Moxifloxacin reduces *Stenotrophomonas maltophilia* adhesion to mouse intestinal tract *in vitro*

Ayaid Khadem Zgair[1], Jenan A Ghafil[1], Hala M. Radif, Shamim Naser Radhi[1], Mohammad H Hafiz[2,3] and Shaymaa Fadhel Abbas Albaayit

[1]Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq
[2]Department of Materials, University of Technology, Baghdad, Iraq
[3]Iraqi university/College of Engineering

Abstract: *Stenotrophomonas maltophilia* is an important opportunistic pathogen that affects immunocompromised individuals. Viable bacterial count method was used to count the number of adhered bacteria. The current study showed the efficiency of *S. maltophilia* (Sm2) adhesion on different parts of mouse intestinal tract (IT), small intestinal tract (SIT), large intestinal tract (LIT) and rectum ($P<0.05$) and this ability was equal for each part of IT [ANOVA test ($P > 0.05$)]. Moxifloxacin (0.03 x MIC) resulted a significant decrease in adhesion of *S. maltophilia* to SIT ($P<0.05$) versus control and other sub-inhibitory moxifloxacin concentrations (0.06 x and 1.2 x MIC). It can be concluded from the current study that the *S. maltophilia* (Sm2) has a good ability to adhere to mouse IT and the lowest concentrations of moxifloxacin (0.03 x MIC) reduced the ability of this bacterium to infect IT by reducing the ability of this bacterium to adhere to IT.

Keywords: Intestinal tract, mouse, *Stenotrophomonas maltophilia*, moxifloxacin, adhesion inhibition.

INTRODUCTION

S. maltophilia is an emerging clinical pathogen. It is not anaturally virulent pathogen, but its capability to colonize biotic and abiotic surfaces makes it a ready colonizer of hospitalized patients (Looney *et al*., 2009). The most common and severe clinical manifestations of *S. maltophilia* infection in humans include bacteremia, endocarditis, respiratory tract diseases, meningitis (Denton and Kerr, 1998; Gales *et al*., 2001; Yemisen *et al*., 2008). A number of studies were conducted on this isolate of bacteria. *S. maltophilia* (Sm2) was named by Dr. Sanjay Chhibber and Dr. Zgair when the ability of different bacteria to adhere to cell line *in vitro* was studied (Chhibber and Zgair. 2009). The ability of Sm2 to adhere to abiotic and biotic surfaces was investigator previously (Zgair and Chhibber, 2010a; Zgair and Chhibber, 2010b; Zgair and Chhibber, 2011a; Zgair and Chhibberb, 2011; Zgair and Chhibber, 2013; Mouhamed *et al.*, 2014).

The information about the ability of *S. maltophilia* to colonize the gastrointestinal tract is very scanty in literature. The recent study of Zhang *et al*., (2012) showed that the patients with biliary tract infections and intestinal infections caused by several gram negative bacteria and *S. maltophilia* were more prone to develop sepsis-associated encephalopathy.

Antibiotic concentrations lesser than MIC are called sub-inhibitory concentrations (sub MICs). Bacteria often grow in the presence of sub MICs, which can-not inactivate microorganisms. Sub MICs of antibiotics are altering the chemical and physical cell-surface features of bacteria and that may reduce the functionality and expression of some virulence properties such as bacterial adherence (Fonseca *et al*., 2004; Wojnicz and Jankowski, 2007; Pompilio *et al*., 2010).

There is no earlier study showing the efficiency of *S. maltophilia* adhesion to intestinal tract. Moreover, no earlier study has covered the effect of moxifloxacin on *S. maltophilia* adhesion on mouse intestinal tract. That is why; the current study will study for the first time the adhesion of *S. maltophilia* on mouse intestinal tract and role of sun-inhibitor dose of moxifloxacin on the ability of this bacterium to adhere to mouse intestinal tract IT.

MATERIALS AND METHODS

Clinical isolate

A clinical isolate of *S. maltophilia* (Sm2) (Department of Medical Microbiology, Post Graduate Institute of Medical Education and Research, Chandigarh, India) was used in current study. Bacteria isolate was maintained at 37°C on Luria Bertani (LB) agar plates (Himedia, India)**.**

Moxifloxacin susceptibility and MICs

The antibiotic discs technique was applied to estimate the susceptibility of *S. maltophilia* (Sm2) to moxifloxacin. Moxifloxacin powder was procured from Himedia, Mumbia, India. MICs (moxifloxacin) for the *S. maltophilia* Sm2 strain were estimated using a broth micro-dilution technique. The standard method of Clinical and Laboratory Standards Institute guidelines (CLSI, 2006) was followed. Results were reported post-overnight incubation at 37^{o}C, with the MIC (Zgair *et al.*, 2014).

**Corresponding author:* e-mail: Ayaid_khadem@yahoo.com

Animals

Male BALB/c mice (4-6 weeks old, weighing 15-20g) procured from the central animal house of University of Baghdad, Baghdad, Iraq. The animals were put in clean polypropylene cages and fed on standard antibiotic-free diet (JBD Agencies, Pvt. Ltd., India). The study was carried out by following approval from the animal ethics committee of University of Baghdad, Baghdad, Iraq.

Bacterial adhesion to mouse intestinal tract (IT)

Bacteria were grown overnight in separate flasks containing trypticase soy broth (TSB; Himedia, Mumbia, India) at 37ºC. The cell pellet was obtained by centrifugation (10000 g in 5 min at 4ºC), washed two times with phosphate buffer saline (PBS, 0.01 M, pH 7.2) and re-suspended in sterile PBS (0.01 M, pH 7.2) to get a bacterial number of $1x10^7$ c.f. u/ml. For adhesion assay, mouse intestinal tract was excised from BALB/c mice and cut into 4 mm pieces in length under sterile conditions. The intestinal tract pieces [small intestinal tract (SIT), large intestinal tract (LIT) and rectum] were put in separate small Petri dishes, covered with respective bacterial growth and incubated at 37ºC for 2 hours. Then, each piece of intestinal tract tissue was rinsed gently with PBS three times to eliminate unattached bacteria. The pieces were homogenized individually in 1 ml PBS and 0.1 ml was serially diluted and plated on duplicate plates of Luria agar. The bacterial number was quantified post-overnight incubation at 37ºC (Zgair and Chhibber, 2010).

Kinetics of bacteria adhesion

The part of intestinal tract that *S. maltophilia* attached on it with maximum bacteria number was used to examine the kinetics of bacterial adherence. Similar procedure of bacterial adhesion on mouse intestinal tract (mentioned above) was followed but the ability of bacterial adhesion to mouse intestinal tract was checked after different time intervals (0, 0.5, 1, 2, 4, 8, 24, 48, 72 h).

Bacterial treatment with moxifloxacin (sub-MICs)

After overnight incubation, the colonies of *S. maltophilia* that grown onto Mueller-Hinton agar were suspended in tryp tone soy broth (TSB; Himedia, Mumbia, India). The bacterial number was adjusted to 1 x $10^7$with Mueller Hinton broth (Himedia, Mumbia, India) and then treated for 18 h at 37ºC with different concentrations of moxifloxacin (0.12, 0.06 and 0.03 x MIC15.625 µg/ml). Bacterial suspension that was not treated with moxifloxacin was considered as a control. Bacteria were then washed three times with PBS (10000*g*, 5min) to eliminate the antibiotic and then bacteria were re-suspended in TSB for adhesion method.

STATISTICAL ANALYSIS

All values were calculated as the means ± SD. Differences between test and control groups were analyzed using Student's t-test. Differences among multiple groups were analyzed by applying the Tukey honestly significant differences test to one-way ANOVA Using Origin version 8.0 software. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Adhering of S. maltophilia to different parts of IT

The ability of *S. maltophilia* to adhere to different parts of intestinal tract (IT) was estimated in current study. The data in fig. 1 showed that there is no significant difference among all parts of intestinal tract (SIT, LIT and rectum) as the ANOVA test followed by tukeys test showed that the P value for all intestinal tract parts was higher than 0.05 (F value: 0.1049, pro P>0.05: 0.901). According to current result, each part can be selected for further study, but we selected small intestinal tract (SIT) as this part is easy to handle and longer than other parts. That is why; we can get a lot of parts of SIT as compare with others (LIT and rectum).

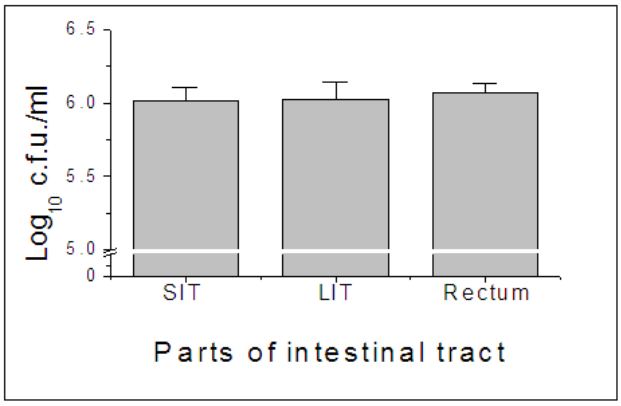

Fig. 1: Adhesion of clinical isolate of *S. maltophilia* to different parts of mouse intestinal tract *in vitro* (c.f.u./ml). No significant difference of bacterial adhesion was observed among all parts of intestinal tract, P>0.05. SIT, small intestinal tract; LIT, large intestinal tract.

Kinetics of bacterial adhesion to SIT

Data in fig. 2 and table 2 showed the ability of *S. maltophilia* (Sm2) to adhere to mouse intestinal tract (SIT). The significant adhering of *S. maltophilia* to SIT started as early as 30min post of incubation with SIT parts (P<0.05). The ability of this bacterium to attach to SIT increased dramatically with time of incubation. Maximum adhesion of Sm2 was observed by hour 72.

Moxifloxacin lethal and sub-lethal (subMICs) concentrations

The present study showed that *S. maltophilia* (Sm2) strain was susceptible to moxifloxacin (MIC 15.625µg/ml). Beginning experiments were carried out to assess the killing of *S. maltophilia* post exposure to different concentrations of moxifloxacin (0.56 x, 0.256 x, 0.126 x,

0.066 x and 0.036 x MIC). Moxifloxacin at 0.56 x and 0.256 x MIC caused significant killing of *S. maltophilia* (ranging from 88.4% and 71.3%, respectively). However, no significant killing was observed post exposing to 0.126 x, 0.066 x and 0.036 x MIC versus control (without moxifloxacin). Thus, further experiments were performed using a standardized *S. maltophilia* inoculum exposed to moxifloxacin at 0.12x, 0.066 x and 0.036x MIC, corresponding to 1.87, 1.03 and 0.46mg/ml, respectively**.**

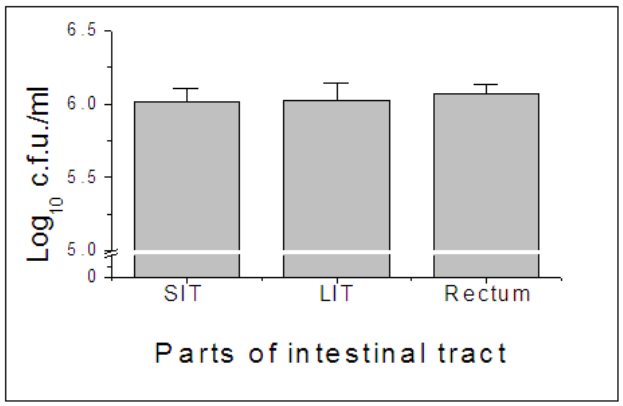

Fig. 2: Kinetics of *S. maltophilia* adhesion to mouse small intestinal tract (SIT) *in vitro*. Significant adhesion of S. maltophilia Sm2 was detected after 0.5 h of incubation. Asterisks indicate a significant difference from 0-h.

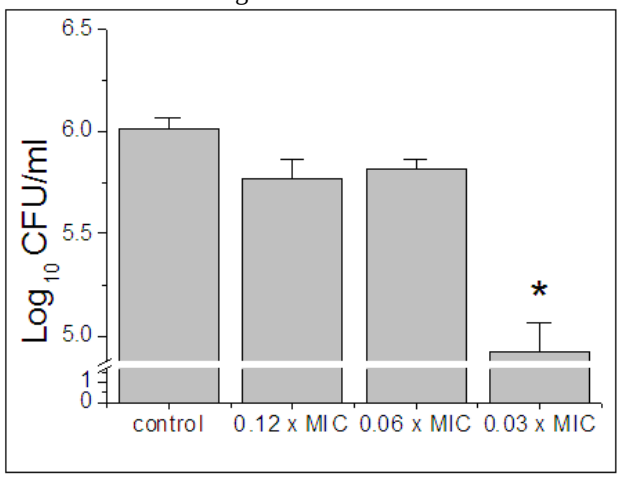

Fig. 3: Effect of moxifloxacin sub MICs on adherence of *S. maltophilia* to mouse intestinal tract *in vitro*. The control contained bacteria that were not exposed to moxifloxacin. Results are expressed as means ±SD. * P<0.005 (versus control).

Effect of moxifloxacin on S. maltophilia adhesion

Fig. 3 and table 3 showed the number of adhered bacteria (*S. maltophilia*) to SIT post exposure to sub-MICs of moxifloxacin. Adhesion of *S. maltophilia* (Sm2) strain was, in comparison with the control, significantly lower (P<0.01) in the presence of 0.036 x MIC with inhibition level of 92.0±5.6%. While, no significant decrease (P>0.05) in bacterial adhesion post treatment with 0.12 and 0.06 x MIC with inhibition levels 30.1±2.8 and 32.2±3.1%, respectively was observed. The inhibition level of adhered bacterial count was 72.9±11.8 post exposing to 0.036 x MIC of moxifloxacin. No significant difference was observed between inhibition levels that caused by 0.066 and 0.036x MIC exposure for Sm2 strain. While, the significant decrease in bacterial adhesion was observed between *S. maltophilia* pretreated with 0.036 x MIC and bacterial pretreated with either 0.12 or 0.06 x MIC (P<0.05).

Table 1: Number of adhered bacteria (*S. maltophilia*) to different parts of mouse intestinal tract (IT) *in vitro* (c.f.u. /ml). No significant difference of bacterial adhesion was observed among all parts of IT, (F value: 0.1049, Prob >0.05: 0.901). SIT, small intestinal tract; LIT, large intestinal tract; SD, standard deviation.

1	5.812913	5.812913	6.176091
2	5.90309	5.977724	6.060698
3	5.977724	6.176091	6.09691
Mean	5.897909	5.988909	6.111233
sd	0.082527	0.181847	0.059015

DISCUSSION

Many previous studies covered the adhesion of S. *maltophilia* to abiotic surfaces such as polystyrene (Di Bonaventura *et al*. 2004) and other studies were demonstrated the capability of *S. maltophilia* to adhere to biotic surfaces such as human and mouse epithelial cells (Chhibber and Zgair, 2009). Moreover the old study of our laboratory proved the ability of *S. maltophilia* to adhere to mouse mucus (Zgair and Chhibber, 2011). In current study we demonstrated the first time the ability of *S. maltophilia* to adhere to mouse IT. Moreover, this ability decreased significantly post exposing to sub-inhibitor dose of moxifloxacin. The interest finding, the lowest sub-inhibitor dose resulted the maximum reduction of bacterial adhesion. Thus, lower doses of moxifloxacin significantly inhibited adhesion of bacteria to IT. It means it is bactericidal at higher doses and bacteriostatic at lower concentrations. Therefore, administrating this antibiotic will reduce intestinal tract infection with *S. maltophilia*.

Adherence is an essential step in bacterial pathogenesis or infection, required for colonizing a new host and causing infection. Sub inhibitory consent rations of antibiotics have been shown to reduce the ability of pathogens to adhere to various substrates (Weiss *et al*., 1998). There is, however, little evidence that sub lethal concentrations of antibiotics are bene¢cial in the therapy of infections. Sub MICs of antibiotics could reduce bacterial adhesion through several mechanisms. Sub MICs can inhibit the synthesis or expression of adhesins on the bacterial cell surface. Moreover, the Sub MICs may affect on protein synthesis and that results abnormal bacterial adhesions,

Table 2: Log 10 number of *S. maltophilia* adhesion to mouse small intestinal tract (SIT) *in vitro*. Significant adhesion of S. maltophilia was detected after 0.5 h of incubation. Asterisks indicate a significant difference from 0-h. SD, standard deviation.

Time	I	II	III	Mean	sd
0 h	1	0.8	1.2	1	0.2
0.5 h	3.34	3	4.16	3.5	0.596322061
1 h	4.22	3	5.38	4.2	1.190126044
2 h	4.65	5.4	7.35	5.8	1.393735986
4 h	4.05	6.35	7.6	6	1.800694311
8 h	5.3	6.3	7.3	6.3	1
24 h	5.4	6.6	7.8	6.6	1.2
48 h	4.75	6.7	8.95	6.8	2.101784956
72 h	5.15	6.8	8.75	6.9	1.802082129

Table 3: Effect of moxifloxacin subMICs on adherence of S. maltophilia to mouse intestinal tract *in vitro*. The control contained bacteria that were not exposed to moxifloxacin. Results are expressed as means±SD. * P<0.005 (versus control). Ttest (2 tails and 2 type 2).

	I	II	III	Mean	SD	P value (t.test with control)	P value (t. test with 0.12 x MIC)	P value (t. test with 0.06 x MIC)
Control	6.049	6.079	5.913	6.014	0.071			
0.12 x MIC	5.623	5.763	5.939	5.77	0.129	0.084		
0.06 x MIC	5.812	5.913	5.7242	5.88	0.077	0.057		
0.03 x MIC	4.903	4.698	5.176	4.9	0.195	0.0017	0.00689	0.003917

bacterial receptors and bacterial shape. All these will affect negatively on bacterial adhesion to biotic and abiotic surfaces (Lorian *et al*., 1989; Pompilio *et al*., 2010).

Moxifloxacin decreases cell-surface hydrophobicity by interfering with the synthesis and expression of outer membrane proteins, LPS or fimbriae, structures known to affect bacterial hydrophobicity (Pompilio *et al*., 2008). That is why; moxifloxacin reduce the ability of S. maltophilia to adhere to biotic surfaces (intestinal tract). Previous study showed suggested that moxifloxacin can modulate the level of hydrophobicity in S. maltophilia, although this effect appears to be strain-dependent (Pompilio *et al*., 2010; Benbouzid *et al*., 2016). Thus, may be the lowest concentration of moxifloxacin appears the highest effect on hydrophobicity this step needs further studies.

Our study suggested that the moxifloxacin is high effective antibiotic against *S. maltophilia* infection especially in the immunosuppressive patients such as cystic fibrosis and cancer patients. The results of the present study could have important clinical implications, providing an additional rationale for the use of sub-inhibitor dose (lower sub-MIC) concentration of moxifloxacinin reducing *S. maltophilia* to adhere to intestinal tract, furthermore, this dose may prevent bacteria to cause infection in host intestinal tract.

DISCLOSURE

The authors have no financial support and no financial or proprietary interests in this study.

REFERENCES

Apisarnthanarak A, Mayfield JL, Garison T, DiPersio JF, Fraser VJ and Polish LB (2003). Risk factors for *Stenotrophomonas maltophilia* bacteremia in oncology patients: a case-control study. *Infect. Control Hosp. Epidemiol.*, **24**: 269-274.

Benbouzid F, Kodjikian L, Hartmann D, Renaud F and Baillif S (2016). Moxifloxacin superior to cefuroxime in reducing bacterial adhesion of *Staphylococcusepidermidis* on hydrophobic intraocular lenses. *Acta. Ophthalmol.*, **94**: e11-e15.

Chhibber S and Zgair AK (2009). Involvement of *Stenotrophomonas maltophilia* flagellin in bacterial adhesion to airway biotic surfaces: An *in vitro* study. *Am. J. Biomed. Sci.*, **1**: 188-195.

CLSI (2006). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 7th edn, approved guideline M7–A7. Wayne, PA: Clinical and Laboratory Standards Institute.

Denton M and Kerr KG (1998). Microbiological and clinical aspects of infection associated with *Stenotrophomonas maltophilia*. *Clin. Microbiol. Rev.*, **11**: 57-80.

Di Bonaventura G, Prosseda G, Del Chierico F, Cannavacciuolo S, Cipriani P, Petrucca A, Superti F,

Ammendolia MG and 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.

Di Bonaventura G, Spedicato I, D'Antonio D, Robuffo I and Piccolomini R (2004). Biofilm formation by *Stenotrophomonas maltophilia*: Modulation by quinolones, trimethoprim-sulfamethoxazole and ceftazidime. *Antimicrob. Agents Chemother.,* **48**: 151-160.

Fonseca AP, Extremina C, Fonseca AF, Sousa JC(2004). Effect of subinhibitory concentration of piperacillin/tazobactam on *Pseudomonas aeruginosa*. *J. Med. Microbiol.*, **53**: 903-910.

Gales AC, Jones RN, Forward KR, Linares J, Sader HS, Verhoef J (2001). Emerging importance of multidrugresistant Acinetobacter species and Stenotrophomonas maltophilia as pathogens in seriously ill patients: Geographic patterns, epidemiological features and trends in the SENTRY Antimicrobial Surveillance Program (1997-1999). *Clin. Infect. Dis*., **32**: S104-S113.

Labarca JA, Leber AL, Kern VL and *et al* (2000). Outbreak of *Stenotrophomonasmaltophilia* bacteremia in allogenic bone marrow transplant patients: role of severe neutropenia and mucositis. *Clin. Infect. Dis.*, **30**: 195-197.

Looney WJ, NaritaM, Mühlemann K (2009). *Stenotrophomonas maltophilia*: An emerging opportunist human pathogen. *Lancet Infect. Dis*., **9**: 312-323.

Lorian V, Ernst J and Amaral L (1989). The post-antibiotic effect defined by bacterial morphology. *J. Antimicrob. Chemother.,* **23**: 485-491.

Mouhamed RS, Jafaar MM, Hafudh MH, Abbas LMR, Aziz MM, Ahmad MJ, Mohsan H, simer H, Ghafil JA, Hassan SH, Zgair AK (2014). Effect of water taken from different environments on the ability of bacteria to form biofilm on abiotic surfaces. *World J. Exp. Biosci*., **2**: 19-23.

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.

Pompilio A, Catavitello C, Picciani C, Confalone P, Piccolomini R, Savini V, Fiscarelli E, D'Antonio D and Di Bonaventura G (2010). Sub inhibitory concentrations of moxifloxacin decrease adhesion and biofilm formation of *Stenotrophomonasmaltophilia* from cystic fibrosis. *J. Med. Microbiol*., **59**: 76-81.

Pompilio A, Piccolomini R, Picciani C, D'Antonio D, Savini V and Di Bonaventura G (2008). Factors associated with adherence to and biofilm formation on polystyrene by *Stenotrophomonas maltophilia*: The role of cell surface hydrophobicity and motility. *FEMS Microbiol. Lett.*, **287**: 41-47.

Weiss EI, Lev-Dor R, Kashman Y, Goldhar J, Sharon N and Ofek I (1998). Inhibiting interspecies co aggregation of plaque bacteria with cranberry juice constituent. *J. Am. Dent. Assoc.*, **129**: 1719-1723.

Wojnicz D and Jankowski S (2007). Effects of subinhibitory concentrations of amikacin and ciprofloxacin on the hydrophobicity and adherence to epithelial cells of uropathogenic Escherichia coli strains. *Int. J. Antimicrob. Agents.*, **29**: 700-704.

Yemisen M, Mete B, Tunali Y, Yentur E and Ozturk R (2008). A meningitis case due to *Stenotrophomonas maltophilia* and review of the literature. *Int. J. Infect. Dis*., **12**: e125-e127.

Zgair AK and Chhibber S (2010a) *Stenotrophomonas maltophilia* flagellin induces a compartmentalized innate immune response in mouse lung. *J. Med. Microbiol.,* **59**: 913-919.

Zgair AK and Chhibber S (2010b). Immunological and pathological aspects of respiratory tract infection with *Stenotrophomonas maltophilia* in BALB/c Mice. *J. Microbiol.Biotechnol*., **20**: 1585-1591.

Zgair AK and Chhibber S (2011a). Adhesion of *Stenotrophomonas maltophilia* to mouse tracheal mucus is mediated through flagella. *J. Med. Microbiol.,* **60**: 1032-1037.

Zgair AK and Chhibber S (2011b), Immunoassay Method to Check the Flagellin Mediated Binding of *Stenotrophomonas maltophilia* to Polystyrene, *Microbiol.*, **80**: 132-134.

Zgair AK, Chhibber S (2013). *Stenotrophomonas maltophilia* Flagellin is Involved in Bacterial Adhesion and Biofilm Formation. *Microbiol.,* **82**: 646-650.

Zgair AK, Radhi SN, Ghafil JA. (2014) Coating urinary catheter with moxifloxacin restricts Stenotrophomonas maltophilia adhesion in vitro. *World J Exp Biosci* **2**: 54-58.

Zhang LN, Wang XT, Ai YH, Guo QL, Huang L, Liu ZY, Yao B (2012). Epidemiological features and risk factors of sepsis-associated encephalopathy in intensive care unit patients: 2008-2011. *Chin. Med. J. (Engl).*, **125**: 828-831.