A Case Report of Non-Healing Surgical Site Infection Caused By Biofilm Producing Methicillin Resistant *Staphylococcus Epidermidis* (MRSE) in A Tertiary Care Hospital

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ABSTRACT

Introduction: Persistence of wound infections at surgical sites impair patient recovery and if that is due to biofilm producing bacteria it becomes even more difficult to manage. Biofilms are difficult to remove and they have increased resistance towards antibiotics and biocides.

Case report: We report a case of surgical site infection with discharging wound sinus by a biofilm producing Methicillin resistant *Staphylococcus epidermidis* (MRSE) along with catheter associated-urinary tract infection with multidrug resistant *Acinetobacter baumanii*, in a nine year old malnourished boy about six weeks after open cystolithotomy which has been rarely reported before. He was refractory to conventional treatment. Antibiotic sensitivity testing(AST) showed sensitive only for vancomycin for MRSE and congo red agar test method for detection of biofilm gave confluent growth of black colonies with crystalline consistency confirming it as biofilm producing MRSE. After proper debridement of wound and regular surgical dressing along with vancomycin infusion, wound started to heal.

Conclusion: If the bacteria that contaminate incision site of surgical wound have the ability to attach on a biological surface, they rapidly expresses new proteins, which becomes sessile and along with generation of protective exopolysaccharide matrix, it changes into biofilm states, which are significantly different from their planktonic counterpart. Antimicrobials and biocides fail to penetrate biofilm, resulting in persistence of wound infections. So, physical removal of biofilm from wound surface, followed by selective use of biocides in conjunction with systemic antibiotics should be the wound management strategy.

Keywords: Surgical site infections, biofilm, Methicillin resistant *Staphylococcus epidermidis*, *Acinetobacter baumanii*

INTRODUCTION

Surgical site infections (SSI) are important cause of hospital acquired infections (HAI) and responsible for significant post operative morbidity, mortality, prolonged hospital stay and increase in cost.[1] Persistence of wound infections at surgical sites can further impair patient recovery and if that is due to biofilm producing bacteria it becomes even more difficult to manage. This is because biofilms are difficult to remove and they generally have 100–1000-fold increased resistance towards antibiotics and biocides than equivalent populations of planktonic bacteria.[2] We report a case of SSI with discharging wound sinus by a biofilm producing Methicillin resistant *Staphylococcus epidermidis* (MRSE) along with catheter associated-urinary tract infection (CA-UTI) with multidrug resistant (MDR) *Acinetobacter baumanii*, in a nine year old malnourished boy about six weeks after open cystolithotomy which has been rarely reported before.

CASE REPORT

A nine year old malnourished boy presented in urology out patient department of a tertiary care hospital with dysuria, intermittently interrupted urinary flow and pain in lower abdomen for one year. There was no history of recurrent urinary tract infections or hematuria. Ultrasonography of abdomen detected single urinary bladder stone of 14.6mm size and on X-ray of kidney and urinary bladder, stone was found to be radioopaque. Complete blood haemogram, urea, creatinine, uric acid, parathyroid hormone, serum calcium and routine examination of urine were within normal limits. There was no significant growth in urine culture pre-operatively. Open cystolithotomy was done under general anaesthesia. He was catheterised and a drain was placed for two days for drainage of retropubic space. Post operative dressing done and amoxicillin-clavulanic acid (375mg) was prescribed thrice daily for ten days. He was discharged with urinary catheter on seventh post operative day and was advised to apply mupirocin ointment topically at surgical site daily. But the surgical wound never healed properly and after six weeks patient presented with discharge of pus from incision site with lower abdominal pain and burning sensation in urethra. On examination there was pus discharging wound sinus at incision site and wound dehiscence. [Figure-1] Patient was referred to microbiology department for opinion. Pus from discharging sinus was squeezed out and collected aseptically in test tube and in sterile swabs. Immediately direct smear preparation and grams staining, ziehl neelsen (ZN) staining and KOH wet mount were done. Direct smear microscopy revealed plenty of pus cells with gram positive cocci in clusters, but no acid fast bacilli or fungal body were seen. Pus sample was inoculated in nutrient agar media, 5% sheep blood agar media and MacConkey agar media and incubated overnight aerobically at 37^0C. As the patient was catheterised, urine sample was collected by sterile needle and syringe directly from the catheter after proper disinfection

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of the site with 70% alcohol. Microscopical examination of wet film of uncentrifuged urine under high power objective lens showed 5-10 pus cells per high power field indicating significant pyuria. Semi quantitative method of urine culture was done by standard loop method. Uncentrifuged urine sample were inoculated in nutrient agar media, 5% sheep blood agar media and MacConkey agar media and incubated overnight aerobically at 37^{0}C.

Culture from pus sample gave smooth, low convex, opaque and white colonies of 2-4 mm diameter in nutrient agar media. Gram staining showed gram positive cocci in clusters. Biochemical tests for identification following standard methods were done.[3] Catalase test was positive but slide and tube coagulase tests were negative. Mannitol fermentation test was negative. Novobiocin sensitivity test with 5µg novobiocin disc gave a sensitive zone diameter of 16mm indicating *Staphylococcus epidermidis*. To exclude skin contamination, pus was again collected from same site and there was repeated isolation of *Staphylococcus epidermidis.* Antibiotic sensitivity testing (AST) were done according to CLSI (Clinical and Laboratory Standards Institute) guidelines by Kirby-Bauer disk diffusion method in Mueller Hinton agar.[4] It showed multidrug resistance to cefoxitin (30µg), amoxicillin-clavulanic acid (20/10µg), clindamycin (2µg), erythromycin (15µg), tetracycline (30µg), cotrimoxazole (1.25/23.75µg), ciprofloxacin (5µg), levofloxacin (5µg), linezolid (30µg) and teichoplanin (30µg). MIC testing by Epsilometer (E test strip) Vancomycin-Cefoxitin Dual Ezy MIC strip (Himedia, India), was done and found to be resistant for cefoxitin (MIC>64µg/ml) and sensitive for vancomycin (MIC=1.5 µg/ml). So SSI pathogen was MRSE which was sensitive only to vancomycin. [Figure-2] Due to the non-healing nature of the wound, in addition to standard microbiological culture identification and sensitivity testing, test for detection of biofilm production by this MRSE strain was also performed by standard Congo Red agar method.[5] A suspension of this MRSE strain was inoculated into plate containing specially prepared solid medium with Brain Heart Infusion broth (BHI) with agar added which was supplemented with 5% sucrose and Congo Red stain.[5] The plates were incubated aerobically for 24-48 hours at 37°C that gave confluent growth of black colonies with crystalline consistency confirming it as biofilm producing MRSE.[5] [Figure-3]

Urine culture gave significant growth of tiny, semi-transluscent, low covex faint pink colonies on MacConkeys agar media which on gram staining gave gram variable diplo-cocco-bacilli and in singles also. After doing standard biochemical tests for identification, it was identified as *Acinetobacter baumanii.* It was catalase positive, oxidase negative, triple sugar iron (TSI) media gave alkanine slant and alkaline butt, ie non-fermentor, Hugh Leifsons O/F test showed oxidative break down of sugar and it was non motile.[3] AST showed resistance to piperacillin (100µg), piperacillin-tazobactam (100/10µg), ceftazidime (30µg), ciprofoxacin (5µg), levofloxacin (5µg) but sensitive only to imipenem (10µg), amikacin (30µg) and colistin (10µg). Test for detection of biofilm production was also performed by standard Congo Red agar method, but no biofilm was produced. So, urinary isolate was multidrug resistant, but non-biofilm producing strain of *Acinetobacter baumanii*.

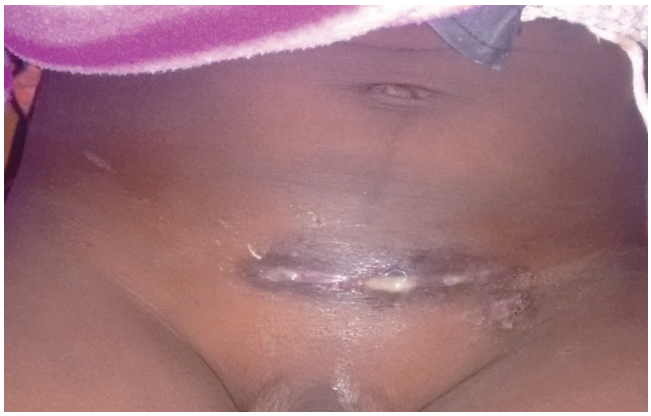

Figure-1: Surgical site infection with discharging pus

Figure-2: Epsilometer (E test strip) Vancomycin-Cefoxitin Dual Ezy MIC strip showing resistance for cefoxitin (MIC>64µg/ml) and sensitive for vancomycin (MIC=1.5 µg/ml). **Figure-3:** Congo Red agar test gave confluent growth of black colonies with crystalline consistency.

Patient was finally treated with imipenem and cilastin infusion 250mg six hourly and urine culture showed no significant growth after seven days of treatment. Wound debridement and regular dressing of surgical site was done and vancomycin infusion 250mg twelve hourly given. The condition of the patient improved after seven days and SSI started to heal. After two weeks there was no purulent discharge. Patient was further lost to follow-up.

DISCUSSION

Surgical site infections (SSI) with delayed wound healing due to infection, inflammation, discharge and wound dehiscence accounts for 5% to 20%.[1] Bacterial biofilm is a wide spread problem nowadays and poses a potentially significant risk of hospital acquired infections. There is high probability that bacteria will contaminate incision site in a surgical wound. Now if the bacteria have the ability to attach on a biological surface they rapidly expresses new proteins, which becomes sessile and along with generation of protective exopolysaccharide matrix, it changes into biofilm states, which are significantly different from their planktonic counterpart. Surgical site infections lack the traditional host response seen in acute infections and have similar characteristics as observed in chronic wounds. The production of pro-inflammatory cytokines and exudates provide a highly nutritious and ideal environment for the bacteria within the biofilm to survive, may be totally unperturbed by activated

macrophages, neutrophils, antibodies, complement or other host defences.[6] Moreover antimicrobials and biocides fail to penetrate biofilm resulting persistence of wound infections and wound dehiscence. Biofilms are often associated with chronic wound infections which is around 60%, whereas only 6% of acute wound infections are also associated with biofilm.[7] We report this rare and interesting case of six weeks old SSI in a nine year old malnourished boy by a biofilm producing MRSE strain, following open cystolithotomy for removal of 14.6mm single urinary bladder stone.

Bladder calculi are one of the commonest health problems in young malnourished children below 10 years with low body mass index in endemic areas.[8] They are related to peculiar feeding habit mainly dependant on cereal based diet and lacking animal proteins along with chronic dehydration with hot, arid and dry climate in the absence of obstruction or any bladder emptying disorders.[8] Surgical management includes open cystolithotomy or percutaneous cystolithotomy in paediatric age group.[8] A study by Satyanarayana V *et al* reported that SSI following open cystolithotomy is only 5.3%,[1] but no case has been reported by biofilm producing organism to the best of our knowledge.

Due to treatment refractoriness of biofilms, it is very difficult to manage and often show recurrences. An *in-vitro* study by Wasfi *et al* on antimicrobial activities against biofilm showed that at higher concentration of antibiotics in multiples of MIC, biofilm biomass and viable cells in biofilms were reduced.[9] Similarly another *in-vitro* study by Chakraborty *et al* on efficacy of hospital disinfectants against biofilms, showed that much higher concentration and greater contact time than that recommended is required for reduction of biofilm bioburden.[10] But none of the *in-vitro* studies showed complete elimination of biofilms by antibiotics and disinfectants. Hence physical removal of biofilm by debridement from the surface of wound is an essential step in management, followed by use of proper biocides and antibiotics. Also rigorous mechanical brushing, cleaning and proper disinfection of reusable medical devices, surgical instruments and hospital environment is essential to prevent transmission of hospital acquired infections due to biofilms.

Second important aspect of our case report is hospital acquired urinary tract infection (UTI) by multidrug resistant *Acinetobacter baumanii*. Catheter associated - urinary tract infections (CA-UTI) are the most common type of hospital acquired infections accounting for 30-40%. Prolonged catheterisation inevitably predisposes UTI. So basic principles of hospital infection control should be practised to limit CA-UTI. Also antibiotic therapy should be given based on their antibiotic susceptibility pattern which is very important for avoiding the hazards of injudicious use of anti-bacterial agents and prevention of emergence of resistant strains.

CONCLUSION

Hence to conclude, any non healing SSI with pus discharging sinus should be evaluated for biofilm colonisation and wound management strategy should include physical removal of biofilm from the surface by opening of wound and aggressive debridement of surgical wound to remove devitalised tissues, followed by using selective biocide like silver or cadexomer iodine in conjunction with appropriate systemic antibiotics.

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