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Research article

Coating urinary catheter with moxifloxacin restricts *Stenotrophomonas maltophilia* adhesion *in vitro*

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ABSTRACT

Ability of *Stenotrophomonas maltophilia* to adhere and form biofilm onto abiotic surfaces is one of important features of clinical and environmental isolates. Ability of *S. maltophilia* to adhere to urinary catheter in vitro was not evaluated clearly. Here, the efficiency of *S. maltophilia* to adhere to coated and uncoated urinary catheter with moxifloxacin was evaluated for first time. Pieces of catheters were coated with moxifloxacin. Spectrophotometric method was used to check the moxifloxacin leached onto urinary catheter. Coated and uncoated catheters were incubated with bacterial growth. Viable of bacterial count was used to check the number of adhered bacteria onto both catheters. Significant adhesion of *S. maltophilia* to urinary catheter (uncoated with moxifloxacin) in vitro was started as early as 15 min post incubation with bacterial suspension (107 c.f.u./ml). Maximum adhesion was observed at 48 h. Pretreatment of urinary catheter in vitro with 50 ug of moxifloxacin per ml for 24 h significantly reduced the adhesion and survival of the clinical isolate *S. maltophilia* (Sm2). The significant reduce ($P < 0.05$) of bacterial adhesion was found at each time point (1, 4, 24 h). The current study showed for the first time high efficiency of *S. maltophilia* to adhere to urinary catheter in vitro. Moreover, the present study demonstrated for the first time that the coated catheter with moxifloxacin reduced significantly ability of *S. maltophilia* adhesion and biofilm formation in vitro.

Keywords: Adhesion, Biofilm formation, Moxifloxacin, Urinary catheter.

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INTRODUCTION

Stenotrophomonas maltophilia has emerged as an important opportunistic pathogen in the debilitated host [1]. In most human patients, *S. maltophilia* infection is acquired in the hospital setting [2]. *S. maltophilia* is a bacterium that can be present in almost any aquatic or humid environment and may persist for extended periods in such locations. *S. maltophilia* survives and multiplies in respiratory secretions, urine or intravenous fluids [3]. Immunocompromised patients are more susceptible to *S. maltophilia* infection [4]. The significance of Stenotrophomonas as an important nosocomial pathogen has risen over the last two decades. *S. maltophilia* can cause bacteraemia, endocarditis, pneumonia, meningitis, infections of bones and joints, urinary tract, soft tissues, and wounds.

Bacterial cells have a strong preference for life on surfaces rather than in planktonic suspension [5]. These cells have an array of adhesins in their cell walls that allow them to colonize many types of substrate, and, on contact with a surface, the cells secrete exopolysaccharides that secure their attachment [6]. Several study demonstrated the ability of *S. maltophilia* to adhere to biotic and abiotic surfaces [7-9]. The ability of *S. maltophilia* to form biofilm onto abiotic surfaces is one of important features of clinical and environmental isolates of *S. maltophilia* [9,10]. Previous studies reported that the *S. maltophilia* attached to different catheters and creates a lot of clinical disasters [11,12]. Several studies have identified major risk factors for the infection with *S. maltophilia* that associated with using of medical devices such as prosthetic heart valves, urinary catheters, and indwelling central venous catheters (CVCs) [11,13]. The experimental work of adhesion of *S. maltophilia* to urinary catheter is not described clearly in literature. Thus, in current study, we demonstrated experimentally the ability of *S. maltophilia* to adhere to urinary catheter.

Urinary catheters, which are used in 15% to 25% of short-term care patients during their hospitalization, confer a predisposition to bacteriuria [14,15]. Catheter associated urinary tract infection (UTI) is the most common type of hospital-acquired infection, accounting for approximately 40% of such infections and for most of the 900 000 patients with nosocomial bacteriuria in U.S. hospitals each year [16]. Adverse consequences include local and systemic morbidity, secondary bloodstream infection, death, a reservoir of drug-resistant microorganisms, and increased health care costs [14-17]. That is why; several previous studies gave many solutions for reducing the infection of urinary catheter with pathogenic bacteria. Reid et al. [18] coated catheter with Ciprofloxacin, Norfloxacin, and Ofloxacin to reduce adhesion of Pseudomonas aeruginosa to catheter in vitro. Four antimicrobial urinary catheters are currently marketed in the United States. They are coated with silver alloy (3 latex- or silicone-base catheters) or nitrofurazone, a nitrofurantoin-like drug (1 silicone-base catheter). Two previous meta-analyses of randomized, controlled trials (RCTs) of antimicrobial catheters concluded that trial quality was modest, that silver oxide– coated catheters (which are no longer marketed) lack efficacy, and that silver alloy– coated catheters are protective, and no conclusions were made about the prevention of symptomatic UTI, bloodstream infection, or death [19,20].

In current study, we demonstrated for the first time and experimentally the adhesion of *S. maltophilia* to urinary catheter. Moreover, the catheter was coated with moxifloxacin and the affectivity of this method to prevent *S. maltophilia* adhesion was evaluated for the first time in present study.

MATERIALS AND METHODS

Clinical isolate

A clinical isolate of *S. maltophilia* (Sm2) was used in this study. This isolate was procured from the Department of Medical Microbiology, Post Graduate Institute of Medical Education and Research, Chandigarh, India. Bacteria was preserved by lyophilization and also maintained at 37 °C on Luria Bertani (LB) agar plates (Himedia), subcultures were made every week.

Bacterial adhesion to urinary catheter

Bacteria were grown overnight in separate flasks containing trypticase soy broth (TSB; Himedia, Mumbia, India) at 37 ºC. The cell pellet was obtained by centrifugation (10,000 g in 5 min at 4 ºC), washed twice with PBS (0.01 M, pH 7.2) and re-suspended in sterile PBS (0.01 M, pH 7.2) to achieve a bacterial concentration of $1x10^7$ c.f.u./ml. For adherence assay, Sections of 1-cm-long silicone latex urinary catheter were placed in small Petri dishes, covered with respective bacterial growth and incubated at 37 ºC for different time intervals (0, 15 min, 30 min, 1, 2, 4, 24, 48 h). After incubation each piece of urinary catheter was rinsed gently with PBS (0.01 M, pH 7.2) three times to remove unbound bacteria. Each piece was scratched homogenized separately in 1 ml PBS and 100 µl was serially diluted and plated on duplicate plates of Luria agar. The bacterial number was quantified after overnight incubation at 37 ºC [21].

Adsorption of Moxifloxacin onto urinary catheter

The standard method of Reid et al. [18] was followed. Briefly, Sections of 1-cm-long silicone latex urinary catheter were placed in a test tube with 50 µg/ml of moxifloxacin for 24 h of incubation at room

temperature. The pre-incubated catheter was gently washed with 2 ml of PBS (pH 7.1 ± 0.1). The wash solution was used to determine the amount of moxifloxacin adsorbed onto the catheter surface by UV determination. The catheter segment was incubated in 2 ml of PBS (pH 7.0 ± 0.1) for an additional 1 h. The solution was analyzed by spectrophotometer to determine the leaching of moxifloxacin from the catheter surface. The amount of moxifloxacin was estimated (Spectrophotometric method) in test tube before and after adding the catheter pieces. All of the solutions were filtered through 0.2-p.m-pore-size acrodisc filter units into Wheaton liquid chromatograph vials. Quantity of moxifloxacin was calculated by using a maximum A290 values. The standard curve was made for moxifloxacin. All experiments were carried out in triplicate.

Adhesion of *S. maltophilia* to coated urinary catheter with moxifloxacin

Similar procedure for the adhesion of *S. maltophilia* to urinary catheter that mention in above (in current stud) was followed to study the ability of *S. maltophilia* to adhere to coated urinary catheter in terms of c.f.u./ml at different time intervals (1, 4, 24 h) post incubation with bacterial suspension (10^7 c.f.u./ml).

Statistical analysis

All values have been used to give a mean value and the standard deviation (s.d.) calculated. The differences were analyzed by using Student's t-test, employing Origin version 8.0 software. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Adhesion of *S. maltophilia* to catheter in vitro

In present study, we performed the ability of *S. maltophilia* to adhere to catheter at different time intervals (15 min, 1, 2, 4, 24, and 48 h). *S. maltophilia* (Sm2) was used for studying the kinetics of *S. maltophilia* adhesion. Kinetics of *S. maltophilia* (Sm2) adhesion to catheter was performed by checking the viable bacterial count (c.f.u./ml) (biofilm formed on catheter). Sm2 isolate adhered to catheter with higher efficiency. The significant bacterial adhesion started as early as 15 minutes post incubation with maximum adhesion at 48 h (**fig. 1**). The present study proved the efficiency of *S. maltophilia* to adhere to urinary catheter in vitro.

Attachment of moxifloxacin to catheter pieces

Overnight incubation of catheter pieces in moxifloxacin suspension was used to coat the catheter with moxifloxacin. To check the efficiency of this method, the absorbance of antibiotic suspension was measured.

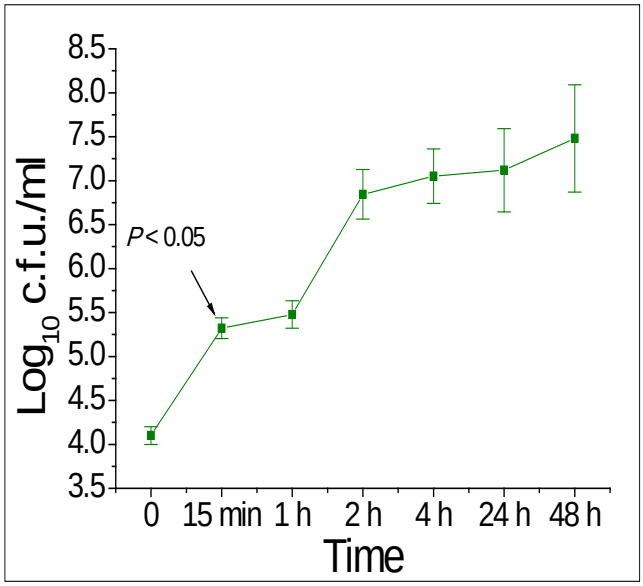

Fig. 1 Kinetics of *S. maltophilia* (Sm2) adhesion to catheter in vitro. Significant adhesion started as 15 minutes post bacterial incubation.

The optimum wavelength that should be used in this experiment was estimated practically post used different wavelengths. **Fig. 2a** showed that the maximum absorbance was observed at wavelength 290 nm. That is why; this wavelength was used in further experiments. To evaluate the efficiency the method of coating catheter with antibiotic, the absorbance of antibiotic suspension before and after adding the catheter pieces was measured. **Fig. 2b** showed the level of antibiotic (absorbance at 290 nm) in suspension post incubation with catheter pieces was lower significantly ($P < 0.5$) than level of antibiotic in suspension before adding the catheter pieces. This finding proves the catheter was adsorbed and coated with antibiotic.

Role of moxifloxacin in reducing bacterial attachment

In current study, ability of *S. maltophilia* to attach to coated and uncoated catheter was evaluated. This experiment was performed to prove the central role of moxifloxacin that coated catheter to reduce bacterial attachment. The effect of moxifloxacin that coated catheter on the ability of *S. maltophilia* attachment was checked at different time intervals (1, 4, and 24 h). **Fig. 3** showed a significant difference between number of adhered bacteria (c.f.u./ml) on coated and uncoated catheters 1, 4 and 24 h post catheter incubation with bacterial suspension (10^7 c.f.u./ml). Current study suggested that the presence of moxifloxacin onto catheter reduced bacterial attachment significantly.

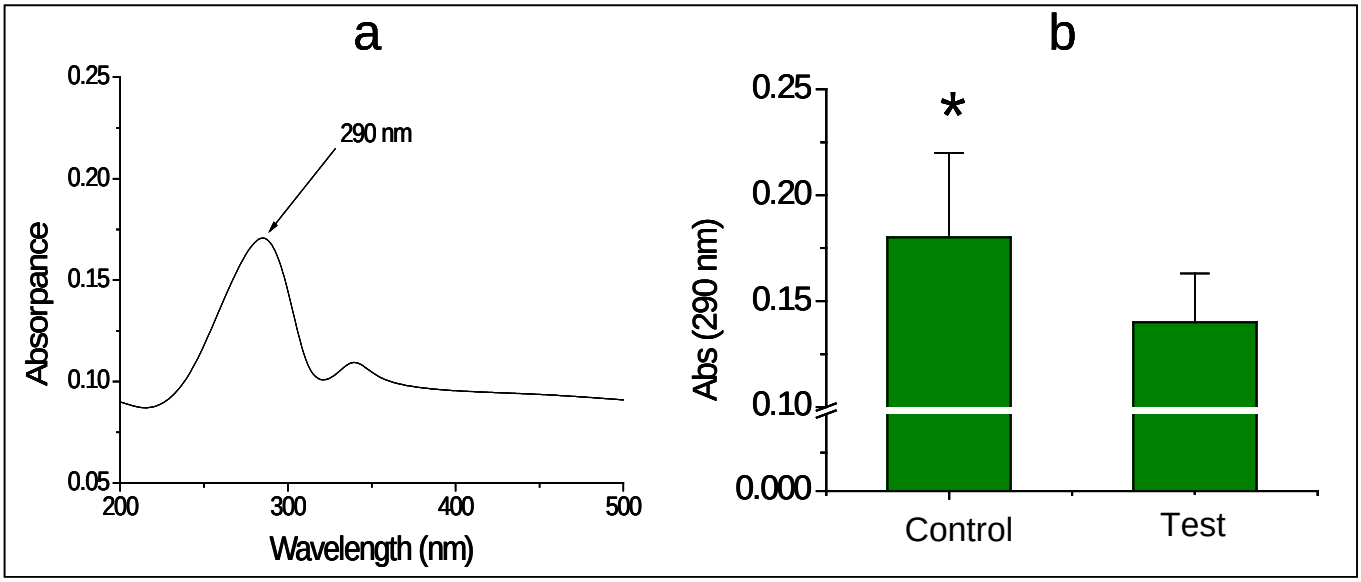

Fig. 3 Bacterial adhesion to coated catheter with moxifloxacin (test) and uncoated catheter with moxifloxacin (control). Asterisks indicate a significant difference from the control.

DISCUSSION

Ability to adhere to abiotic and biotic substrates is a prerequisite for infection so that the microorganism can colonize and thus cause infection in the host. One of the major risk factors associated with *S. maltophilia* is the presence of prosthetic devices [22]. Previous study demonstrated that the ability to adhere to polystyrene is highly conserved in *S. maltophilia* isolates [9,23]. Other studies demonstrated the ability of *S. maltophilia* to adhere to different kinds of catheters [11,12]. Previous study showed several defects associated with bacterial biofilm formation onto urinary catheter [6]. To find solution for this problem is one of great aims for scientist.

In current study, we find a good method to eradicate the adhesion and biofilm formation of *S. maltophilia* to urinary catheter. The method was dependent on coating catheter with moxifloxacin. It was found in the current study that the adhesion of bacteria and biofilm formation was less significantly onto the coated catheter with moxifloxacin as compared with the catheter that uncoated with antibiotic (control). Our study is the first study that demonstrated practically the adhesion of *S. maltophilia* onto urinary catheter in vitro. Moreover, the current study showed for the first time the coated catheter with moxifloxacin and used this method to eradicate *S. maltophilia* adhesion and biofilm formation onto urinary catheter.

Antibiotics could inhibit bacterial adhesion through different mechanisms. They may inhibit the synthesis or expression of adhesins on the bacterial cell surface,

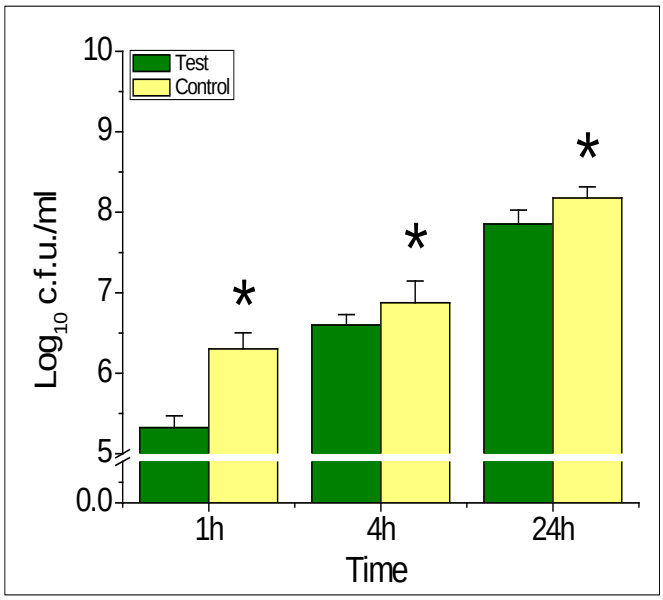

Fig. 3 Bacterial adhesion to coated catheter with moxifloxacin (test) and uncoated catheter with moxifloxacin (control). Asterisks indicate a significant difference from the control.

lead to the formation of functionally aberrant adhesins, cause the release of adhesions from the surface of bacterial cells or modify the bacterial shape in a such way as to interfere with the ability of the microorganisms to approach host cell-surface receptors [24].

The new technique aims to minimize the likelihood of patients developing infection, thus reducing hospital

stays, hospital readmissions and the need for surgical interventions. Ultimately, this would also minimize the loss of life. This method can deliver drugs to the source of infection, known to be on the device surface, in a controlled way. One of the major advantages of this new technology is that it allows much higher drug concentrations at the infection site, in comparison to conventional routes of drug therapy. Although this is highly likely to prevent patients from developing infections, there is a safeguard designed into these new materials that will initiate should this prevention mechanism fail. The coating catheters with antibiotic will self-cleanse once they have become infected with bacteria and ultimately the patients, to remain free from infection during usage catheters. The results obtained thus far are preliminary and more research is required to ensure these materials meet clinical demands.

Present study showed the ability of *S. maltophilia* to adhere to urinary catheter. Moreover, here, the coating catheter with moxifloxacin reduced significantly the ability of *S. maltophilia* to adhere and to form biofilm onto catheter. The current study is the pioneer study that used moxifloxacin in coating catheter and applied this technique to eradicate bacterial adhesion and biofilm formation onto urinary catheter.

Conflict of interest:

The authors declare that they have no conflict of interests.

REFERENCES

[1] **Looney WJ, Narita M, Mühlemann K.** (2009) *Stenotrophomonas maltophilia*: an emerging opportunist human pathogen. *Lancet Infect Dis* **9**: 312–323.

[2] **Laying FPY, Ramotar K, Read RR**. (1995) Alfieri N, Kureishi A, Henderson EA, Louie TJ: Molecular epidemiology of *Xanthomonas maltophilia* colonization and infection in the hospital environment. *J Clin Microbiol* **33**: 513–518.

[3] **Falagas ME, Kastoris AC, Vouloumanou EK, Dimopoulos G.** (2009) Community-acquired *Stenotrophomonas maltophilia* infections: a systematic review. Eur J Clin Microbiol Infect Dis **28**: 719–730.

[4] **Spicuzza L, Sciuto C, Vitaliti G, DiDio G, Leonardi S, La Rosa M**. (2009) Emerging pathogens in cystic fibrosis: ten years of follow-up in a cohort of patients. *Eur J Clin Microbiol Infect Dis* **28**:191–195.

[5] **Donlan RM.** (2002) Biofilms: microbial life on surfaces. *Emerg Infect Dis* **8**: 881–890.

[6] **Stickler DJ.** (2008) Bacterial biofilms in patients with indwelling urinary catheters. *Nature Clin Practice Urology* **5**: 598-608.

[7] **Zgair, AK.** (2013) Role of Stenotrophomonas flagella in bacterial adhesion on human epithelial cells. *World J Exp Biosci.* **1**: 19-21.

[8] **Zgair AK, Al-Adressi AMH.** (2013) *Stenotrophomonas maltophilia* fimbrin stimulates mouse bladder innate immune response. *Eur J Clin Microbiol Infect Dis* **32**:139–146.

[9] **Zgair AK, Chhibber S**. (2011) Immunoassay method to check the flagellin mediated binding of *Stenotrophomonas maltophil*ia to polystyrene. *Microbiol* **80**:136–138.

[10] **Di Bonaventura G, Spedicato I, D'Antonio D, Robuffo I, Piccolomini R.** (2004) Biofilm formation by *Stenotrophomonas maltophilia*: modulation by quinolones, trimethoprim-sulfamethoxazole, and ceftazidime. *Antimicrob Agents Chemother* **48**: 151-60.

[11] **Vartivarian SE, Papadakis KA, Anaissie EJ.** (1996) *Stenotrophomonas* (*Xanthomonas*) *maltophilia* urinary tract infection. A disease that is usually severe and complicated. *Arch Intern Med* **156**: 433–435.

[12] **Boktour M, Hanna H, Ansari S, Bahna B, Hachem R, Tarrand J, Rolston K, Safdar A, Raad I.** (2006) Central Venous Catheter and *Stenotrophomonas maltophilia* Bacteremia in Cancer Patients. *Cancer* **106**: 1967–73.

[13] **Khan IA, Mehta NJ.** (2002) *Stenotrophomonas maltophilia* endocarditis: a systematic review. *Angiology* **53**: 49–55.

[14] **Saint S, Chenoweth CE.** (2003) Biofilms and catheter-associated urinary tract infections. *Infect Dis Clin North Am* **17**: 411-32.

[15] **Maki DG, Tambyah PA.** (2001) Engineering out the risk for infection with urinary catheters. *Emerg Infect Dis* **7**: 342-7.

[16] **Warren JW.** (1991) The catheter and urinary tract infection. *Med Clin North Am* 75: 481-93.

[17] **Saint S, Lipsky BA, Goold SD.** (2002) Indwelling urinary catheters: a one-point restraint? [Editorial]. *Ann Intern Med* **137**: 125-7.

[18] **Reid G, Sharma S, Advikolanu K, Tieszer C, Martin RA, Bruce AW.** (1994) Effects of Ciprofloxacin, Norfloxacin, and Ofloxacin on *In Vitro* Adhesion and Survival of Pseudomonas aeruginosa AK1 on Urinary Catheters. *Antimicrob Agents Chemother* **38**: 1490-1495.

[19] **Saint S, Elmore JG, Sullivan SD, Emerson SS, Koepsell TD.** (1998) The efficacy of silver alloy-coated urinary catheters in preventing urinary tract infection: a meta analysis. *Am J Med* **105**: 236-41.

[20] **Brosnahan J, Jull A, Tracy C.** (2004) Types of urethral catheters for management of short-term voiding problems in hospitalized adults. *Cochrane Database Syst Rev* **1**: CD004013.

[21] **Zgair AK, Chhibber S.** (2010) Immunological and Pathological Aspects of Respiratory Tract Infection with *Stenotrophomonas maltophilia* in BALB/c Mice. *J Microbiol Biotechnol* **20**: 1585–1591.

[22] **Muder RR, Harris AP, Muller S, Edmond M, Chow JW, Papadakis K, Wagener MW, Bodey GP, Steckelberg JM.** (1996) Bacteremia due to *Stenotrophomonas (Xanthomonas) maltophilia*: a prospective, multicenter study of 91 episodes. *Clin Infect Dis* **22**: 508–512.

[23] **Di Bonaventura G, Prosseda G, Del Chierico F, Cannavacciuolo S, Cipriani P, Petrucca A, Superti F, Ammendolia MG, Concato C. et al**. (2007) Molecular characterization of virulence determinants of *Stenotrophomonas maltophilia* strains isolated from patients affected by cystic fibrosis. *Int J Immunopathol Pharmacol* **20**: 529–537.

[24] **Pompilio A, Catavitello C, Picciani C, Confalone P, Piccolomini R, Savini V, Fiscarelli E, D'Antonio D, Di Bonaventura G.** (2010) Subinhibitory concentrations of moxifloxacin decrease adhesion and biofilm formation of *Stenotrophomonas maltophilia* from cystic fibrosis. *J Med Microbiol* **59**: 76–81.

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