Journal of Animal Research: v.9 n.5, p. 775-781. October 2019

DOI: 10.30954/2277-940X.05.2019.22

Comparative Evaluation of Methicillin Resistant and Methicillin Sensitive *Staphylococcus aureus* of Livestock Origin for Antibiotic Sensitivity, Biofilm Formation and Virulence in *Galleria mellonella*

Sirsant Bhoomika[1]*, Ragini Hazari[2], Jay Prakash Yadav[1], Richa Pathak[1], Kaushik Satyaprakash[1], Diksha Gourkhede[1], Satya Veer Singh Malik[1], Sukhadeo B. Barbuddhe[3] and Deepak B. Rawool[1]

[1]*Division of Veterinary Public Health, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P., INDIA*
[2]*Division of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Anjora, Durg, C.G., INDIA*
[3]*ICAR-National Research Centre on Meat, Chengicherla, Hyderabad, INDIA*

**Corresponding author: S Bhoomika; E. mail: sirsant.mannat.09@gmail.com*

Received: 19 Aug., 2019 **Revised:** 30 Sept., 2019 **Accepted:** 08 Oct., 2019

ABSTRACT

The objectives of the study were to isolate and identify livestock associated methicillin resistant *Staphylococcus aureus* (LA-MRSA) and methicillin sensitive *S. aureus* (LA-MSSA) from clinical mastitis cases and to compare their antibiotic susceptibility, biofilm formation and *in vivo* pathogenicity in *Galleria mellonella* larva model. A total of 60 milk samples were collected from cows suffering from mastitis and processed for isolation and identification of *S. aureus* using standard conventional methods. All the recovered *S. aureas* isolates were subjected for detection of MRSA and/or MSSA employing phenotypic (Cefoxitin disc assay) and genotypic (the *mec*A gene PCR) assays. Antibiotic susceptibility pattern of LA-MRSA and LA-MSSA test isolates was determined using disc diffusion method, biofilm formation by 96 well microtiter plate assay and pathogenicity testing in *G. mellonella* larvae. On microbiological, biochemical and PCR analyses, 14 *S. aureus* isolates were confirmed. Of these, 4 were tested as LA-MRSA and the remaining 10 isolates were LA-MSSA. Comparative evaluation suggested that MRSA isolates were resistant to different classes of antibiotics and were equally lethal to G. *mellonella* larvae. However, bioflim forming ability was significantly higher ($p < 0.001$) in the MSSA test isolates. An association of biofilm formation and pahogenicity testing was not observed between LA-MRSA and LA-MSSA test isolates. Further, LA-MRSA were resistant to different classes of antibiotic and were more lethal to *G. mellonella* larvae. These preliminary observations are of great concern as the LA-MRSA infections in the community have been documented and warrant in depth research for such pathogens.

Keywords: Antibiotic sensitivity pattern, Biofilm, *Galleria mellonella*, LA-MRSA, Virulence

Methicillin-resistant *Staphylococcus aureus* (MRSA) is well known pathogen in health care facilities and it has emerged as an important pathogen in the community and livestock settings (Parvez *et al*., 2018). In general, MRSA isolates are resistant to β-lactam antibiotics, however, they can also exhibit resistance to other antimicrobial agents, such as macrolides, tetracycline, aminoglycosides, chloramphenicol, and fluoroquinolones (Piechota *et al*., 2018). Moreover, MRSA are associated with a wide range of infections ranging from superficial skin infections to life threatening conditions such as bacteremia, endocarditis, pneumonia or toxic shock syndrome (Piechota *et al*., 2018). Their persistence leading to infection within the host depends largely on their ability to express the battery of virulence factors (virulence associated and biofilm formation genes), which promote their adhesion, acquisition of nutrients, and evasion of host immunologic responses (Ahmadrajabi *et al*., 2017).

How to cite this article: Bhoomika, S., Hazari, R., Yadav, J.P., Pathak, R., Satyaprakash, K., Gourkhede, D., Malik, S.V.S., Barbuddhe, S.B. and Rawool, D.B. (2019). Comparative evaluation of methicillin resistant and methicillin sensitive *Staphylococcus aureus* of livestock origin for antibiotic sensitivity, biofilm formation and virulence in *Galleria mellonella*. *J. Anim. Res.,* **9**(5): 775-781.

Globally, MRSA is responsible for most of the clinical bacteriaemia cases. Also, the infections associated with MRSA have poor clinical outcomes. Besides, several clinical studies have predicted that MRSA significantly contributes to higher mortality rates (Shurland *et al*., 2007). Contrary to the above statements, there are some clinical studies which have postulated that MRSA and MSSA strains are equally pathogenic in nature (Marty *et al*., 1992). In this context, several comparative studies (phenotypic and genotypic) have been undertaken to investigate their biofilm formation (Grinholc *et al*., 2007; Ghasemian *et al*., 2016; Piechota *et al.,* 2018) and virulence potential (Kocsis *et al*., 2010; Wang *et al*., 2010). However, most of the studies have been restricted to either hospital and/or community acquired isolates (Ghasemian *et al*., 2016; Piechota *et al*., 2018) albeit, comparative studies pertaining to livestock associated MRSA (LA-MRSA) and/or LA-MSSA clinical isolates are lacking. In recent past, several reports have stated the emergence of LA-MRSA in foods of animal origin (Mohammed and Nigatu, 2015). In fact, LA-MRSA are known for their sporadic human infections in several countries and may lead to widespread disease episodes, if get adapted to humans (Cuny *et al*., 2015). In this context, the present study was undertaken to isolate and identify LA-MRSA and LA-MSSA strains from cows suffering from mastitis using standard cultural methods. The identified livestock origin isolates (MRSA/MSSA) were further comparatively studied for their antibiotic susceptibility, biofilm formation and virulence testing in *Galleria mellonella* larvae model.

MATERIALS AND METHODS

Sample collection

Milk samples were aspectically collected from clinical mastitic cows (n=60) that were referred to Referral Veterinary Polyclinic, Durg, Chhattisgarh. The samples were transported to the laboratory under chilled condition. All the milk samples were processed for the isolation of *S. aureus* using standard microbiological and biochemical methods. The culturally identified *S. aureus* isolates were confirmed by PCR targeting the *nuc* gene (Brakstad *et al*., 1992) and the *coa* gene (Hookey *et al*., 1998), respectively.

Identification of MRSA

All the confirmed *S. aureus* were then subjected to phenotypic and genotypic detection of MRSA. Adopting CLSI guidelines, phenotypic detection of MRSA was performed with cefoxitin (30µg) disc assay (CLSI, 2018). The *S. aureus* isolates exhibiting a zone of inhibition of < 21 mm were presumptively identified as MRSA positive. The genotypic identification of MRSA was performed by PCR targeting the *mec*A gene (310 bp) as described earlier (Sahebnasagh *et al*., 2014). The amplified PCR products were sequenced and confirmed by the NCBI BLASTn tool.

Antibiotic susceptibility Test

The LA-MRSA (n=4) isolates and LA-MSSA (n=10) isolates were assessed for their antimicrobial susceptibility against 12 different antibiotics. The antimicrobial susceptibility testing (AST) was performed using a disc diffusion method. *Staphylococcus aureus* MTCC 737 (*S. aureus* ATCC 25923) was used as quality control strain. The data generated was interpreted as per the CLSI guidelines (CLSI, 2018).

Biofilm formation

The biofilm forming ability of the test isolates was estimated as described earlier (Torlak *et al*., 2017) with slight modification. In our study, we used Brain heart Infusion broth (BHI) in place of Tryptic soya broth (TSB) supplemented with 2% (w/v) glucose. The BHI broth without bacterial cell served as a negative control. The quantitative biofilm assay was performed in triplicate for all the test isolates. The biofilm forming ability of the test isolate was classified as described earlier (Stepanovic *et al*., 2007). In brief, the $O.D._{595}$ values recorded by the bacterial films were used to classify isolates as either non biofilm producers, weak, moderate or strong biofilm producers. The cut-off O.D. (O.D.c) was defined as three standard deviations above the mean O.D. of the negative control. In brief, strains were classified as follows: O.D.c > O.D = non biofilm producer, (O.D.c < O.D. ≤ 2 O.D.c) = weak biofilm producer, (2 O.D.c) < O.D. ≤ (4 O.D.c) = moderate biofilm producer and (4 O.D.c) < O.D. = strong biofilm producer.

G. mellonella virulence assay

The virulence potential of LA-MRSA and LA-MSSA

test isolate was investigated in *G. mellonella* larvae, as described earlier (Debosis and Coote, 2011). In brief, the larvae (ca. 200–250 mg) were inoculated with 10 µl of log phase grown culture (10^7 CFU) of test isolates (LA-MRSA and LA-MSSA) using the digital syringe applicator (Holmarc, India) and a Hamilton syringe (capacity 50µl; 24 G; point style 2 with beveled tip). The culture was inoculated in the hindmost left proleg at fifth or sixth instar. Inoculated larvae along with appropriate control were incubated at 37°C and were observed for their mortality at 6 h intervals up to 120 h post inoculation. Throughout the experimentation, the larvae were kept in a germ free environment and were provided with *ad libitum* food.

Statistical analysis

The differences in the degree of biofilm formation between LA-MRSA and LA-MSSA isolates were examined by one-way analysis of variance (ANOVA) with Tukey's multiple comparison tests. P values of < 0.05 were considered as significant.

RESULTS AND DISCUSSION

Isolation and identification of LA-MRSA

On microbiological, biochemical and PCR analyses, 14 *S. aureus* were confirmed (Table 1). All the *S. aureus* isolates revealed amplification of the *nuc* gene (279 bp), however, of the 14 *S. aureus* isolates, 10 were tested positive for the *coa* gene (600 to 800 bp), while, the remaining 4 failed to amplify the *coa* gene (Table 1). Almost similar phenotype results were observed in tube coagulase test (Table 1). Of the 14 *S. aureus* isolates, 4 were tested positive for MRSA by both phenotypic and genotypic methods, while, the remaining 10 *S. aureus* isolates were presumed as MSSA. Usually the detection of MRSA in food producing animals is low (Huber *et al*., 2010). Earlier, studies from Asian countries have reported their occurrence ranging between 6.3 to 47 percent in food producing animals (Mohammed and Nigatu, 2015).

Antibiotic Susceptibility profile of LA-MRSA and LA-MSSA

Previous studies have reported that hospital acquired-MRSA (HA-MRSA) are more multidrug resistant in comparison to community acquired-MRSA (CA-MRSA), however, a recent study predicted that CA-MRSA isolates were more resistant to tested antibiotics than HA-MRSA (Belbase *et al*. 2017; Parvej *et al*., 2018). Such information pertaining to LA-MRSA is lacking. In this study, all the MSSA (n=10) isolates were found to be sensitive to all the tested antibiotics except for penicillin (8/10) and ampicillin (1/10) (Table 2). Similarly, all the MRSA (n=4) isolates were also found sensitive to most of the tested antibiotics except for penicillin (4/4), ampicillin (4/4), vancomycin (2/4) and gentamicin (2/4) (Table 2). Comparatively, almost all LA-MSSA isolates were sensitive to all the tested antibiotics that were commonly prescribed in livestock, whereas LA-MRSA test isolates exhibited resistant against βeta lactam, cephalosporins, aminoglycosides and glycopetides antibiotics. Usually LA-MRSA are resistant and in part to fluoroquinolones and cotrimoxazole (Cuny *et al*., 2015). Besides, a number of studies have reported more than 80% of MRSA to be resistant against erythromycin, ciprofloxacin, gentamicin, tetracycline and clindamycin (Chuang and Huang, 2015). Although, LA-MRSA were often susceptible to glycopeptides, daptomycin, tigecyclin, rifampicin, fusidic acid, fosfomycin, and also to linezolid, the difference observed in susceptibility towards antibiotics in the present study might be due to strain variability and also due different antibiotic usage in different countries (Chuang and Huang, 2015). Moreover, in this study, the LA-MRSA test isolates (n=2) were also resistant to vancomycin, which is of great public health concern, as the LA-MRSA infections in the community have been documented earlier (Cuny *et al*., 2015).

Biofilm forming ability of LA-MRSA and LA-MSSA

The emergence of MRSA in food producing animals has provoked a great concern in associated food stuff (Doulgeraki *et al*., 2017). In fact, both MRSA and MSSA have an inherent ability to form biofilm on biotic and abiotic surfaces. These biofilms protects the bacterial cells from host immune responses as well as from antimicrobial and disinfectant agents. The results of biofilm formation are presented in Table 2. In brief, majority of the LA-MRSA isolates (3/4) were weak biofilm producers, whereas, most of the LA-MSSA isolates revealed strong (3/10) to moderate (4/10) biofilm formation, except three LA-MSSA isolates which exhibited weak to non-biofilm

Table 1: identification details of LA-MRSA and LA-MSSA isolates recovered from cattle mastitis cases

Sl. No	Isolate code	Biochemical tests					Molecular detection		MRSA Identifcation	
		Gram Staining	Catalase	Oxidase	Coagulase	β haemolysis on blood agar	nuc gene	coa gene	Cefoxitin disc assay	PCR targeting mecA gene
1	SA-1	+	+	_	+	+	+	-	_	_
2	SA-2	+	+	_	+	+	+	+	_	_
3	SA-3	+	+	_	+	+	+	+	+	+
4	SA-4	+	+	_	+	+	+	+	_	_
5	SA-5	+	+	_	+	+	+	+	_	_
6	SA-6	+	+	_	+	+	+	+	+	+
7	SA-7	+	+	_	+	+	+	+	+	+
8	SA-8	+	+	_	+	+	+	+	+	+
9	SA-12	+	+	_	+	+	+	+	_	_
10	SA-13	+	+	_	+	+	+	_	_	_
11	SA-14	+	+	_	+	+	+	+	_	_
12	SA-15	+	+	_	+	+	+	_	_	_
13	SA-16	+	+	_	+	+	+	_	_	_
14	SA-17	+	+	_	+	+	+	+	_	_

Table 2: Comparative antimicrobial susceptibility, biofilm formation and virulence potential of LA-MRSA and LA-MSSA isolates

Isolates	Antibiotic Susceptibility		Biofilm formation	*In vivo* virulence in *Galleria mellonella* larvae	
	Sensitive	Resistant		Survival (%)	Median Survival time (h)
MSSA					
SA-1	GM, TE, C, CIP, AMP, CX, VA, LZD, OX, CD, E	PEN	Moderate	100	NA
SA-2	GM, TE, C, CIP, AMP, CX, VA, LZD, OX, CD, E	PEN	Non-Biofilm former	100	NA
SA-4	GM, TE, C, CIP, CX, VA, LZD, OX, CD, E	PEN, AMP	Moderate	88.88	54
SA-5	GM, TE, C, CIP, AMP, CX, VA, LZD, OX, CD, E	PEN	Moderate	88.88	60
SA-12	PEN , GM, TE, C, CIP, AMP, CX, VA, LZD, OX, CD, E	—	Weak	88.88	60
SA-13	PEN ,GM, TE, C, CIP, AMP, CX, VA, LZD, OX, CD, E	—	Weak	55.56	40
SA-14	GM, TE, C, CIP, AMP, CX, VA, LZD, OX, CD, E	PEN	Strong	100	NA
SA-15	GM, TE, C, CIP, AMP, CX, VA, LZD, OX, CD, E	PEN	Strong	100	NA
SA-16	GM, TE, C, CIP, AMP, CX, VA, LZD, OX, CD, E	PEN	Moderate	66.66	72
SA-17	GM, TE, C, CIP, AMP, CX, VA, LZD, OX, CD, E	PEN	Strong	55.56	64
MRSA					
SA-3	GM, TE, C, CIP, AMP, VA, LZD, OX, CD, E	PEN, CX	Weak	0	18
SA-6	TE, C, CIP, LZD, OX, CD, E	PEN, CX, GM, AMP, VA	Weak	0	12
SA-7	TE, C, CIP, LZD, OX, CD, E	PEN, CX, GM, AMP, VA	Weak	0	12
SA-8	GM, TE, C, CIP, AMP, VA, LZD, OX, CD, E	PEN, CX	Moderate	0	24

AMP- Ampicillin, GM – Gentamicin, C- Chloramphenicol, CIP -Ciprofloxacin, TE –Tetracyccline, PEN- Peniillin, CX- Cefoxitin, OX -Oxacillin, CD- Clindamycin, E -Erythromycin, LZD -Linezolid, VA –Vancomycin, NA – Not applicable.

formation (Table 2). Comparatively, LA-MSSA test isolates exhibited significant ($p<0.05$) biofilm formation, when compared with LA-MRSA test isolates. Studies addressing comparative biofilm formation between LA-MRSA and LA-MSSA are sparse; however, earlier study with hospital acquired strains have shown higher biofilm forming ability among HA-MRSA strains (Piechota *et al*. 2018). On the contrary, significant difference in the biofilm formation and distribution of the biofilm encoding genes were not observed among HA-MRSA and HA-MSSA strains (Ghasemian *et al*., 2016). Generally, *in vitro* phenotypic expression of *S. aureus* biofilm appears to be often inconsistent. Also, biofilm production largely depends on media composition, essential nutrient requirements, surrounding temperature, strain type etc (Arciola *et al*., 2012). Moreover, MRSA and MSSA strains produce biofilm with a different mechanism. MSSA strains produce biofilm using the *ica* (intercellular adhesion)-dependent manner (polysaccharide intercellular adhesion (PIA)-dependent), whereas, MRSA forms biofilm through *ica*-independent manner (PIA-independent) (Doulgeraki *et al*., 2017). The difference observed in the biofilm formation among LA-MRSA and LA-MSSA test isolates in the present study could be attributed either one or more facts discussed above. Besides, it would be worth investigating the exact mechanism for its effective management in clinical and food processing settings.

Virulence testing of LA-MRSA and LA-MSSA

Earlier studies on the detection of virulence factors or susceptibility to phagocytosis assays or virulence in animal models were quite conflicting (Kocsis *et al*., 2010). Also, most of the comparative virulence studies between MRSA and MSSA strains were investigated with community and hospital acquired *S. aureus* isolates (Montgomery *et al*., 2008; Wu *et al*., 2012; Perez-Montarelo *et al*., 2017; Mannala *et al*., 2018). We did not come across any study addressing the *in vivo* virulence testing of LA-MRSA and LA-MSSA strains.

G. mellonella larvae are increasingly being used as an infection model to study virulence factors and pathogenesis of many prominent bacterial and fungal human pathogens. In fact, these larvae are easier and cheaper to procure, establish and maintain. Also any special laboratory equipment and ethical approval are not required. The short life span and ability of larvae to mimic the human host while investigating the clinically relevant human pathogens at 37°C suites the larvae to be an ideal in vivo model for high throughput studies (Tsai *et al*., 2016). The results of survival analysis are summarized in Table 2. Overall comparison revealed that all the MRSA test isolates were highly virulent in *G. mellonella* larvae model than MSSA test isolates (Table 2). The median survival time for MRSA test isolates varied between 18-24 h, whereas, for MSSA test isolates it ranged between 40-72 h, respectively (Table 2).

An earlier study reported significant mortality among MRSA strains (strains recovered from clinical complications) in *G. mellonella* larvae than in MSSA strains (Perez-Montarelo *et al*., 2018). Besides, several other studies have stated that HA-MRSA strains appeared to be more virulent in guinea pigs and in mice compared to the congenic MSSA (Cutler, 1979; Kocsis *et al*., 2010). On the contrary, there are few studies which had shown that the lethal dose (LD_{50}) of HA-MRSA strains was significantly higher than that for HA-MSSA (Rozgonyi *et al*., 1984; Kocsis *et al*., 2010). Moreover, some investigators have revealed no difference between the two populations (Peacock *et al*., 1981). In the present study, the significant difference observed in survival of MSSA strains in comparison to that of MRSA strains suggested that LA-MRSA were more virulent than LA-MSSA strains. Since the study was performed with limited number of isolates, we emphasize that more number of LA-MRSA and LA-MSSA strains should be tested for their complete virulence profile to draw a meaningful conclusions.

CONCLUSION

To conclude, the present study appears to be first of its kind to compare LA- MRSA and LA-MSSA for their antibiotic susceptibility, biofilm formation (phenotype) and virulence testing in *Galleria mellonella* larvae model. LA-MRSA were found to exhibit resistance against βeta lactam, cephalosporins, aminoglycosides and glycopeptides, whereas, LA-MSSA isolates were sensitive to almost all the tested antibiotics except penicillin. LA-MSSA test isolates exhibited significantly higher ($p<0.001$) biofilm formation than LA-MRSA isolates. Although numerous studies have predicted Biofilm formation was not associated with the actual pathogenicity observed in

G. mellonella larvae. In the context, it would be worth to investigate the exact biofilm formation mechanism for its effective management in livestock and food processing settings. The virulent nature of the LA-MRSA test isolates is cause of great concern, as antibiotic consumption in farm animals has been the driving force in the emergence and spread of LA-MRSA. Concurrently, the zoonotic aspects of LA-MRSA need to elucidate. Therefore, further in depth studies on every important facet of the LA-MRSA are warranted.

ACKNOWLEDGMENTS

The authors thank Director, Indian Veterinary Research Institute, Izatnagar for providing necessary facilities to undertake the research. We express our gratitude towards Dr Sanjay Shakya, Professor & Head, Department of VPH & Epidemiology, College of Veterinay Science and A.H. Anjora, Durg (CG) for providing necessary facility for the isolation of *S. aureus* and Dr. Vinay Kalia, Senior Scientist, Division of Entomology, IARI, Delhi for providing *Galeria mellonella* culture for our research work. We also thank Mr. K.K. Bhatt for his excellent technical assistance.

REFERENCES

Ahmadrajabi, R., Layegh-Khavidaki, S., Kalantar-Neyestanaki, D. and Fasihi, Y. 2017. Molecular analysis of immune evasion cluster (IEC) genes and intercellular adhesion gene cluster (ICA) among methicillin-resistant and methicillin-sensitive isolates of *Staphylococcus aureus*. *J. Prev. Med. Hyg*., **58**(4): E308.

Arciola, C.R., Campoccia, D., Speziale, P., Montanaro, L. and Costerton, J.W. 2012. Biofilm formation in *Staphylococcus* implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials. *Biomaterials*., **33**(26): 5967-5982.

Belbase, A., Pant, N.D., Nepal, K., Neupane, B., Baidhya, R., Baidya, R. and Lekhak, B. 2017. Antibiotic resistance and biofilm production among the strains of *Staphylococcus aureus* isolated from pus/wound swab samples in a tertiary care hospital in Nepal. *Ann. Clin. Microb. Anti*., **16**(1): 15.

Brakstad, O.G., Aasbakk, K. and Maeland, J.A. 1992. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. *J. Clin. Microbiol*., **30**(7): 1654-1660.

Chuang, Y.Y. and Huang, Y.C. 2015. Livestock-associated meticillin-resistant *Staphylococcus aureus* in Asia: an emerging issue?. *Int. J. Antimicrob. Ag*., **45**(4): 334-340.

Clinical Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility testing: Twenty Second Informational Supplement M100-S28. 2018 Wayne, PA, USA.

Cuny, C., Wieler, L. and Witte, W. 2015. Livestock-associated MRSA: the impact on humans. *Antibiotics*, **4**(4): 521-543.

Cutler, R.R. 1979. Relationship between antibiotic resistance, the production of "virulence factors", and virulence for experimental animals in *Staphylococcus aureus*. *J. Med. Microbiol*., **12(1)**: 55-62.

Desbois, A.P. and Coote, P.J. 2011. Wax moth larva (*Galleria mellonella*): an *in vivo* model for assessing the efficacy of antistaphylococcal agents. *J. Antimicrob. Chemoth*., **66**(8): 1785-1790.

Doulgeraki, A.I., Di Ciccio, P., Ianieri, A. and Nychas, G.J.E. 2017. Methicillin-resistant food-related *Staphylococcus aureus*: A review of current knowledge and biofilm formation for future studies and applications. *Res. Microbiol*., **168**(1): 1-15.

Ghasemian, A., Peerayeh, S.N., Bakhshi, B. and Mirzaee, M. 2016. Comparison of biofilm formation between methicillin-resistant and methicillin-susceptible isolates of *Staphylococcus aureus*. *Iran. Biomed. J*., **20**(3): 175.

Grinholc, M., Wegrzyn, G. and Kurlenda, J. 2007. Evaluation of biofilm production and prevalence of the *icaD* gene in methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains isolated from patients with nosocomial infections and carriers. *FEMS Immunol. Med. Microbiol*., **50**(3): 375-379.

Hookey, J.V., Richardson, J.F. and Cookson, B.D. 1998. Molecular typing of *Staphylococcus aureus* based on PCR restriction fragment length polymorphism and DNA sequence analysis of the coagulase gene. *J. Clin. Microbiol*., **36**(4): 1083-1089.

Huber, H., Koller, S., Giezendanner, N., Stephan, R. and Zweifel, C. 2010. Prevalence and characteristics of meticillin-resistant *Staphylococcus aureus* in humans in contact with farm animals, in livestock, and in food of animal origin, Switzerland, 2009. *Eurosurveillance*., **15**(16): 19542.

Kocsis, E., Kristof, K., Hermann, P. and Rozgonyi, F. 2010. A comparative review on the pathogenicity and virulence factors of meticillin-resistant and meticillin-susceptible *Staphylococcus aureus*. *Rev. Med. Microbiol*., **21**(2): 31-37.

Mannala, G.K., Koettnitz, J., Mohamed, W., Sommer, U., Lips, K.S., Sproer, C., Bunk, B., Overmann, J., Hain, T., Heiss, C. and Domann, E. 2018. Whole-genome comparison of high and low virulent *Staphylococcus aureus* isolates inducing implant-associated bone infections. *Int. J. Med. Microbiol*., **308**(5): 505-513.

Marty, L., Flahault, A., Suarez, B., Caillon, J., Hill, C. and Andremont, A. 1993. Resistance to methicillin and virulence of *Staphylococcus aureus* strains in bacteriemic cancer patients. *Intensive Care Med*., **19**(5): 285–289.

Mohammed, S. and Nigatu, S. 2015. Review on Livestock Associated Methicillin Resistant *Staphylococcus aureus* and its Zoonotic Importance. *Int. J. Microbiol. Res*., **6**: 164-174.

Montgomery, C.P., Boyle-Vavra, S., Adem, P.V., Lee, J.C., Husain, A.N., Clasen, J. and Daum, R.S. 2008. Comparison of virulence in community-associated methicillin-resistant *Staphylococcus aureus* pulsotypes USA300 and USA400 in a rat model of pneumonia. *J. Infect. Dis*., **198**(4): 561-570.

Parvez, M.A.K., Ferdous, R.N., Rahman, M.S. and Islam, S. 2018. Healthcare-associated (HA) and community-associated (CA) methicillin resistant *Staphylococcus aureus* (MRSA) in Bangladesh–Source, diagnosis and treatment. *J. Gene. Eng. Biot*., **16**(2): 473-478.

Peacock Jr., J.E., Moorman, D.R., Wenzel, R.P. and Mandell, G.L. 1981. Methicillin-resistant *Staphylococcus aureus*: microbiologic characteristics, antimicrobial susceptibilities, and assessment of virulence of an epidemic strain. *Jpn. J. Infect. Dis*., **144**(6): 575-582.

Perez-Montarelo, D., Viedma, E., Murcia, M., Muñoz-Gallego, I., Larrosa, N., Branas, P., Fernandez-Hidalgo, N., Gavalda, J., Almirante, B. and Chaves, F. 2017. Pathogenic characteristics of *Staphylococcus aureus* endovascular infection isolates from different clonal complexes. *Front. Microbiol*., **8**: 917.

Piechota, M., Kot, B., Frankowska-Maciejewska, A., Gruhewska, A. and Wofniak-Kosek, A. 2018. Biofilm formation by methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* strains from hospitalized patients in Poland. *Biomed. Res. Int*.: 1-7.

Rozgonyi, F., Laczko, J.O. and Vaczi, L. 1982. Phenotypic expression of resistance to penicillinase-stable beta-lactams in *Staphylococcus aureus*: growth rate, cross wall morphogenesis, and cell division cycle. *FEMS Microbiol. Lett*., **14**(4): 237-240.

Sahebnasagh, R., Saderi, H. and Owlia, P. 2014. The Prevalence of Resistance to Methicillin in *Staphylococcus aureus* Strains Isolated from Patients by PCR Method for Detection of *mecA* and *nuc* Genes. *Iran J. Public Health*, **43**(1): 84.

Shurland, S., Zhan, M., Bradham, D.D. and Roghmann, M.C. 2007. Comparison of mortality risk associated with bacteremia due to methicillin resistant and methicillin-susceptible *Staphylococcus aureus*. *Infect. Control. Hosp. Epidemiol*., **28**(3): 273–279.

Stepanovic, S., Vukovic, D., Hola, V., Bonaventura, G.D., Djukic, S., Cirkovic, I. and Ruzicka, F. 2007. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by *Staphylococci. Apmis*., **115**(8): 891-899.

Torlak, E., Korkut, E., Uncu, A.T. and Şener, Y. 2017. Biofilm formation by *Staphylococcus aureus* isolates from a dental clinic in Konya, Turkey. *J. Infect. Public. Heal*., **10**(6): 809-813.

Tsai, C.J.Y., Loh, J.M.S. and Proft, T. 2016. *Galleria mellonella* infection models for the study of bacterial diseases and for antimicrobial drug testing. *Virulence*., **7**(3): 214-229.

Wang, L., Yu, F., Yang, L., Li, Q., Zeng, X.Z. and Xu, Y. 2010. Prevalence of virulence genes and biofilm formation among *Staphylococcus aureus* clinical isolates associated with lower respiratory infection. *Afr. J. Microbiol. Res*., **4**(23): 2566-2569.

Wu, K., Conly, J., Surette, M., Sibley, C., Elsayed, S. and Zhang, K. 2012. Assessment of virulence diversity of methicillin-resistant *Staphylococcus aureus* strains with a *Drosophila melanogaster* infection model. *BMC Microbiol*., **12**(1): 274.