraq by using conventional and PCR techniques. Iraqi Journal of Veterinary Sciences, 34(1), 15-22. doi:10.33899/ijvs.2019.125757.1145
2. Bakeet, A.M. & Darwish, S.F. doi (2014). Phenotypic and genotypic detection of methicillin- resistant staphylococcus aureus (mrsa) in broiler chickens. *Assiut Veterinary Medical Journal*. 60(143):142-151. doi. 10.33899/ijvs.2019.125757.1145
3. Becker, A., Forster, D. H., & Kniehl, E. (2002). Oxacillin resistance screening agar base for detection of methicillin-resistant Staphylococcus aureus. Journal of clinical microbiology, 40(11), 4400-4401.doi: 10.1128/JCM.40.11.4400-4401.2002
4. Broens, E. M., Graat, E. A. M., Van der Wolf, P. J., Van de Giessen, A. W., & De Jong, M. C. M. (2011). Prevalence and risk factor analysis of livestock associated MRSA-positive pig herds in The Netherlands. Preventive veterinary medicine, 102(1), 41-49. doi.org/10.1016/j.prevetmed.2011.06.005
5. Deurenberg, R. H., Vink, C., Kalenic, S., Friedrich, A. W., Bruggeman, C. A., & Stobberingh, E. E. (2007). The molecular evolution of methicillin-resistant Staphylococcus aureus. Clinical Microbiology and Infection, 13(3), 222-235. doi.org/10.1111/j.1469-0691.2006.01573.x
6. Feßler, A. T., Kadlec, K., Hassel, M., Hauschild, T., Eidam, C., Ehricht, R., ... & Schwarz, S. (2011). Characterization of methicillin-resistant Staphylococcus aureus isolates from food and food products of poultry origin in Germany. Applied and environmental microbiology, 77(20), 7151-7157. doi: 10.1128/AEM.00561-11
7. Geenen, P. L., Graat, E. A. M., Haenen, A., Hengeveld, P. D., Van Hoek, A. H. A. M., Huijsdens, X. W., ... & Van De Giessen, A. W. (2013). Prevalence of livestock-associated MRSA on Dutch broiler farms and in people living and/or working on these farms. Epidemiology & Infection, 141(5), 1099-1108. DOI: 10.1017/S0950268812001616
8. Kluytmans, J. A. J. W. (2010). Methicillin-resistant Staphylococcus aureus in food products: cause for concern or case for complacency?. Clinical microbiology and infection, 16(1), 11-15. doi.org/10.1111/j.1469-0691.2009.03110.x
9. Lancette, G.A. and Bennett, R.W. (2001). In: Downes, F.C., Ito, K. (eds.), Compendium of Methods for the Microbiological Examination of Foods. (4th Ed.). American Public Health Press, pp. 387-403.
10. Luteijn, J.M., Hubben, G.A.A., Pechlivanoglou, P., Bonten, M.J. & Postma, M.J., (2011). Diagnostic accuracy of culture-based and PCR-based detection tests for methicillin-resistant Staphylococcus aureus: a meta-analysis. *Clinical Microbiology and*

Infection, *17*(2), pp.146-154. DOI: 10.1111/j.1469-0691.2010.03202.x

11. Nworie, A,, Madubuko, E.F., Eze, U.A. (2013). Nasal carriage of methicillin-Resistant Staphylococcus aureus amongst meat sellers in Abakaliki Metropolis, Ebonyi State, Nigeria. *Microbiology Research International*. 1(3):48-53.Persoons, D., Van Hoorebeke, S., Hermans, K., Butaye, P., De Kruif, A., Haesebrouck, F., & Dewulf, J. (2009). Methicillin-resistant Staphylococcus aureus in poultry. Emerging infectious diseases, 15(3), 452. doi: 10.3201/eid1503.080696
12. Sergelidis, D., & A. S. Angelidis. (2017). Methicillin-resistant Staphylococcus aureus (MRSA): a controversial food-borne pathogen. *Lett. Appl. Microbiol*. 64:409–418. doi.org/10.1111/lam.12735
13. Lee, A. S., de Lencastre, H., Garau, J., Kluytmans, J., Malhotra-Kumar, S., Peschel, A., & Harbarth, S. (2018). Methicillin-resistant Staphylococcus aureus. *Nature reviews Disease primers*, *4*(1), 1-23. doi.org/10.1038/nrdp.2018.33