INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

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Case Study

BIOFILM PRODUCTION OF *S. AUREUS* ASSOCIATED WITH CANINE URINARY TRACT INFECTION

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ABSTRACT
Biofilm has a tremendous impact in the field of veterinary medicine leading to a serious economic loss. Over the years, little attention has been given to biofilm in canines with most of the research geared toward human biofilm diseases. A male dog was presented to the University Veterinary Hospital; Mannuthy with history of stranguria followed by haematuria and aseptically urine was collected and cultured. Our results showed presence of *S. Aureus* organism in this case formed biofilms in-vitro and these were highly tolerant to all the drugs tested, consistent with the treatment failures observed in practice. *S. aureus* was found to be biofilm positive with absorbance value of 1.4 fold more than mean value of control.

KEYWORDS: *Staphalococcus aureus*, Biofilm, Dog, Urinary tract infection.

INTRODUCTION

Bacteria are renowned for their ability to tolerate and adapt to a wide range of adverse environmental conditions. The primary mechanism that facilitates these adaptations is thought to be the capacity to form and maintain biofilms. The organisms within biofilms are notorious for their resistance towards the host immune response and antibacterial agents compared to their free-living planktonic counterparts. Canine uropathogenic *Escherichia coli* infection which affects the human urinary system; this bacterial biofilm has been experimentally demonstrated to induce cytotoxicity in human bladder epithelial cells. It is suggested to be responsible for about 80% of infectious diseases affecting animal and human, hence the impact in veterinary medicine cannot be ignored. Restriction of antimicrobial agents from gaining access to the pathogens due to the impervious nature of the biofilm encased extracellular matrix, plays a pivotal role in biofilm antimicrobial resistance. The present case deals with *Staphalococcus aureus* associated biofilm antimicrobial resistance in dog with urinary tract infection.

MATERIALS & METHODS

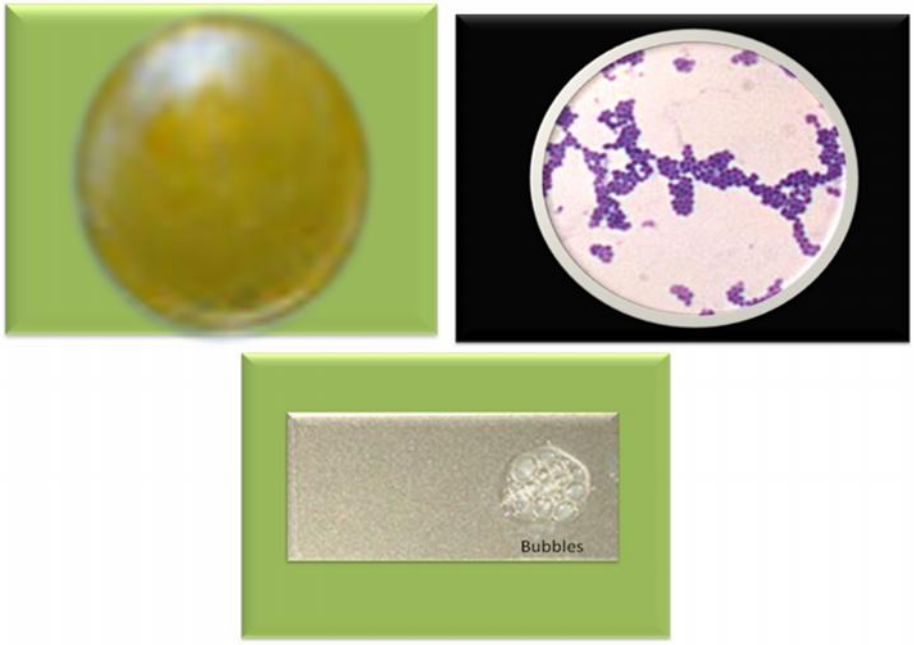
Dogs presented to the University Veterinary Hospitals at Kokkalai and Mannuthy with clinical signs suggestive of urinary tract infections were subjected to detailed clinical examination and confirmation were made after urinalysis. The diagnosis UTIs was made primarily on the basis of observation of clinical signs of various combinations, including dysuria, pollakiuria, haematuria, and voiding outside the litter box. Additional diagnostic testing such as ultrasonography, radiography, quantitative bacteriologic culture of urine was performed. Urine was collected by ante pubic cystocentesis on the day of admission and 10th day before discontinuing the treatment, into 50 ml sterile urical vials and kept under refrigeration until further analysis.

Relevant clinical materials were collected at the time of admission. Five ml of whole blood was collected from saphenous or cephalic vein of the selected dogs in dry glass vials containing EDTA@ 1-2mg/ ml as anticoagulants (Benjamin, 1985). About 10 ml of blood was collected in another vial on the day of admission to separate serum for biochemical examination. Sera thus separated were stored at -20°C till further analysis. Total erythrocyte count, hemoglobin, volume of packed red cells, total leukocyte count, differential leukocyte count and platelet count were estimated as per the method (Schalm *et al.*, 1975). Blood urea nitrogen, serum creatinine were estimated on first day using semiautomatic analyzer, as per the manufacturer's instructions and using standard kits.

RESULTS & DISCUSSION

A male Labrador dog of 5 year age was presented to the University Veterinary Hospital, Mannuthy with history of stranguria and haematuria. On detailed clinical examination, there was increased rectal temperature and enlarged prostate by per rectal examination. Urine was collected by cystocentesis and processed for isolation and identification of bacteria (Cruickshank *et al.*, 1975). Individual colonies were identified on the basis of colony morphology (Fig. 1). Gram staining and standard biochemical reactions were used to identify the organisms (Barrow and Feltham, 1993). The isolate was identified as

S. aureus (50,000 colony forming unit /ml) and cultures maintained for the further investigation.



Catalase test – positive

FIGURE1: Colony morphology and corresponding Gram's stains of *S. aureus*

Physical examination of urine revealed dark and yellow, turbid appearance, alkaline pH and specific gravity of 1.024. Chemical examination of urine sample showed positive occult blood and 2 + proteinuria. Microscopic examination revealed significant pyuria (80-90 white blood cells/ high power field (HPF), haematuria (field packed red blood cells/ HPF), microburia (+++/ HPF). Haemato-biochemical analysis of blood sample collected on arrival in clinic revealed leucocytosis (22,600/cumm) with neutrophilia (90%), increased erythrocyte sedimentation rate (80 mm/hour) with normal serum urea and creatinine values. Ultrasonographic examination showed hyperechoic areas with prostatic enlargement (Fig. 2). Further this *S. aureus* were grown on the glass slides and in 96-well plates as per the standard methods described by the Varma *et al.* (2011). The ability of *S. aureus* isolates to produce biofilm in vitro was determined as per the method with slight modifications done by Vasudevan *et al.* (2003) and strains producing a blank corrected mean absorbance value of >0.1 were considered as biofilm producers. *S. aureus* was found to be biofilm positive with absorbance value of 1.4 fold more than mean value of control.

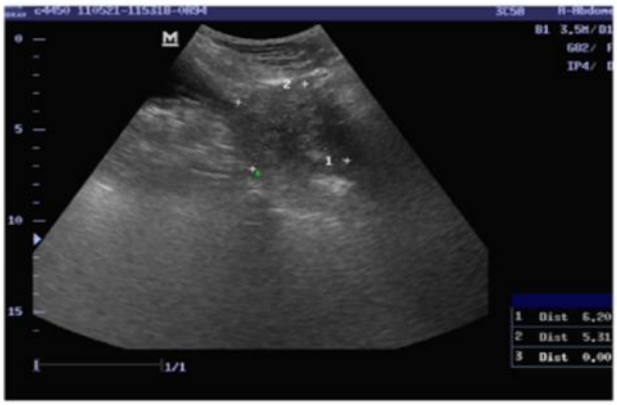


FIGURE 2. Ultrasonographic examination prostatic enlargement

Despite treating for urinary tract infections, this case looks at the characteristics of biofilm infections in dogs with urinary tract infections. Isolate was sensitive to levofloxacin and cefpodoxime proxetil. Treatment was started with once daily oral levofloxacin @10 mg/kg b wt. Biofilm producing strains of *S. aureus* were able to attach to mucosal surfaces and cause infections better than strains that did not produce biofilm. It has been proposed that biofilm, like capsule, protects *S. aureus* against phagocytosis. In addition, it may help *S. aureus* to resist antibiotic therapy (Fox *et al.*, 2005). Difficulty in eradicating a infection associated with biofilm formation lies in the fact that biofilm bacteria were able to resist higher antibiotic concentrations than bacteria in suspension (Amorena *et al.*, 1999). This might be reason for the treatment failure in that particular case. Increasing

evidence suggested that antibiotics were not only less effective against bacterial biofilms, but also might stimulate the biofilm formation further. This also might responsible for failure in antimicrobial therapy. Biofilm associated bacteria showed an innate resistance to antibiotics, disinfectants and clearance by host defence mechanisms (Melchior *et al.*, 2006).

This also might be reason for death of the animal and failure to antimicrobial therapy in this case. It was concluded that the biofilm associated bacteria showed an innate resistance to antibiotics, disinfectants and clearance by host defence mechanisms (Melchior *et al.*, 2006).

CONCLUSION

Additional research is needed to unravel the mystery of biofilm. Present trend of biofilm in veterinary medicine suggest persistence of animal and human health challenges in the future, leading to greater economic loss. Application of novel therapeutic approach such as, phage therapy and the use of some mucolytic agents that is capable of inhibiting biofilm formation, are highly recommended in veterinary medicine.

ACKNOWLEDGMENTS

The authors acknowledge the Dean, College of veterinary and animal sciences for their support.

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