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Role of *Stenotrophomonas maltophilia* flagella in adhesion to human epithelial cells *in vitro*

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ABSTRACT

Stenotrophomonas maltophilia is an important opportunistic pathogen that affects immunocompromised individuals and has high ability to adhere to different surfaces. In the present study its ability to adhere to human epithelial cells that collected from mouths of healthy volunteers was checked. Clinical isolate (Sm2) used in this study was able to adhere to the human epithelial cells. The involvement of flagella in the adhesion process was evaluated by employing anti-flagellin antibodies inhibitors of adhesion. To achieve this, flagellin was purified from clinical isolate (Sm2) anti-sera were raised in rabbit. The flagellin receptors were blocked by treatment of bacteria with anti-flagellin. Reduced bacterial adherence in presence of flagellin ant-isera confirmed the role of flagella in adhesion to biotic surfaces. The effect of anti-flagellin was in a dose dependent manner. These evidences prove the involvement of flagella in the adhesion of *S. maltophilia* to human epithelial cells.

Keywords: Anti-flagellin, Flagellin, oral epithelial cells, *Stenotrophomonas maltophilia*.

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INTRODUCTION

S. maltophilia has emerged as an important opportunistic pathogen affecting primarily the hospitalized and debilitated hosts. *S. maltophilia* does not appear to be inherently virulent, and it is an uncommon cause of invasive infections [1]. *S. maltophilia* is an emerging human pathogen that increasingly posses a challenge to clinicians, microbiologists, and infection control specialists.

The earlier studies have reported the ability of both clinical and environmental *S. maltophilia* isolates to adhere to abiotic surfaces [2, 3]. This is nonspecific adhesion as the interaction occurs between the bacterial surface and synthetic surface without involving the participation of specific receptors. However, information about ability of *S. maltophilia* to adhere to biotic surfaces is scanty in literature.

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This kind of adhesion is called specific adhesion as the interaction occurs between specific receptors on bacterial surface and biotic surface [4]. Flagella are responsible for adhesion of *S. maltophilia* to biotic and abiotic surface [3,4] but the adhesion of this bacteria to epithelial cell and role of flagellin in this process was not investigated yet. In current study, we evaluate the ability of clinical isolates of *S. maltophilia* to adhere to human epithelial cell and estimate the direct role of flagellin in this process.

MATERIALS AND METHODS

Bacterial isolate

S. maltophilia clinical isolate (Sm2) was used in this study. This isolate was procured from the Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India. The bacterial isolate was preserved by lyophilization and routinely cultured at 37 °C on Luria Bertani (LB) agar plates. Subcultures were made every week.

Epithelial cells collection

Ten ml of sterile normal saline was used to wash mouths of ten volunteers. The collected normal saline was centrifuged at 1500 g for 10 minute. The sediment was washer three time with normal saline. Finally, the cells count was adjusted to105/ml.

Flagellin and anti-flagellin preparation

Flagellin was isolated from *S. maltophilia* (Sm2) according to the procedure described earlier [4,5]. Briefly, *S. maltophilia* (Sm2) was grown in LB broth overnight and pelleted by centrifugation. Pellet was suspended in potassium phosphate buffer (0.01 M, pH 7.0) and sheared for 1 min in commercial blender. The sheared suspension was centrifuged for 30 min at 5000 × g and then centrifuged for 15 min at 16000 × g. The supernatant was centrifuged at 100000 × g for 3 h. The pellet was collected and kept at –80°C. Anti-flagellin antiserum was prepared by immunizing rabbit with flagellin. Complement was inactivated by incubating sera at 56°C for 30 min and presence of anti-flagellin was detected by immunoblotting.

Experiment

Bacterial growth was washed (5000 g for 10 min) three times with sterile phosphate buffer saline (0.1 M, pH 7.2) and palate was resuspended with PBS and number of bacteria was adjusted to 107/ml. 0.1 ml of bacterial suspension was mixed with 0.1 ml of prepared epithelial cells (10^5/ml) and incubated for 1 h at 37 ^oC after that the mixture was centrifuged for 10 min at 1000 g. The palate was smeared on clean slides and fixed with methanol for 5 min and dried. The slides were stained with Leishman stain. For bacterial adhesion inhibition the bacterial suspension was pretreated with different dilutions of anit-flagellin (1/80, 1/160, 1/320, 1/640) and tested for adhesion to epithelial cells.

Statistical analysis

All values have been used to give a mean value and the standard deviation calculated. The correlation coefficient test [Pearson correlation (r)] was used to check the relationship between two groups. The differences were analyzed using Student's t test, and one-way ANOVA test (followed by Tukey's test) with Origin version 8.0 software. A value of $P<0.05$ was considered to be statistically significant.

RESULTS

Fig. 1 shows the ability of *S. maltophilia* to adhere to epithelial cell that collected from mouth of healthy volunteers. Moreover, the figure depicted the effect of different dilutions of anti-flagellin on bacterial adhesion. *S. maltophilia* has a good ability to adhere to human epithelial cells in vitro. In addition the anti-flagellin reduced significantly, the ability of this bac-

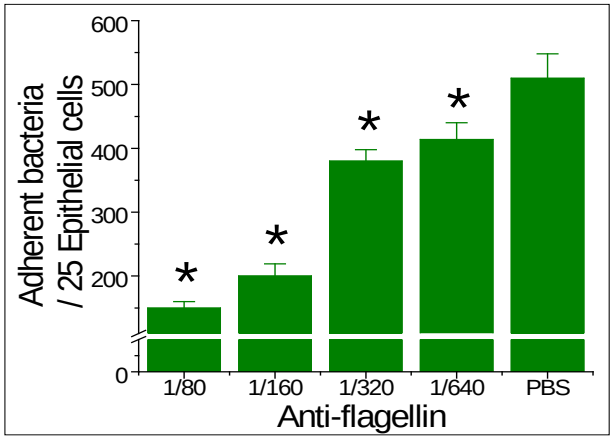

Fig. 1. Adhesion of *S. maltophilia* to human epithelial cells that collected from oral cavity of healthy volunteers upon pretreatment with different dilution of anti-Flagellin. The data represents mean ± SD of independent experiments.

terium to adhere to human epithelial cells. The anti-flagellin reduced bacterial adhesion in a dose dependent manner. This finding proved the direct role of flagella in adhesion of *S. maltophilia* to epithelial cells *in vitro*. **Fig. 2** shows the ability of *S. maltophilia* to adhere to epithelial cells under high power light microscope. In addition the picture depict clearly that the pretreatment of *S. maltophilia* with anit-flagellin reduced the ability of *S. maltophilia* to adhere to human epithelial cells significantly.

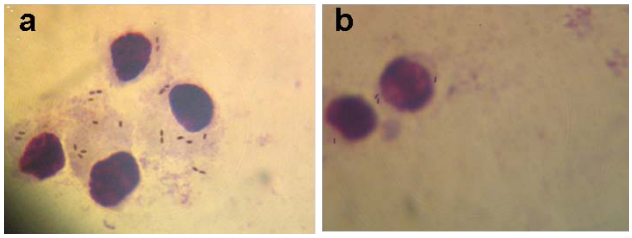

Fig. 2. Leishman stained slides depicted adhesion of *S. maltophilia* to human epithelial cells. a, human epithelial cells treated with *S. maltophilia*; b, human epithelial cells treated with *S. maltophilia* pretreated with anti-flagellin.

DISCUSSION

Adhesion is an important step in the bacterial colonization to biotic surfaces to establish of infection and generate the innate immune response [5]. It is considered that without attachment *S. maltophilia* is unable to colonize *in vivo* and is likely to be removed by non-specific host defenses [6]. In this study, Sm 2 isolate of *S. maltophilia* showed the significant ability to adhere to biotic surface (human epithelia cells). To define the precise role of flagella in adhesion process, the flagellin was purified from this isolate and antisera raised against this preparation. The role of flagellin in adhesion to biotic surfaces was established by using anti-flagellin antibodies. The results showed that treatment of bacteria with antisera reduced adhesion of *S. maltophilia* to human epithelial cells confirming the role of flagella in adhesion process. This may be due to the dual mode of action of anti-flagellin antibodies. The antibody either directly blocked the binding of flagellar protein by occupying the specific sites on the flagella or indirectly affected the adhesion process by inhibiting the motility of the bacteria, making them unable to reach the surface to bind [7].

The motility of bacteria is considered to be important for the initiation of contact between bacteria and epithelial cells [8]. Earlier studies have reported the presence of specific receptors on the epithelial cell surfaces, to which flagellin C binds [9]. The most important receptors specific for flagellin are TLR5 and asialoGM1 [10]. Though in this study no attempt was made to identify the adhesion sites for *S. maltophilia* on these biotic surfaces but the receptors were blocked by treating human epithelial cells with pure flagellin before exposing them to bacteria. That is why; flagellum plays a direct role in the pathogenicity of *S. maltophilia* as it mediates the attachment of bacteria to epithelial cells, the essential step in the initiation of infection.

Conflict of interest

The author declares that he has no conflict of interests.

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