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The interaction between the antimicrobial compounds and the biofilms of *Staphylococcus* species isolated from bovine mastitis

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Abstract
Bovine mastitis is a dreadful disease of cattle majorly caused by *Staphylococcus* species. In the present study, a total of 20 *Staphylococcus* species were isolated from mastitis milk samples of dairy Cattle. Out of 20 samples, 10 samples were quantitatively characterized as biofilm producers. The highest density of the biofilm was recorded at 32 hrs post incubation in comparison to that of 18 hours culture. Five commonly used antibiotic drugs by the field Veterinarians (Ciprofloxacin, Amoxicillin Na, Gentamicin, Cefaperazone and Enrofloxacin) for treating the mastitis, were tested. The minimum inhibitory concentration (MIC) of the drugs has been altered between 18 hrs post incubation culture and 36 hrs post incubation culture. The sample S6 was sensitive to the drug Enrofloxacin, and the end point of the MIC is 25 µg for 18 hours culture, which has increased to 50 µg for 36 hours culture. For the drug Amoxycillin Na, the MIC required for S11 was 12.52 µg for 18 hrs culture while it was enhanced to 50 µg after 36 hrs of post incubation culture. For the drug Enrofloxacin, the MIC for S11 was 3.125 µg for 18 hrs culture and 6.25 µg for 36 hrs culture and for Ciprofloxacin it was 25 µg for 18 hrs culture and 50 µg for 36 hrs culture. The drug Ciprofloxacin against S13, the variation in MIC was reported as 3.625 µg for 18 hrs and 50 µg for 36 hrs. The S19 sample was sensitive to the drug Amoxycillin Na and the MIC for 18 hrs culture was 25 µg and for 36 hrs culture was 50 µg.. The results have proved that the MIC of antibiotics against the *Staphylococcus* has got altered depending on the density of the biofilm formation by the bacterium. The bacteria encased in biofilms are being protected from the action of antibiotics at therapeutic concentrations, evade the host immune responses and become a potential threat for the health communities.

Keywords: Bovine mastitis, biofilm, anti-microbial compounds, minimum inhibitory concentration

1. Introduction
Bovine mastitis is one of the economically significant diseases of cattle that has huge impact on the diary sector. The major etiological agent that is involved in bovine mastitis is *Staphylococcus* species (Ruegg PL, 2017) [33], which is a gram-positive cocci. In bovine mastitis cases, *Staphylococcus* species mainly targets the mammary tissues and damages the tissue through their wide array of potential virulence factors which contributes for the invasion of the bacterium (Moormeier *et al*., 2017) [25]. Amongst them, the in-vitro biofilm forming ability of the *Staphylococcus* species, further aggravate the pathogenicity of organism in developing bovine mastitis (Rossi BF *et al*., 2019) [31].
The biofilm is produced as an extracellular mucopolysaccharide (Slime layer) that aids in adhesion and colonization of the bacterium in the mammary glandular epithelium to form biofilm colonies. The colonies of the bacterium embedded in the biofilm offers an enhanced tolerance to the opsonophagocytosis, host defence mechanisms, and conventional antibiotics, being 100-1000 times less susceptible to antibiotics when compared with the non-biofilm producing bacterium (Burmolle *et al*., 2010) [6]. In addition to the physical tolerance offered by the biofilm, it may enhance the production of host inflammatory factors like nitrous oxide, lysozymes, peroxides etc. which can damage the epithelial cells adjoining the biofilm (Pérez *et al*., 2020; Hamel *et al*., 2020) [29, 16] making the condition a chronic disease with impact on economic gains.
The biofilm is composed of a high molecular weight polysaccharide called adhesin, and its formation begins with the interaction of the surface oriented adhesion factors such as MSCRAMMs (Microbial surface components recognizing adhesive matrix molecules), autolysin, adhesin, protein SasG, eDNA, fibronecting binding proteins and clumping factors

(Parsek and Singh 2003; Lopez *et al*. 2010) [28, 22]. The *staphylococcus* mediated mastitis infections are very difficult to treat therapeutically and in most cases resurgence of infections are common. Many authors have studied several factors in order to explain the tolerance of the biofilm producing *Staphylococcus* species to antibiotic therapies (Hoiby *et al*., 2011; Hoiby *et al*., 2015) [19, 18]. Some of the mechanisms responsible for this tolerance include physical and chemical diffusion barrier formed by the components of the biofilm that hinders the penetration of the antimicrobial drugs to the site of action (Melchior *et al*., 2006; Gedif, 2020) [24, 14]. The excessive secretion of exopolysaccharide and its associated factors may bring about change in the microenvironment of the mammary tissue that favours the bacterial tolerance to antimicrobial action (Gedif, 2020) [14]. The change in microenvironment may also alter the physiology of the *Staphylococcus* species due to inefficient diffusion of nutrients through the biofilm that results in slow and steady growth of the bacterium (Stoodley *et al*., 2004) [14]. Hence, the biosynthesis mechanisms of bacterial replication may not be reachable for the action of antimicrobial drugs. In addition to these direct antimicrobial counter mechanisms, if the same *Staphylococcus* species is strong biofilm producer may worsen the disease conditions. In the present study, the biofilm producing ability and antimicrobial drug resistance of *Staphylococcus* species was simultaneously studied at different intervals of growth curve of the bacterium. The consequence of simultaneous expression of the mechanisms, (antimicrobial resistance and biofilm production), in *Staphylococcus* species of bovine mastitis was explored through this study.

2. Materials and Methods

2.1 Collection and isolation of *Staphylococcus* species

An aliquot of 20 milk samples were collected from mastitis affected cattle, with the clinical signs of inflammation of the udder, swelling of the teats, abnormal colour of the milk, flakes in the milk, pain on palpation of the udder and reduction in milk production. The milk samples (200 µl) were inoculated into 5ml BHI broth (Hi-Media) and incubated overnight. A loopful of culture was streaked onto the Mannitol salt agar medium (MSA) (Hi-Media) and incubated for 24 hrs. The pin-point colonies developed on MSA agar were selected and the morphology was confirmed by Gram's staining. The pure cultures were then preserved in Glycerol at -20 °C until further use.

2.2 Detection of biofilm forming ability of *Staphylococcus* species

For the detection of biofilm forming ability, qualitative (tube method) and quantitative (tissue culture plate method) methods were followed.

2.2.1. Tube method: The tube method as described by Christensen *et al*. (1982) [7] was used with little modification. Briefly, the protocol followed, 5ml of the BHI broth was inoculated with 20 µl of the overnight *Staphylococcal* culture and incubated at 37 °C for 24 hrs in test tubes. The broth culture was discarded aseptically and the tubes were washed twice with sterile PBS (pH -7.6) The tubes were then stained with Crystal Violet stain for 5 minutes, washed with PBS and air dried by inverting the tubes.

2.2.2. Tissue culture plate method: The *Staphylococcus* culture was inoculated into 5ml of BHI broth and incubated for overnight at 37 °C. The overnight culture was adjusted to 0.5 Mc Farland scale with sterile PBS added with 0.5% glucose to achieve a concentration of $1.5x10^8$ CFU/ml. This culture was 10-fold diluted using BHI broth added with 0.5% glucose and 200 µl was aliquoted into sterile 96-well microtitre plate. The plates were incubated at 37 °C for 24 hrs. The bacterial culture was discarded aseptically, plates were washed twice with PBS, pH -7.6 and air dried for 1 hr. The plates were stained with crystal violet stain 200 µl/well for 15 minutes at room temperature, washed twice with PBS, air dried and absorbance was measured at 490nm in ELISA plate reader (Biorad). All samples were tested in triplicate and average of the absorbance was considered for the final quantitative classification of the bacteria as biofilm producer. Uninoculated BHI broth added with 0.5% glucose was kept as controls. The bacteria was categorized based on the OD values as: ≥0.3 OD- strong biofilm producer; ≤0.3 to 0.2 OD – moderate; ≤0.2 to 0.1-weak and ≤0.1 OD as non-biofilm producing bacteria.

2.3 Determination of incubation period required for minimum and maximum biofilm production

The time required for minimum and maximum production of biofilm was assessed by tissue culture plate method. For each sample of the *Staphylococcus* species, the 0.5 Mc Farland scale matched culture was incubated at 37 °C in duplicates in 96-well tissue culture plates with 200 µl of BHI broth. Each plate was labelled as 6 hrs, 12 hrs, 18 hrs, 24 hrs, 30 hrs, 36 hrs and 42 hrs and incubated for corresponding time intervals. The biofilm production at specified time intervals was determined by measuring the absorbance at 490nm in ELISA plate reader, after staining with Crystal violet. The incubation period required for minimum and maximum biofilm production was established for each sample by analyzing the absorbance at regular intervals of incubation period.

2.4 Impact of biofilm on Minimum Inhibitory Concentration of selected drugs

Each sample of the *Staphylococcus* species was cultured in 5ml of Mueller Hinton broth (MH broth) at 37 °C for overnight and the culture was adjusted to 0.5 McFarland scale by diluting with fresh MH broth in order to obtain $5x10^5$ CFU/ml. The culture was prepared15minutes prior to start the MIC. Among the most commonly used antibiotic drugs by the field veterinarians to treat the bovine mastitis five were selected for the present study, they include: Gentamicin, Ciprofloxacin, Cefaperazone, Amoxicillin, & Enrofloxacin. Two fold serial dilutions of the antibiotics were prepared in MH broth with an initial concentration of 2mg/ml (2*x*) in 96-well microtitre plates (100 µl/well). Meanwhile, two 96-well microtitre plates were labelled as 18 hrs and 36 hrs. For the plate labelled as 18 hrs, 100 µl of the $5x10^5$ cfu/ml of the *Staphylococcus* cultures were added per well and then 100 µl of each dilution of the drug was added to its corresponding well to obtain the 1*x* concentration of the drug per well achieving concentrations of 100 µg, 50 µg, 25 µg, 12.5 µg, 7.25 µg, 3.62 µg, 1.81 µg and 0.9 µg. The plates were incubated for 18 hrs at 37 °C and one hour prior to the completion of the corresponding incubation time, the p-nitrotetrazolium blue dye (p-nitro bromo tetra zoline, Sigma N6876) was added to each well.

For the 36 hrs culture plate, initially the 100 µl of $5x10^5$ cfu/ml culture was incubated for 18 hrs in 96 well microtitre plate, (minimum incubation time required for start of biofilm production) followed by 100 µl of 2*x* drug dilutions were added gently along walls into corresponding well to achieve 100 µg, 50 µg, 25 µg, 12.5 µg, 7.25 µg, 3.62 µg, 1.81 µg and 0.9 µg drug concentration. The plates were incubated for another 18 hrs (total 36 hrs incubation period, maximum biofilm production time) and one hour prior to the completion of the corresponding incubation time, the p-nitrotetrazolium blue dye (p-nitro bromo tetra zoline, Sigma N6876) was added to each well. All biofilm producing samples were tested in duplicate. The biofilm producing samples were included as positive controls for each sample and the negative control was included with MH broth only without the inoculums. The MIC for each drug for 18 hrs as well as 36 hrs cultures were analyzed.

3. Results and Discussion

Mastitis is one of most economically significant disease of cattle and buffaloes caused by a variety of microorganisms like viruses, Bacteria and fungi. Among the bacterial etiological agents, *Staphylococci* species are the predominant bacteria that are involved in the bovine mastitis. The *staphylococcus* has the ability to form biofilms, which are assemblages of the microbial mass either attached to the surface or presented as an unattached biomass that secreted as an extracellular matrix composed of Polysaccharides, extracellular DNA, Proteins and lipids. These biofilms offer physical resistance to the cells encased within the biofilm from adverse environmental occults like desiccation, defence mechanisms of the host, and protection form therapeutic drugs by hindering the penetration of the drugs to their site of action. In addition, the suboptimal concentration of the drugs and improper usage of the drugs will lead to the enhanced production of biofilms through the development of drug resistant phenotypes. In the present study the effect of biofilm production on minimum inhibitory concentration of the antibiotic drugs was evaluated in-*vitro* by using the bovine mastitis causing *Staphylococcus* species. Five most commonly used antibiotic drugs by the field veterinarians for the control of bovine mastitis were studied in the present research work.

A total of 20 *staphylococcus* species were isolated from clinical cases of bovine mastitis. On the selective MSA medium small pinpoint yellowish colonies were observed and cocci arranged in bunch of grape morphology, was noticed on gram staining, which is a characteristic feature of the *Staphylococcus* species.

3.1 Qualitative assessment of Biofilm by tube method: Out of the 20 *Staphylococcus* species only nine samples were identified as biofilm producers by tube method. However, the intensity of stained region of the tube was varied between the samples and were qualitatively categorized (Table No.1) as strong biofilm producer (S2), as moderate biofilm producer (S1, S7, S13 and S19), S5, S6, S11& S18 as weak biofilm producer (+) and remaining samples as non-biofilm producers (-). Quantitatively these biofilm producing *staphylococci* may be categorized into strong, moderate, weak, and non-biofilm producers. Several methods are available for characterization of the biofilm viz. Tube method, congo red agar method, Tissue culture plate method (TCP), quantification of sessile bacteria after detachment from the surface by scraping, Vortexing and sonication techniques and observation by microscopy methods (Christensen *et al*., 1982; Freeman *et al*., 1989; Stiefel *et al*., 2016; Azeredo *et al*., 2017) [7, 11, 34, 4]. Traditionally tube method was used for qualitative assessment of the biofilm production and TCP method was used for quantitative assessment of biofilm production. By tube method only 45% of the isolates (9 isolates out of 20) were identified as biofilm producers in the present study. In general, 25% to 75% of *staphylococcus* sps. isolated from bovine mastitis cases have the ability to form biofilm as evaluated by tube method (Dhanawade *et al*., 2010; Aslanta and Demir, 2016; Shah *et al*., 2019) [9, 3, 33].

Table 1: Biofilm Production assessed by Tube Method and Tissue Culture Plate method

Sample I.D.	Tube Method	18 Hours	32 Hrs
S1	++	0.217	0.434
S2	+++	0.405	0.705
S3	-	0.058	0.099
S4	-	0.005	0.051
S5	+	0.126	0.247
S6	+	0.127	0.260
S7	++	0.146	0.652
S8	-	0.108	0.186
S9	-	0.072	0.091
S10	-	0.036	0.05
S11	+	0.121	0.185
S12	-	0.003	0.06
S13	++	0.187	0.406
S14	-	0.045	0.058
S15	-	0.063	0.082
S16	-	0.008	0.015
S17	-	0.077	0.086
S18	+	0.129	0.204
S19	++	0.152	0.347
S20	-	0.009	0.012

3.2 Quantitative assessment of biofilm production by Tissue culture plate method: In the present study the tissue culture plate method was adopted to identify the biofilm producing *Staphylococci*, categorization of the samples, as well as for the determination of incubation time required for minimum and maximum biofilm production. The maximum biofilm production was considered for categorization of the samples into strong biofilm producer as absorbance of >0.3; ≤0.3 to 0.2 OD – moderate; ≤0.2 to 0.1-weak and ≤0.1 OD as non-biofilm producing *Staphylococcus* species. On analyzing the results, 10 samples, out of 20 were found to be positive for biofilm production. Among the positive samples, 5 samples (S1, S2, S7,S13 and S19) were strong biofilm producers with an absorbance of more than 0.3, three samples (S5,S6 and S18) are moderate biofilm producers, two samples (S8 and S11) were weak biofilm producers and remaining all are characterized as non-biofilm producers (Table No.1). However, the variation was observed in quantity of biofilm production for the samples at different intervals of time (Graph 1).Interestingly, for all 10 positive samples the minimum biofilm production was estimated at 18 hours of post incubation and maximum was observed at 32 hours of post incubation. Further incubation, beyond 32 hours did not bring any observable change in quantity of biofilm production. The TCP method has advantage over tube method in that it quantitatively detected 5 isolates as strong, 3 isolates

as moderate and 2 isolates as weak biofim producers. Hence this TCP method was used for assessing the incubation time required by each biofilm positive isolate for the production of minimum and maximum biofilm, which in turn used for studying its effect on minimum inhibitory concentration of the drugs.

3.3 Assessment of impact of biofilm on minimum inhibitory concentration of selected drugs

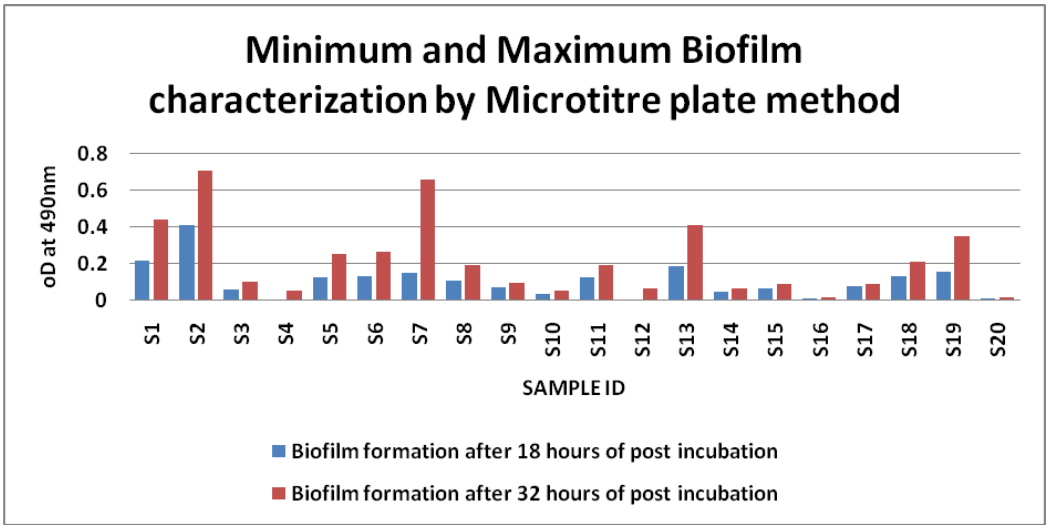
The effect of biofilm on minimum inhibitory concentration (MIC) of the five most commonly used antibiotic drugs by the field veterinarians for treating the bovine mastitis was assessed. The difference between the MIC of the drugs on 18 hrs culture of *staphylococcus* (before formation of the biofilm) and on 36 hrs culture of *staphylococcus* (during the formation of the biofilm) revealed the effect of biofilm formation *in-vitro* on the MIC of the drugs. The biofilm producing samples S11, S18 and S19 were sensitive to the drug Amoxicillin & Cloxacillin Na, while remaining biofilm producing samples were resistant. The biofilm producing samples S5, S6, and S7 were sensitive to the drug Cefaperazone, while the remaining biofilm producing samples were resistant to the drug. The MIC of the drug Gentamicin revealed that all the samples are resistant to gentamicin drug. The biofilm producing samples S6, S8, S11 and S18 were sensitive to the drug enrofloxacin while the remaining samples are resistant. All the biofilm producing samples were sensitive to the drug Ciprofloxacin.

The effect of biofilm production on MIC end point of the drugs for 18 hrs culture and 36 hrs culture was not altered for all the samples, except the samples S6, S11, S13 and S19. The sample S6 was sensitive to the drug Enrofloxacin, and the end point of the MIC 25 µg for 18 hours culture has enhanced to 50 µg for 36 hours culture (Graph 2; table 2). The S11 sample was susceptible to the drugs Amoxycillin Na & Cloxacillin Na, Enrofloxacin, and Ciprofloxacin. For the drug Amoxycillin Na & Cloxacillin Na, the MIC required for S11 was 12.52 µg for 18 hrs culture while it was enhanced to 50 µg after 36 hrs of post incubation culture. (Graph 3; table 2), for Enrofloxacin, the MIC was 3.125 µg for 18 hrs culture and 6.25 µg for 36 hrs culture (Graph 2; table 2) and for Ciprofloxacin the MIC end point variation was 25 µg for 18 hrs culture and 50 µg for 36 hrs culture (Graph 4; table 2). The sample S13 was susceptible to the drug Ciprofloxacin and the variation in MIC end point was reported as 3.625 µg for 18 hrs culture and 50 µgfor 36 hrs culture (Graph 4; table 2). The S19 sample was sensitive to the drug Amoxycillin Na & Cloxacillin Na and the MIC required for 18 hrs culture was 25 µg and for 36 hrs culture was 50 µg (Graph 3 table 2). For the samples S1, S2, S5, S6, S7, S8, S18 and S19 the MIC was required for 18 hrs culture and 36 hrs culture was unchanged for the drug Ciprofloxacin.

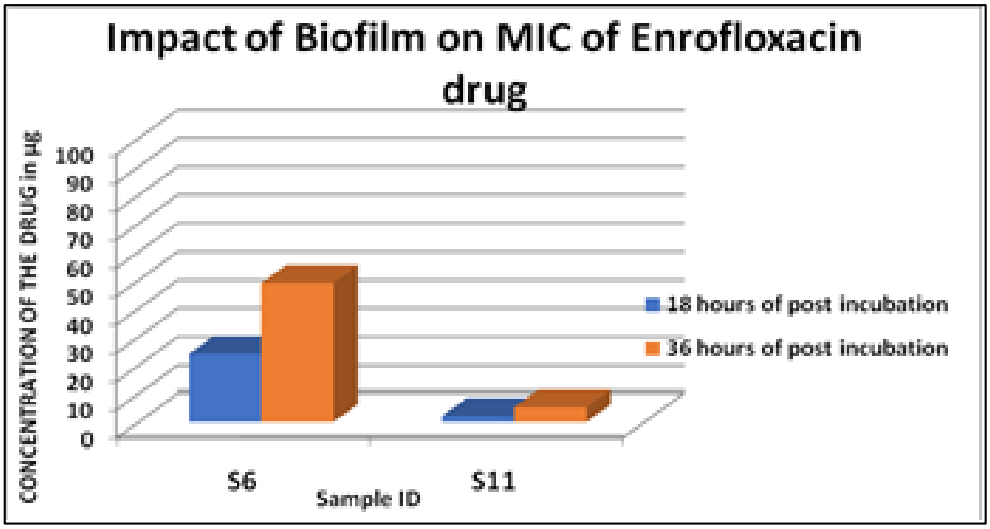
Table 2: Minimum Inhibitory Concentration of Antibiotics for *Staphylococcus* Species

Sample I.D.	Commonly Used Antibiotic Drugs In Field Conditions									
	Amoxicillin Na & Cloxacillin Na		Cefaperazone		Gentamicin		Enrofloxacin		Ciprofloxacin	
	18 Hours	36 Hours	18 Hours	36 Hours	18 Hours	36 Hours	18 Hours	36 Hours	18 Hours	36 Hours
S1	R	R	R	R	R	R	R	R	50 µg	50 µg
S2	R	R	R	R	R	R	R	R	50 µg	50 µg
S5	R	R	50 µg	50 µg	R	R	R	R	50 µg	50 µg
S6	R	R	25 µg	25 µg	R	R	25 µg	50 µg	50 µg	50 µg
S7	R	R	50 µg	50 µg	R	R	R	R	50 µg	50 µg
S8	R	R	R	R	R	R	50 µg	50 µg	50 µg	50 µg
S11	12.525 µg	50 µg	R	R	R	R	3.125 µg	6.25 µg	25 µg	50 µg
S13	R	R	R	R	R	R	R	R	3.125 µg	50 µg
S18	50 µg	50 µg	R	R	R	R	50 µg	50 µg	50 µg	50 µg
S19	25 µg	50 µg	R	R	R	R	R	R	50 µg	50 µg

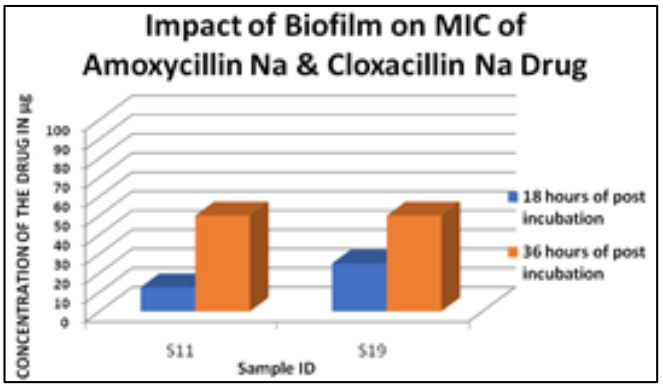
R = Resistant



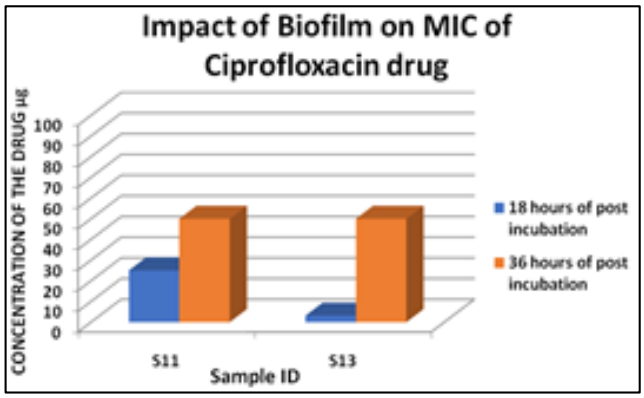
Graph 1: Minimum and Maximum biofilm characterization by Microtitre plate method



Graph 2: Impact of biofilm on MIC of the drug Enrofloxacin



Graph 3: Impact of biofilm on MIC of the drug Amoxycillin Na & Cloxacillin Na



Graph 4: Impact of biofilm on MIC of the drug Ciprofloxacin

In this study, a novel approach was used to study the impact of biofilm on the biological activity of the drugs. It was well known fact that the incubation period required for minimum and maximum biofilm production was varied, which enabled us to test the antibiotic activity against the biofilm producing *Staphylococcus* before formation of the biofilm and after formation of the biofilm. The minimum incubation period required for the production of in-*vitro* detectable biofilm was observed as 18 hrs and maximum production was noticed at 36 hrs of post incubation period for all the 10 biofilm positive isolates. The maximum biofilm production was not increased even after prolonging the incubation period beyond 36 hrs. This may be due to the depletion of nutrients, accumulation of waste products, alterations in the microenvironment of the biofilm and limited space of the microtiter plate well.

All the biofilm producing samples are resistant against the drug Gentamicin. The isolates S6. S11, S13 and S19 showed evidence that the activity of the antibiotics were altered after the formation of the biofilm i.e. after 36 hrs of post incubation period in comparison to that of before formation of the biofilm (18 hrs of post incubation). The MIC of the drug Amoxicillin Na and cloxacillin Na was determined as 12.5 µg (S11) and 25 µg (S19) for 18 hrs culture while for the same samples (S11, & S19) after the formation of maximum biofilm, the MIC was enhanced to 50 µg. Similar variation was noticed for the drug Enrofloxacin, where MIC for the culture embedded in fully developed biofilm 50 µg (S6), & 6.25 µg (S11), was higher in comparison to the bacteria not embedded in biofilm, i.e. 25 µg (S6) and 3.125 (S11). The MIC of the drug Ciprofloxacin, required for growth inhibition for bacteria, when not encased in biofilm was 25 µg (S11) & 3.125 (S13) while it was increased to 50 µg (S11) & 6.25 (S13) when it was protected from biofilm. These results furnished the evidence for the effect of biofilms on the activity of the chemotherapeutic agents, where the biofilm formation drastically increased the MIC of the drugs against bovine mastitis causing staphylococci. Several studies have proven that biofilms offers resistance against the chemotherapeutic agents by several mechanisms, which became the burning problem for the effective implementation of control measures against infectious diseases (Verderosa *et al*., 2019) [1]. There is a correlation between the bacterial cells encased in a biofilm and the antibiotic resistance patterns exhibited by them (Scoffone *et al*., 2019) [36].

It has been proved that the tolerance to the antimicrobials by the bacteria encased in biofilms is mediatied by the enhanced enzymatic activity of the enzymes like β-lactamases (Dibdin *et al*., 1996, Ciofu *et al*., 2000) [10, 8] and altered metabolic activity mediated by the quorum sensing phenomenon (Bjarnsholt *et al*., 2005) [5]. The bacterial defense mechanisms like enzymes taargetting the antimicrobial compounds, efflux pumps and other biological compounds which interfere with the activity of the antimicrobials may secreted by the bacterium are accumulated in high concentrations within the layers of the biofilm. These compounds interfere with the antimicrobials before they reach their actual drug targets (Høiby *et al*., 2010) [15].Quorum sensing is a phenomenon by

which bacteria produce signal molecules and coordinates with its fellow communities and alters its behaviour in a density dependent manner (Brackman and T. Coenye, 2014) [12]. The quorum sensing mechanism contributes for the enhanced resistance of the bacteria against the chemotherapeutic agents (Plusa T, 2019) [35]. In addition to these communication signals these biofilm communities and its environment favours the exchange of genetic materials, as close contact exists between the microbes in the biofilm (Madsen *et al*., 2012) [19]. This exchange of genetic materials may occur at high frequency with in the biofilms in compared to that of freely existing colonies (Roberts *et al*., 2004) [2]. One among such genetic elements are the resistance genes against the antimicrobial compounds. It has been reported that the biofoilm formation by a bacterium is one of the mechanism to resist the hostile environmental influences, like antimicrobial activities of antibiotics (Navon-Venezia *et al*., 2017; Chen *et al*., 2013; L. Lu *et al*., 2019; Høiby *et al*., 2010) [32, 22, 20, 16]. In recent years this was identified as emerging problem that results in persistant and recurring infections worldwide (Verderosa *et al*., 2019) [1]. These biofilm structural components, extracellular polymeric substances, prevents the penetration of the antimicrobial compounds at its bactericidal concentrations (Pinto *et al*., 2020) [29]. Through this study, it is understood that the minimum inhibitory concentration of antibiotics against the bovine mastitis causing *staphylococcus* has got altered depending on the density of the biofilm formation by the bacterium. It was noticed that the minimum inhibitory concentration of some antibiotics has got increased for the culture with high density of biofilm in comparison to that of the culture with low or minimal biofilm formation. This needs to be addressed as a major threat of resistant and recurring infections, where the dose dependent drug-target interactions may be explored for modification in formulations of drugs to reach its targets, like nanotechnology, and increasing the dose to overcome the biofilm or extracellular matrix barriers.

4. Conclusion

In conclusion, the biofilm forming bacteria are emerging as a potential threat for the health communities, where the bacteria encased in the biofilm matrix enables them to expel the antibiotics, evade host immune responses and protects them from its environmental occults. This physiological barrier have to overcome for effective implementation of therapeutic measures and control of antimicrobial resistance.

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