Assessment of TLR2 & TLR4 in urine of Iraqibacter isolated from urinary Tract infection of Baghdad hospitals

Heba Fezaa Abdalhussin[1], Nihad Abdulhussain Jafar[2], Sinan B. Alrifai[3]
[1]Tikrit University /college science microbiology /Iraq
[2]Tikrit university / college of veterinary medicine/Iraq
[3]Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, Iraq

Abstract

Acinetobacter baumannii has grown to be a major source of worry. It has gained particular notoriety in the recent desert conflicts in Iraq, earning the nickname "Iraqibacter." Although most A. baumannii infections have been studied in the context of pneumonia and bloodstream infections. Urinary tract infections (UTIs) are occasionally caused by A. baumannii . Three hundred and forty-six urine samples were collected from several Baghdad hospitals in Karkh. Samples were taken from different ages and genders. This study has been done during the period from the first of November 2021 to the end of March 2022. Among all 3466 urine samples with positive culture, only 14 cases revealed Acinetobacter baumannii isolates (about 4%) , the isolates of positive cultured urine were identification by conventional methods for identifying Acinetobacter baumanniii (i.e. Iraqibacter). The non-lactose and partially lactose fermenting colonies on MacConkey agar and non-hemolytic vague creamy colonies on blood agar, and cultured on CHROM agar selective medium for A.baumanniii, and the isolates were definitely identified by using the VITEK- Toll like receptors 2(TLR2) and Toll like resptors 4 (TLR4) concentration in urine was estimated by specific commercial sandwich enzyme-linked immune sorbent assay ELISA kit . The present results demonstrated that the TLR2 level elevated in urine of patients in comparsion to control with a high statistical significance The current results also showed that TLR4 concentration in urine of patients was slightly elevated with non-significant difference .

Keyword: A.baumannii ,Urinary tract infection ,Toll like receptors 2(TLR2) , Toll like receptor4 (TLR4).

Introduction

Acinetobacter baumannii is a gram-negative coccobacillus initially considered to be an opportunistic pathogen, which plays a vital role as a major cause of healthcare-associated infections. In recent years, A.baumannii has become resistant to most effective antimicrobial agents and causing a high incidence rate of morbidity and mortality especially in the intensive care unit in many countries (Moulana et al.,2020). Acinetobacter baumannii strains have the ability to colonize several ecological niches including soil, water, and animals, including humans. They also survive under extremely harsh environmental conditions thriving on rare and recalcitrant carbon compounds (Yakkala et al., 2019). Acinetobacter baumannii is frequently described as the etiologic agent for ventilator-associated pneumonia, urinary infections, wound infections, and bloodstream infections (Yadav et al., 2020).

In conflict zones, *Acinetobacter baumannii* has grown to be a major source of worry. It has gained particular notoriety in the recent desert conflicts in Iraq, earning the nickname "Iraqibacter." Particularly among US Army personnel, high rates of multi drug resistant (MDR) bacteremia have been reported.High rates of MDR bacteremia have been reported, particularly among US Army personnel. Urinary tract infections (UTIs) are occasionally caused by *A. baumannii*, particularly when indwelling urinary catheters are used (Dijkshoorn, L. *et al.*,2007), and one study found that *A. baumannii* was responsible for 1.6 percent of ICU-acquired UTIs (Gaynes, R. *et al.*,2005). Mediated innate immune response in the urinary tract includes various resident and recruited cells that express a wide range of pattern recognition receptors (PRRS)such as toll-like receptor 2 (TLR2), Toll-like receptor 4 (TLR4).Uropathogenic microorganisms can occasionally enter the human urinary tract successfully despite the presence of robust barriers created by urothelial cells. The innate immune responses will be triggered by the entry of uropathogenic microorganisms into the urinary tract through expression of the associated TLRs in the urothelial cells of the bladder (cystitis) and kidneys (nephritis). Therefore, the release of chemokines, interferons, interleukins, antimicrobial agents, and proinflammatory cytokines are just a few of the cascading reactions triggered by the expression of related TLRs (Behzadi & Sirmatel, 2009).

Host Innate Immune Responses to Acinetobacter baumannii

Generally speaking, A. baumannii poses no threat to healthy people, but it can seriously infect patients who are immunosuppressed or in intensive care. In experiments, the host immune system identified A. baumannii very quickly after infection (within hours), which significantly influenced the outcome of the infection for the host (Harris et al., 2013; Bruhn et al., 2015). By interacting with the host pattern recognition receptors (PRRs), A. baumannii infection activates the host's innate immune responses. This results in the production of proinflammatory

doi.org/10.31838/hiv22.02.639 Received: 06.04.22, Revised:18.06.22, Accepted: 12.08.22

cytokines and chemokines as well as the local recruitment of macrophages and neutrophils (Garcia-Patino et al., 2017; Wong et al., 2017). When dealing with highly virulent strains of A. baumannii, those reactions might not always be sufficient to control the infection. Additionally, in order to successfully infect the host, A. baumannii may manipulate the host's immune system.

Receptors for Pathogen Pattern Recognition

The majority of innate immune cells express proteins called pattern recognition receptors (PRR). By sensing and identifying both damage-associated molecular patterns (DAMPs), which are composed of host cell components released during infection-induced cell damage or death, and pathogen-associated molecular pattern (PAMP) components, such as lipopolysaccharide (LPS), OMVs, and capsule, PRRs play a crucial role in the host innate immune system. Specific PAMPs of A. baumannii are recognized by host innate immune cells via membrane-bound PRRs like Toll-like receptors (TLRs) and cytoplasmic PRRs like nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) (Chen, W.,2020).

Toll-Like Receptors

One of the most important membranes bound PPRs is TLR and are a key component in launching an innate immune response to the microbial challenge, TLR4 and TLR2 are recognized as specific receptors for components of Gram-negative and Gram-positive bacteria, respectively (Nanda, J., et al.,2006). As a Gram-negative bacterium, A. baumannii interacts with host cells mainly by engaging TLR2 and TLR4, with CD14 as a co-receptor (Garcia-Patino et al., 2017; Wong et al., 2017; Morris et al., 2019).

TLR4

Tlr4 is the key receptor for host recognition of Gram-negative bacteria, including A. baumannii. TLR4 interacts with the lipid A moiety of LPS, the major cell wall component of Gram-negative bacteria. the studies have confirmed the important role of TLR4 in the host innate defense against respiratory and systemic A. baumannii infection (Knapp et al., 2006; Lin et al., 2012; Wang et al., 2016; Peng et al., 2018; Zhang et al., 2018). Virulent A. baumannii strains shed more LPS during bacterial replication than less virulent strains, which resulted in enhanced TLR4 activation and hyperinflammatory response (Lin et al., 2012).

TLR2

Tlr2 senses bacterial lipoproteins, peptidoglycan, and mycobacterial lipoarabinomannan. (Kim et al., 201) .

Material and method

fourteen isolation of A. baumannii were used in this study

Isolation and identification of Acinetobacter baumannii

isolates urine was identification by conventional methods for identifying A*cinetobacterbaumannii* (i.e. Iraqibacter). The non-lactose and partially lactose fermenting colonies on MacConkey agar and non-hemolytic vague creamy colonies on blood agar . These colonies were then cultured on CHROM agar selective medium for *A.baumannii* . Finally, the isolates were definitely identified by using the VITEK-2 system. which is also used for detection of antibiotic sensitivity .Urine sample were stored at freezer (-12 to-16c) to be used for assessment of TLR2 and TLR4 by using the ELISA Technique (Sandwich Method) later

Immunology method

Measurement Human Toll-Like Receptor 2 (TLR2) and Toll like receptor 4 (TLR4) in urine by the quantitative sandwich enzyme immunoassay technique Antibody specific for TLR2 &TLR4 ELISA Kit using ELISA. isolation of A.baumannii (i.e.14) Compared with control (non-infection) (i.e.12) in both (TLR2&TLR4) and this Compared with anther bacterial infection.

Results and Discussion

Assessment of TLR2 & TIR4 in urine

It was confirmed by the discovery of TLRs as a component of innate immunity and their function in host defense that innate immunity operates through more complex mechanisms than previously thought. TLRs are widely expressed in both immune and non-immune cells and are regarded as the nerve center of the immune response (K. Takeda, T. et al.,2003; Y. Tabel *et al* 2007) .

Recent research on TLR polymorphism in human patients provide evidence that TLRs play a significant role in the host's resistance to A. baumannii infection. (He et al., 2016; Chatzi et al., 2018).

TLRs are primarily expressed on cells with a high chance of coming into contact with microbes , and each TLR type recognizes a distinct set of PAMPs. A. baumannii, a Gram-negative bacterium , primarily engages TLR2 and TLR4 in its interactions with host cells, with CD14 serving as a co-receptor (Garcia-Patino et al., 2017; Wong et al., 2017; Morris et al., 2019).

TLR2 and TLR4 appear to have a connection to UTI. Lipopeptides,

peptidoglycans, and lipote ichoic acid in Gram-positive bacteria, as well as lipoproteins in mycoplasmas and mycobacteria, are just a few examples of the various microbial components that TLR2 recognizes and interacts with. The lipopolysaccharide (LPS) signaling receptor, on the other hand, is TLR4 .

it has been determined that certain TLRs play a crucial role in the host's innate immunity against A. baumannii infection:

Assessment of TLR2 in urine

Detection of TLR2 in urine by ELISA kit showed that there is a highly significant increase of mean value of *A. baumannii* group in compare with that of control group(Table1)

Table (1): comparison of TLR2 mean values between *A.baumannii* infected patients and control group in current study.

Study groups	Mean ± SD	SEM	T- test	DF	95% CI	P- value
A. baumannii infected cases	2.534 ± 1.988	0.531	2.8927	24	0.47817 to 2.85973	0.008*
Control (non- infected) cases	0.865 ± 0.139	0.04				
** Highly Significant*						

The mean value of TLR2 for other gram-negative bacilli group differs non-significantly in compare with that of control group (table1).
TLR 2 detects mycobacterial lipoarabinomannan, peptidoglycan, and bacterial lipoproteins TLR2's function in the host's innate immune response to *A. baumannii* is less clear than TLR4's. (Knapp *et al* 2018).

In current results the TLR2 was measured for all (14) isolates of *A. baumannii* and it was compared with control (uninfected) samples (i.e. 12) sample.
The results showed that the percentage of TLR2 in UTIs caused by *A.baumanni* was highly significant .($p < 0.001$) , parable due to the effect of capsule in all of isolates in current study .

in Table (2): comparison of TLR2 mean values between other gram negative infected patients and control group current study.

Study groups	Mean ± SD	SEM	T- test	DF	95% CI	P- value
Other gram-negative infected cases	1.637 ± 1.519	0.406	1.7483	24	-0.13932 to 1.68294	0.0932
Control (non- infected) cases	0.86533 ± 0.13961	0.04				

On comparison between other gram negative bacterial infection of urine sample and control (non-infection), the TLR2 was measured for (14) isolates of *(i.e. E.coli , Enterococcus faecalis and Klibsela)* and it was compared with control (uninfected) samples was (i.e. 12) sample
Although that the mean rate of TLR in UTIs caused by Other gram negative infection was slightly elevated, it was statistically non-significant .

This ensures of TLR2 in gram-negative bacteria goes with (AL- Swadi,2020), also coincident with the result of (Karanareu et al. 2016)

Assessment of TLR4 in urine

Detection of TLR4in urine by ELISA kit showed that there is a non- significant increase of mean value in compare with that of control group. (Table3)

Table (3): comparison of TLR4 mean values between *A. baumannii* infected patients and control group in current study.

Study groups	Mean ± SD	SEM	T- test	DF	95% CI	P- value
A. baumannii infected cases	1.141 ± 2.571	0.687	0.7414	24	-1.02334 to 2.17077	0.4656
Control (non- infected) cases	0.567 ± 0.790	0.228				

The primary receptor for Gram-negative bacteria, such as *A. baumannii*, to recognize their hosts is Tlr4. The main part of Gram-negative bacteria's cell wall, the lipid A moiety of LPS, and TLR4 interact (Kim *et al.*, 2013).
In this study, the TLR4 has measured for all (i.e. 14) isolates of A. baumannii and it was compared with control (uninfected) samples (i.e. 12) sample.

The mean value was increased in the study group non-significantly, probably due to the effect of the capsule in all of the current isolates, which sequestered the immunology reaction with A.baumannii.
The mean value of TLR4 for other gram-negative bacilli group compared with that of control group as the showed in (table 4).

Table (4): comparison of TLR4 mean values between other gram-negative infected patients and control group in current study.

Study groups	Mean ± SD	SEM	T- test	DF	95% CI	P- value
Other gram negative infectedcases	0.55800 ± 0.57844	0.154	0.0664	24	-0.57235 to 0.53668	0.9476
Control (non- infected) cases	0.57583 ± 0.78883	0.227				

On comparison between other gram negative bacterial infection of urine sample and control (non-infection), the TLR4 was measured for (i.e. 14) sample of (E. coli , Enterococcus faecalis and Klibsela) and it was compared with control (uninfected) samples was (i.e. 12) sample.
There is no significant difference between mean TLR4 Other gram-negative infection and control

group although mean the level of TLR4 was higher.

References

- ALswadi ,A .J . (2020) . Study of Some Innate Immune Parameters Associated with Urinary Tract Infections (UTIs). College of Science, University of Basrah .

Behzadi, E., Behzadi, P., & Sirmatel, F. (2009). Identification of 30 Da heat shock protein gene in Trichophyton rubrum. *Mycoses*, *52*(3), 234-238.

- Bruhn, K. W., Pantapalangkoor, P., Nielsen, T., Tan, B., Junus, J., Hujer, K. M., et al. (2015). Host fate is rapidly determined by innate effector-microbial interactions during Acinetobacter baumannii bacteremia. J. Infect. Dis. 211, 1296–1305. doi: 10.1093/infdis/jiu593
- Chatzi, M., Papanikolaou, J., Makris, D., Papathanasiou, I., Tsezou, A., Karvouniaris, M., et al. (2018). Toll-like receptor 2, 4 and 9 polymorphisms and their association with ICU-acquired infections in Central Greece. J. Crit. Care 47, 1–8. doi: 10.1016/j.jcrc.2018.05.012.
- Chen, W. (2020). Host innate immune responses to Acinetobacter baumannii infection. *Frontiers in Cellular and Infection Microbiology*, *10*, 486.
- Chen, X., Meng, X., Gao, Q., Zhang, G., Gu, H., and Guo, X. (2017). Meropenem selection induced overproduction of the intrinsic carbapenemase as well as phenotype divergence in Acinetobacter baumannii. Int. J. Antimicrob. Agents 50, 419–426. doi: 10.1016/j.ijantimicag.2017.04.015
- Dijkshoorn, L.; Nemec, A.; Seifert, H. An increasing threat in hospitals: Multidrug-resistant Acinetobacter baumannii. Nat. Rev. Microbiol. 2007, 5, 939–951. [CrossRef] [PubMed]
- Garcia-Patino, M. G., Garcia-Contreras, R., and Licona-Limon, P. (2017). The immune response against Acinetobacter baumannii, an emerging pathogen in nosocomial infections. Front. Immunol. 8:441. doi: 10.3389/fimmu.2017.00441.
- Garcia-Patino, M. G., Garcia-Contreras, R., and Licona-Limon, P. (2017). The immune response against Acinetobacter baumannii, an emerging pathogen in nosocomial infections. Front. Immunol. 8:441. doi: 10.3389/fimmu.2017.00441
- Harris, G., Kuo Lee, R., Lam, C. K., Kanzaki, G., Patel, G. B., Xu, H. H., et al. (2013). A mouse model of Acinetobacter baumannii-associated pneumonia using a clinically isolated hypervirulent strain. Antimicrob. Agents Chemother. 57, 3601–3613. doi: 10.1128/AAC.00944-13
- He, L., Lin, M., Fan, W., Liu, Y., Suo, J., Xing, Y., et al. (2016). Association of single nucleotide polymorphisms in toll-like receptors with Acinetobacter baumanii infection in a Chinese population. Iran. J. Public Health 45, 20–26
- K. Takeda, T. Kaisho, and S. Akira, "Toll-like receptors," Annual Review of Immunology, vol. 21, pp. 335–376, 2003.
- Karananou, Panagiota et al. 2016. "Altered Expression of TLR2 and TLR4 on Peripheral CD14." BioMed research international 2016.
- Kim, C. H., Kim, D. J., Lee, S. J., Jeong, Y. J., Kang, M. J., Lee, J. Y., et al. (2014). Tolllike receptor 2 promotes bacterial clearance during the initial stage of pulmonary infection with Acinetobacter baumannii. Mol. Med. Rep. 9, 1410–1414. doi: 10.3892/mmr.2014.1966
- Kim, C. H., Kim, D. J., Lee, S. J., Jeong, Y. J., Kang, M. J., Lee, J. Y., et al. (2014). Tolllike receptor 2 promotes bacterial clearance during the initial stage of pulmonary infection with Acinetobacter baumannii. Mol. Med. Rep. 9, 1410–1414. doi: 10.3892/mmr.2014.1966
- Knapp, S., Wieland, C. W., Florquin, S., Pantophlet, R., Dijkshoorn, L., Tshimbalanga, N., et al. (2006). Differential roles of CD14 and toll-like receptors 4 and 2 in murine Acinetobacter pneumonia. Am. J. Respir. Crit. Care Med. 173, 122–129. doi: 10.1164/rccm.200505-730OC
- Lin, L., Tan, B., Pantapalangkoor, P., Ho, T., Baquir, B., Tomaras, A., et al. (2012). Inhibition of LpxC protects mice from resistant Acinetobacter baumannii by modulating inflammation and enhancing phagocytosis. mBio 3:e00312-12. doi: 10.1128/mBio.00312-12
- Morris, F. C., Dexter, C., Kostoulias, X., Uddin, M. I., and Peleg, A. Y. (2019). The mechanisms of disease caused by Acinetobacter baumannii. Front. Microbiol. 10:1601. doi: 10.3389/fmicb.2019.01601
- Moulana, A., Anderson, R. E., Fortunato, C. S., & Huber, J. A. (2020). Selection is a significant driver of gene gain and loss in the pangenome of the bacterial genus Sulfurovum in geographically distinct deep-sea hydrothermal vents. *Msystems*, *5*(2), e00673-19.
- Peng, C., Han, J., Ye, X., and Zhang, X. (2018). IL-33 treatment attenuates the systemic inflammation reaction in Acinetobacter baumannii pneumonia by suppressing TLR4/NF-kappaB signaling. Inflammation 41, 870–877. doi: 10.1007/s10753-018-0741-7
- Suri, S. S., Janardhan, K. S., Parbhakar, O., Caldwell, S., Appleyard, G., & Singh, B. (2006). Expression of toll-like receptor 4 and 2 in horse lungs. *Veterinary research*, *37*(4), 541-551.
- Weinstein, R. A., Gaynes, R., Edwards, J. R., & National Nosocomial Infections Surveillance System. (2005). Overview of nosocomial infections caused by gram-negative bacilli. *Clinical infectious diseases*, *41*(6), 848-854..
- Wong, D., Nielsen, T. B., Bonomo, R. A., Pantapalangkoor, P., Luna, B., and Spellberg, B. (2017). Clinical and pathophysiological overview of Acinetobacter infections: a century of challenges. Clin. Microbiol. Rev. 30, 409–447. doi: 1
- Wong, D., Nielsen, T. B., Bonomo, R. A., Pantapalangkoor, P., Luna, B., and Spellberg, B. (2017). Clinical and pathophysiological overview of Acinetobacter infections: a century of challenges.

Clin. Microbiol. Rev. 30, 409–447. doi: 10.1128/CMR.00058-16.

- Yadav, G., Kumar, A., Luthra, S., Garza-Reyes, J. A., Kumar, V., & Batista, L. (2020). A framework to achieve sustainability in manufacturing organisations of developing economies using industry 4.0 technologies' enablers. *Computers in industry*, *122*, 103280.
- Yakkala, H., Samantarrai, D., Gribskov, M., & Siddavattam, D. (2019). Comparative genome analysis reveals niche-specific genome expansion in Acinetobacter baumannii strains. *PLoS One*, *14*(6), e0218204
- Zhang, X., Zhou, X., Lin, M., & Sun, J. (2018). Shufflenet: An extremely efficient convolutional neural network for mobile devices. In *Proceedings of the IEEE conference on computer vision and pattern recognition* (pp. 6848-6856).