

## Turnabout is fair play

Krachler, Anne-Marie; Ham, Hyeilin; Orth, Kim

DOI:

[10.4161/viru.3.1.18172](https://doi.org/10.4161/viru.3.1.18172)

License:

Creative Commons: Attribution-NonCommercial (CC BY-NC)

*Document Version*

Publisher's PDF, also known as Version of record

*Citation for published version (Harvard):*

Krachler, A-M, Ham, H & Orth, K 2012, 'Turnabout is fair play: use of the bacterial Multivalent Adhesion Molecule 7 as an antimicrobial agent', *Virulence*, vol. 3, no. 1, pp. 68-71. <https://doi.org/10.4161/viru.3.1.18172>

[Link to publication on Research at Birmingham portal](#)

### **Publisher Rights Statement:**

Eligible in repository under Creative Commons license.

Checked January 2015

### **General rights**

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

### **Take down policy**

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

## Turnabout is fair play

### Use of the bacterial Multivalent Adhesion Molecule 7 as an antimicrobial agent

Anne Marie Krachler, Hyeilin Ham and Kim Orth\*

Department of Molecular Biology; UT Southwestern Medical Center; Dallas, TX USA

**P**athogen attachment to host tissues is one of the initial and most crucial events during the establishment of bacterial infections and thus interference with this step could be an efficient strategy to fight bacterial colonization. Our recent work has identified one of the factors involved in initial binding of host cells by a wide range of Gram-negative pathogens, Multivalent Adhesion Molecule (MAM) 7. Interference with MAM7-mediated attachment, for example by pre-incubation of host cells with recombinant MAM7, significantly delays the onset of hallmarks of infection, such as pathogen-mediated cytotoxicity or the development of other adhesive structures such as actin pedestals. Thus, we are trying to develop tools based on MAM7 that can be used to prevent or diminish certain Gram-negative bacterial infections. Herein, we describe the use of bead-coupled MAM7 as an inhibitor of infection with the clinically relevant pathogen *Pseudomonas aeruginosa*.

cell contact and are often only expressed at low levels upon the bacteria's first encounter with its host.<sup>1</sup> Often times, their expression only commences after the bacteria receive specific environmental cues they encounter inside the host, such as elevated temperature, higher or lower than usual ion concentrations, or reactive oxygen species generated as a result of the induction of the host's immune response to infection.<sup>2,3</sup> In many cases, the pathogen's capacity to exert its full virulence is directly dependent on surface contact and therefore relies to a high degree on its ability to establish a stable interaction with the host cells.<sup>4</sup>

For example, many pathogens express and secrete large autotransporter toxins into the surrounding medium. Delivery of these toxins often requires their localized binding to the host membrane, where they cross into the host cell cytoplasm to exert their cytotoxic effect.<sup>5</sup> Other toxins penetrate the host cell membrane by assembling into a multimeric pore complex, a feature which is highly dependent on local toxin concentration and thus only possible if secreting bacteria and host cells are in sufficient proximity to each other to avoid loss of toxin by diffusive processes.<sup>6,7</sup> Many Gram-negative pathogens possess an arsenal of effector proteins which target host proteins to take over the regulation of host signaling events and manipulate them to the pathogens advantage. For example, effector proteins can inhibit immune responses, induce autophagy and trigger reorganization of a cell's cytoskeleton.<sup>8,9</sup> The delivery of effector proteins into host cells is accomplished by type III, type IV or type VI secretion systems, needle-like complexes which translocate

**Keywords:** infection, attachment, adhesin, phosphatidic acid, fibronectin, Gram-negative pathogen, anti-adhesion therapy

Submitted: 08/15/11

Revised: 09/21/11

Accepted: 09/21/11

<http://dx.doi.org/10.4161/viru.3.1.18172>

\*Correspondence to: Kim Orth;  
Email: [Kim.Orth@utsouthwestern.edu](mailto:Kim.Orth@utsouthwestern.edu)

Krachler AM, Ham H, Orth K. Outer membrane adhesion factor multivalent adhesion molecule 7 initiates host cell binding during infection by Gram-negative pathogens. *Proc Natl Acad Sci USA* 2011; 108:11614–9; PMID:21709226; <http://dx.doi.org/10.1073/pnas.1102360108>

Bacterial pathogens are faced with a number of problems when they try to colonize their host—they have to evade immune recognition, invade tissues and modulate cellular signaling events to promote their own survival. A growing number of virulence factors are known to contribute to each of these steps, and more are being discovered continually. However, the initial step giving rise to an infection, the stable attachment of the pathogen to host cells, is often overlooked and as a result a lot less well understood than subsequent events. Many virulence factors are upregulated dependent on host

bacterial proteins directly from the bacterial cytoplasm into the host's cytoplasm.<sup>10,11</sup> Establishment of these translocation machines and correctly timed transfer of proteins relies on direct contact with the host cells.<sup>12</sup>

As such, the initial attachment of pathogens to their eukaryotic host is a logical target of therapeutic interference with bacterial infections. However, developing therapeutic tools interfering with bacterial adhesion remains a major challenge, partly due to the complexity of synthetic receptor or adhesin analogs and variability in receptor-adhesin interactions between different pathogens and tissue types.<sup>13,14</sup> Although many adhesins are known in both Gram-positive and Gram-negative bacteria, their expression is often upregulated as a result of host cell contact and most of them are highly species-specific. In addition, many adhesion factors are large proteins or even multi-subunit protein complexes requiring dedicated cellular machinery for their transport to and assembly at the bacterial outer membrane.<sup>15,16</sup>

Our recent work identified an adhesion factor, termed Multivalent Adhesion Molecule (MAM) 7, which is widely distributed in Gram-negative bacteria, and especially animal pathogens. MAM7 consists of an N-terminal hydrophobic region which is required for outer membrane targeting and anchoring of the protein. This region is followed by a stretch of six to seven mammalian cell entry (mce) domains which are responsible for host cell binding. Mce domains have been described previously—a family of proteins containing one mce domain followed by a domain of unknown function has been identified as a factor promoting cellular attachment and invasion of macrophages by mycobacteria, although the mechanism underlying the invasion process was unknown.<sup>17,18</sup> In Gram-negative bacteria, the mce domain seems to have undergone multiple duplication events to form a distinct family of mce-containing proteins which consist of an N-terminal hydrophobic membrane targeting region, followed by six or seven mce domains.

In contrast to other adhesins, MAM7 is relatively small and constitutively expressed

by bacteria, even prior to host cell contact. Upon encountering host cells, it gives the pathogen a distinct advantage, enabling immediate binding to a broad range of host cell types independent of the induction of virulence factors.<sup>19</sup> Outer membrane localization and anchoring of MAM7 does not require any dedicated transport machinery—this enabled us to express the protein at the surface of non-pathogenic *E. coli*. The non-pathogenic *E. coli* laboratory strain BL21 does not possess attachment factors for mammalian host cells, which allowed us to analyze the contribution of MAM7 to host cell attachment independent of other factors promoting pathogen adhesion and dissect the interactions between MAM7 and the host cell surface.

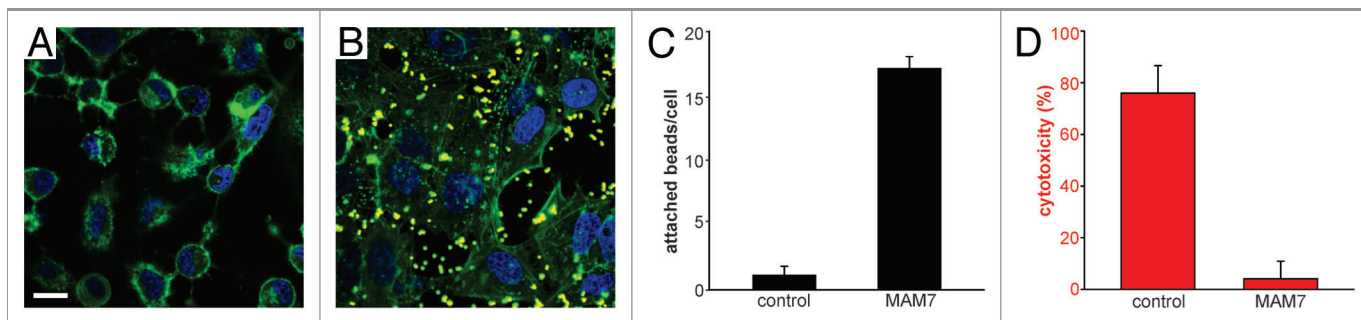
We identified two different host cell receptors for bacterial MAM7—the first receptor is the extracellular matrix protein fibronectin, which has previously been described to serve as a receptor for a variety of bacterial adhesins. In contrast to other adhesins, which exclusively bind fibronectin and have evolved to bind this ligand with exceptionally high affinity,<sup>20-22</sup> MAM7 seems to bind to fibronectin relatively weakly ( $K_D$  of 15  $\mu$ M). This low affinity interaction is complemented by a second receptor, phosphatidic acid, resulting in an overall affinity of MAM7 for host cells that is very high (approx. apparent  $K_D$  of 200 nM). The functionality of mce domains as phosphatidic acid binding domain seems to have been conserved across different families—the Arabidopsis chloroplast protein Tgl2, which contains only one mce domain, also binds PA albeit with lower affinity.<sup>23</sup> We hypothesize that the duplication of mce domains in MAM7 leads to an acquisition of an entirely new functionality, the capability to bind to fibronectin. By enabling pathogens to establish interactions with two distinct types of host cell receptors, pathogens have increased their capacity for initial attachment.

Since attachment to fibronectin seems to initialize host cell binding by a wide range of pathogens, we suspected blocking the host cell receptors with MAM7 would potentially inhibit infection by other pathogens. Indeed, we showed that pre-incubation of mammalian cells with

non-pathogenic *E. coli* expressing MAM7 could competitively inhibit attachment of a range of Gram-negative pathogens and stop them from exerting cytotoxic effects on host cells (as in the case of *Yersinia pseudotuberculosis*, *Vibrio cholerae* or *Vibrio parahaemolyticus*) and from establishing permanent mechanisms of attachment, such as through formation of actin pedestals (enteropathogenic *E. coli*, EPEC). Our current efforts are directed toward developing improved MAM7-derived tools that may be used as competitive inhibitors of bacterial attachment and thus as agents to attenuate a wide range of Gram-negative bacterial infections.

So far, we have successfully inhibited infection of cultured mammalian cells by a number of important enteropathogenic bacteria. *V. cholerae* is the causative agent of cholera, a severe gastrointestinal disease which is responsible of an estimated 5 million deaths annually and still is one of the leading causes of infant death.<sup>24</sup> *V. parahaemolyticus* is a seafood-borne pathogen which usually causes diarrheal disease but can also lead to wound infections and septicemia, particularly in immunocompromised patients.<sup>25</sup> *Y. pseudotuberculosis* is also a food-borne pathogen and mainly causes enteritis or tuberculosis-like symptoms in infected animals.<sup>26,27</sup> EPEC is another major agent of infantile diarrhea and is associated with high mortality rates.<sup>28</sup> For all these pathogens, we observed a 30 to 70% decrease in pathogenicity when host cells were pre-treated with BL21-MAM7.<sup>19</sup>

Our recent efforts to further develop a MAM7-derived agent to attenuate Gram-negative infections have therefore focused on two issues: First, we are seeking to expand the repertoire of pathogens susceptible to MAM7-based inhibition. As discussed in our previous work, we have identified MAM7 homologs in a wide range of Gram-negative pathogens and we currently are testing a selection of them for their susceptibility to MAM7-based inhibition. One of the pathogens we focus on is *Pseudomonas aeruginosa*. *P. aeruginosa* thrives in most environments, including water, soil and on human skin. In immunocompromised patients, it can cause catheter-associated lung and urinary tract infections, but it is also a



**Figure 1.** Inhibition of *Pseudomonas aeruginosa*-mediated cytotoxicity using bead-immobilized MAM7. HeLa epithelial cells (80% confluency) were pre-incubated with bead-immobilized GST (A) or GST-MAM7 (B) for 30 min prior to infection with *P. aeruginosa* strain PAO1 at a multiplicity of infection of 20 for four hours. Cells were fixed and stained for DNA (blue) and actin (green). Attached beads, yellow. Scale bar, 20  $\mu$ m. Attached beads per cell were determined by counting (C, black) and cytotoxicity was determined by measuring LDH released into the culture medium (D, red). Error bars indicate s.e.m. ( $n \geq 9$ ). Cloning of expression constructs for GST and GST-MAM7 fusion protein as well as protein purifications have been described elsewhere.<sup>19</sup> Purified proteins were immobilized on 1  $\mu$ m fluorescent orange latex beads (Sigma) as described by El Shazly et al.<sup>31</sup> For inhibition experiments, a total amount of 7.5  $\mu$ g protein/ $10^6$  beads/well in PBS were used.

major burden for cystic fibrosis patients and can cause persistent wound infections, for example in burn patients.<sup>29,30</sup> Due to its clinical importance, we studied if *P. aeruginosa*-mediated cytotoxicity could be attenuated by MAM7 in a tissue culture model of infection (Fig. 1). The second focus of our current studies in MAM7-based inhibitors is dedicated to finding alternative modes of delivery. While non-pathogenic bacteria expressing surface-bound MAM7 may be a suitable vehicle for gastrointestinal delivery of MAM7 to prevent or combat enteric pathogens, their use on an open wound would most likely exacerbate inflammatory responses and could therefore have adverse effects on wound healing. We are therefore studying alternative modes of delivery for MAM7 to the site of infection. One such approach is to immobilize recombinant MAM7 on the surface of inert polymer beads, which are similar in size to the bacteria we have previously used (1  $\mu$ m). We tested the efficacy of bead-immobilized MAM7 against *P. aeruginosa* infection of epithelial cells and compared it to control beads

displaying GST, which do not bind to host cells (Fig. 1). In each case, we counted the number of bound beads per cell (fluorescent beads were used for ease of visualization) and determined the cytotoxic effect of *P. aeruginosa* using lactate dehydrogenase (LDH) release assays. Upon infection, host cells lyse and release LDH into the culture medium, which can be detected colorimetrically and compared with a standard of detergent-lysed cells (100% lysis). GST-beads did not show any significant attachment to host cells and failed to inhibit infection (Fig. 1A and C). In contrast, MAM7 beads bound to host cells ( $17.1 \pm 0.9$  beads/cell) and, as a consequence, attenuated *P. aeruginosa*-mediated cell killing (cytotoxicity decreased from 76% to 4%).

These studies demonstrate that MAM7-based inhibition may potentially be developed as a tool to attenuate not only enteric pathogens but also hospital-acquired and wound-associated infections, such as those caused by *P. aeruginosa*. The adhesin can be expressed at the surface of non-pathogenic bacteria but may also be

delivered by alternative routes, such as immobilized on beads, which may aid in future applications in decreasing risks associated with the introduction of live bacteria into a living organism. In the future, we hope to be able to extend the application of MAM7 to include other clinically relevant Gram-negative pathogens and develop tools for its efficient delivery to the site of potential infection.

#### Acknowledgments

We would like to thank the Orth lab, especially A.R. Woolery and A. Sreelatha, for critical reading and comments on the manuscript. We also thank C. Cannon (UT Southwestern Medical Center) for providing *P. aeruginosa* strains and technical assistance with infection experiments. K.O., H.H. and A.M.K. are supported by grants from NIH-Allergy and Infectious Disease (R01-AI056404 and R01-AI087808) and the Welch Foundation (I-1561). K.O. is a Burroughs Wellcome Investigator in Pathogenesis of Infectious Disease and a W.W. Caruth Jr. Biomedical Scholar.

#### References

- Roe AJ, Hoey DE, Gally DL. Regulation, secretion and activity of type III-secreted proteins of enterohaemorrhagic *Escherichia coli* O157. *Biochem Soc Trans* 2003; 31:98-103; PMID:12546663; <http://dx.doi.org/10.1042/BST0310098>
- Gode-Potratz CJ, Chodur DM, McCarter LL. Calcium and iron regulate swarming and type III secretion in *Vibrio parahaemolyticus*. *J Bacteriol* 2010; 192:6025-38; PMID:20851895; <http://dx.doi.org/10.1128/JB.00654-10>
- Fukuto HS, Svetlanov A, Palmer LE, Karzai AW, Bliska JB. Global gene expression profiling of *Yersinia pestis* replicating inside macrophages reveals the roles of a putative stress-induced operon in regulating type III secretion and intracellular cell division. *Infect Immun* 2010; 78:3700-15; PMID:20566693; <http://dx.doi.org/10.1128/IAI.00062-10>
- Gode-Potratz CJ, Kustusch RJ, Breheny PJ, Weiss DS, McCarter LL. Surface sensing in *Vibrio parahaemolyticus* triggers a programme of gene expression that promotes colonization and virulence. *Mol Microbiol* 2011; 79:240-63; PMID:21166906; <http://dx.doi.org/10.1111/j.1365-2958.2010.07445.x>

5. Matsuda S, Kodama T, Okada N, Okayama K, Honda T, Iida T. Association of *Vibrio parahaemolyticus* thermostable direct hemolysin with lipid rafts is essential for cytotoxicity but not hemolytic activity. *Infect Immun* 2010; 78:603-10; PMID:19933828; <http://dx.doi.org/10.1128/IAI.00946-09>
6. Kim YR, Lee SE, Kook H, Yeom JA, Na HS, Kim SY, et al. *Vibrio vulnificus* RTX toxin kills host cells only after contact of the bacteria with host cells. *Cell Microbiol* 2008; 10:848-62; PMID:18005241; <http://dx.doi.org/10.1111/j.1462-5822.2007.01088.x>
7. Zrimi J, Ng Ling A, Giri-Rachman Arifin E, Feverati G, Lesieur C. Cholera toxin B subunits assemble into pentamers—proposition of a fly-casting mechanism. *PLoS ONE* 2010; 5:e15347; PMID:21203571; <http://dx.doi.org/10.1371/journal.pone.0015347>
8. Trosky JE, Liverman AD, Orth K. *Yersinia* outer proteins: Yops. *Cell Microbiol* 2008; 10:557-65; PMID:18081726; <http://dx.doi.org/10.1111/j.1462-5822.2007.01109.x>
9. Broberg CA, Orth K. Tipping the balance by manipulating post-translational modifications. *Curr Opin Microbiol* 2010; 13:34-40; PMID:20071215; <http://dx.doi.org/10.1016/j.mib.2009.12.004>
10. Cambronne ED, Roy CR. Recognition and delivery of effector proteins into eukaryotic cells by bacterial secretion systems. *Traffic* 2006; 7:929-39; PMID:16734660; <http://dx.doi.org/10.1111/j.1600-0854.2006.00446.x>
11. Filloux A, Hachani A, Bleves S. The bacterial type VI secretion machine: yet another player for protein transport across membranes. *Microbiology* 2008; 154:1570-83; PMID:18524912; <http://dx.doi.org/10.1099/mic.0.2008/016840-0>
12. Winnen B, Schlumberger MC, Sturm A, Schupbach K, Siebenmann S, Jenny P, et al. Hierarchical effector protein transport by the *Salmonella* Typhimurium SPI-1 type III secretion system. *PLoS ONE* 2008; 3:e2178; PMID:18478101; <http://dx.doi.org/10.1371/journal.pone.0002178>
13. Svensson M, Platt FM, Svanborg C. Glycolipid receptor depletion as an approach to specific antimicrobial therapy. *FEMS Microbiol Lett* 2006; 258:1-8; PMID:16630247; <http://dx.doi.org/10.1111/j.1574-6968.2006.00175.x>
14. Ofek I, Hasty DL, Sharon N. Anti-adhesion therapy of bacterial diseases: prospects and problems. *FEMS Immunol Med Microbiol* 2003; 38:181-91; PMID:14522453; [http://dx.doi.org/10.1016/S0928-8244\(03\)00228-1](http://dx.doi.org/10.1016/S0928-8244(03)00228-1)
15. Fernández LA, Berenguer J. Secretion and assembly of regular surface structures in Gram-negative bacteria. *FEMS Microbiol Rev* 2000; 24:21-44; PMID:10640597; [http://dx.doi.org/10.1016/S0168-6445\(99\)00026-1](http://dx.doi.org/10.1016/S0168-6445(99)00026-1)
16. Palomino C, Marin E, Fernandez LA. The fimbrial usher FimD follows the SurA-BamB pathway for its assembly in the outer membrane of *Escherichia coli*. *J Bacteriol* 2011. In press. PMID:21784935; <http://dx.doi.org/10.1128/JB.05585-11>
17. Saini NK, Sharma M, Chandolia A, Pasricha R, Brahmachari V, Bose M. Characterization of Mce4A protein of *Mycobacterium tuberculosis*: role in invasion and survival. *BMC Microbiol* 2008; 8:200; PMID:19019220; <http://dx.doi.org/10.1186/1471-2180-8-200>
18. Chitale S, Ehrst S, Kawamura I, Fujimura T, Shimono N, Anand N, et al. Recombinant *Mycobacterium tuberculosis* protein associated with mammalian cell entry. *Cell Microbiol* 2001; 3:247-54; PMID:11298648; <http://dx.doi.org/10.1046/j.1462-5822.2001.00110.x>
19. Krachler AM, Ham H, Orth K. Outer membrane adhesion factor multivalent adhesion molecule 7 initiates host cell binding during infection by Gram-negative pathogens. *Proc Natl Acad Sci USA* 2011; 108:11614-9; PMID:21709226; <http://dx.doi.org/10.1073/pnas.1102360108>
20. Meenan NA, Visai L, Valtulina V, Schwarz-Linek U, Norris NC, Gurusiddappa S, et al. The tandem beta-zipper model defines high affinity fibronectin-binding repeats within *Staphylococcus aureus* FnBPA. *J Biol Chem* 2007; 282:25893-902; PMID:17606607; <http://dx.doi.org/10.1074/jbc.M703063200>
21. Schwarz-Linek U, Hook M, Potts JR. Fibronectin-binding proteins of gram-positive cocci. *Microbes Infect* 2006; 8:2291-8; PMID:16782385; <http://dx.doi.org/10.1016/j.micinf.2006.03.011>
22. Kingsley RA, Abi Ghanem D, Puebla-Osorio N, Keestra AM, Berghman L, Baumber AJ. Fibronectin binding to the *Salmonella enterica* serotype Typhimurium ShdA autotransporter protein is inhibited by a monoclonal antibody recognizing the A3 repeat. *J Bacteriol* 2004; 186:4931-9; PMID:15262930; <http://dx.doi.org/10.1128/JB.186.15.4931-4939.2004>
23. Lu B, Benning C. A 25-amino acid sequence of the *Arabidopsis* TGD2 protein is sufficient for specific binding of phosphatidic acid. *J Biol Chem* 2009; 284:17420-7; PMID:19416982; <http://dx.doi.org/10.1074/jbc.M109.016014>
24. Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bull World Health Organ* 2003; 81:197-204; PMID:12764516
25. Daniels NA, MacKinnon L, Bishop R, Altekruse S, Ray B, Hammond RM, et al. *Vibrio parahaemolyticus* infections in the United States, 1973-1998. *J Infect Dis* 2000; 181:1661-6; PMID:10823766; <http://dx.doi.org/10.1086/315459>
26. Naktin J, Beavis KG. *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. [vi.]. *Clin Lab Med* 1999; 19:523-36; PMID:10549424
27. Tauxe RV. Salad and pseudoappendicitis: *Yersinia pseudotuberculosis* as a foodborne pathogen. *J Infect Dis* 2004; 189:761-3; PMID:14976590; <http://dx.doi.org/10.1086/381806>
28. Fagundes-Neto U, Scaletsky IC. The gut at war: the consequences of enteropathogenic *Escherichia coli* infection as a factor of diarrhea and malnutrition. *Sao Paulo Med J* 2000; 118:21-9; PMID:10685124; <http://dx.doi.org/10.1590/S1516-31802000000100006>
29. Høiby N. Recent advances in the treatment of *Pseudomonas aeruginosa* infections in cystic fibrosis. *BMC Med* 2011; 9:32; PMID:21463524; <http://dx.doi.org/10.1186/1741-7015-9-32>
30. Branski LK, Al-Mousawi A, Rivero H, Jeschke MG, Sanford AP, Herndon DN. Emerging infections in burns. *Surg Infect (Larchmt)* 2009; 10:389-97; PMID:19810827; <http://dx.doi.org/10.1089/sur.2009.024>
31. El-Shazly S, Ahmad S, Mustafa AS, Al-Attayah R, Krajci D. Internalization by HeLa cells of latex beads coated with mammalian cell entry (Mce) proteins encoded by the mce3 operon of *Mycobacterium tuberculosis*. *J Med Microbiol* 2007; 56:1145-51; PMID:17761475; <http://dx.doi.org/10.1099/jmm.0.47095-0>