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Pathological Markers for Brucellosis, a Bacterial Challenge: A Review

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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Review Article

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ABSTRACT

The Brucellosis, caused by *Brucellae* species is an infectious disease infecting animals and human population. Unlike other bacteria *Brucella* does not secrete toxin but is pathogenic because of prolonged stay and replication in host. *B. melitensis* is most virulent among six classified species. In this paper the proteins responsible for virulence, survival and replication like two-component regulatory system BvrR/BvrS (TCS BvrRS) , type IV secretary system and effectors molecules have been discussed from different perspective to target and inhibit *Brucellae* inter cellular growth. The various defined effectors may be possible target for inhibitors for future. Genetically the *Brucella* chromosome II having pathogenic *virB* operon maybe engineered for regulation and expression .The available vaccines and inhibitors against bacterial infections are highlighted with side effects . There is urgent need to redesign fool proof vaccines and drugs to protect animals and human population before it challenges to be another pandemic like cholera or plague.

Keywords: T4SS: type IV secretary system; BCV; Brucellae containing vacuole.

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1. INTRODUCTION

Brucellosis is a zoonotic bacterial infectious disease of animals like goat, sheep, cattle, swine or dogs. Human population receives this infection either by direct touch of infected animals or by eating contaminated dairy food products or through air polluting agents [1]. The disease is prevalent in Southern and eastern Europe, United states, Asia, Africa, Caribbean and The Middle East. Total 5,000,000 to 12,500,000 confirmed cases are reported every year globally [2]. Recently 3245 confirmed cases of the Brucellosis or Malta fever or Mediterranean fever are reported in north east china's Gansu province.[3} Brucellosis is caused by gram negative brucellae bacteria named after David Bruce (1855–1931). The Brucellae bacteria size ranges from 0.5 to 0.7 by 0.6 to 1.5 µm and is non encapsulated, nonmotile, facultatively intracellular *coccobacilli* [4]. These bacteria do not multiply in the environment but survive in hosts and are transmitted directly from one host to another hosts. Some of the Brucellae species more virulent are classified as category B pathogens and identified as compliant for BioTerrorism by US centre for disease control (CDC) [5]. Human population has suffered because of cholera or *plague* outbreak and epidemic in the past and recent report from china about brucellosis needs attention for future. This review summarize the present status of understanding about *Brucellae* pathogenic factors, mode of transmission ,targets in host cell ,mechanism of their survival ,proliferation and pathogenesis Recent reports of brucellosis at large in china has alarmed us to understand *infectious disease* from larger perspective.

2. CLASSIFICATION, ISOLATION AND IDENTIFICATION

Genus: Brucellae Family: Brucellaceae (family III)

Class: Alphaproteobacteria. Phylum: Proteobacteria

Order: Rhizobiales

(Axel Thomson, 1934& a, Bergy's an [6,7,8]

There are total 10 species of Brucellae, isolated on the basis of host specificity and according to pathological differences, preference for hosts and phenotypic characteristics, Brucellae genus are classified into six classic species: *B. abortus, B. melitensis, B. suis, B. canis, B. ovis,* and *B. neotomae* by bacterial taxonomy International Committee in 2013.

For isolation of bacteria, Farrell selective media [9] containing Brucella medium base (OXOID) supplemented with 5% fetal bovine serum (Invitrogen) and antibiotics like vancomycin, colistin, nystatin, nitrofurantoin, and Amphotericin B to avoid gram positive bacteria contamination [10,11]. The other media is modified Thayer-Martin medium (mTM)V specific for B. suis because of Farrell Media inhibition for this strain growth [12]. Brucella colonies appear on solid media in 2-3 days. A newer selective medium (CITA) for all Brucellae species is also used for assessing colony morphology under stereoscopic magnification [13]. The use of CITA with FM or mTMA in combination give better results *B. melitensis* and *B. suis* or *B. ovis* isolation and identification [14].

The Brucellae colonies found in culture may be either smooth as found in *B. melitensis, B. suis* and *B. abortus* because of full LPS molecule (S-LPS) molecule anchored to outer membrane (OM) (O antigen) or rough like *B. ovis* and *B. canis* [15,16]. The rough or mucoid colonies do not have O-antigen. The O- antigen is virulence factor [17]. The O antigen containing *B. melitensis, B. suis* and *B. abortus* are very pathogenic to human population [18].

The Brucella bacteria can be differentiated by biochemical, serological and molecular procedures along with colony morphology and growth characteristics etc. In recent years, polymerase chain reaction (PCR) test is being used for identification. Cell wall antigens and phage sensitivity assay is also done for identification [19]. Hemolysis, indole, litmus and Hydrogen peroxide tests for bacteria usually are negative while oxidase enzyme activity, nitrate reduction and Urease enzyme tests were found positive. The Fermentation of Erythritol, L glucose, L alanine, asparagine and oxidation of glutamine p-Nitraphenyl phosphatase and p-nitro phenyl alpha -d-galacto-pyranosidase enzyme test are found positive only in *B. melitensis* [20,21].

3. DIAGNOSIS FOR BRUCELLOSIS

In animals (primary host for brucellae bacteria), the bacterial infection diagnosis is done either by direct microbial culture sensitivity or by polymerase chain reaction (PCR) on 16srRNA basis. The other indirect methods are used like enzyme linked immunoassay (ELISA) in milk or

blood (*'in vitro*' or *'in vivo*' [22]. In human population, the brucellosis is diagnosed by bacteria presence in blood or bone marrow by using antibodies raised against Brucella bacteria. Brucellosis long term infection might cause change in bone and joints so X rays is done to confirm these changes. Computerized tomography (CT) scan or magnetic resonance imaging (MRI) may identify inflammation or lesion in the tissues as confirmatory diagnosis .The Clinical analysis of cerebrospinal fluid (CFS) from brain and spinal cord can be done to know infection causing meningitis and encephalitis. The brucella bacteria slow and long term infection sometimes may cause heart damage, so Echocardiography (based on sound waves to create images) is also used for heart [23]. MALDI-TOF mass spectrometry is another method for Brucella species identification [24].

4. *Brucellae* Genome

The *Brucella* genome has two circular chromosomes. The size of chromosome is 2.2Mb for chromosome I and 1.1 Mb for chromosome II. While the first chromosome has genes for metabolic pathways enzymes, the second smaller chromosome has several genes responsible for pathogenicity ,and it also includes Type IV secretion system *virB* operon of 12 genes from *virB1–12* [25,26]. The genomes of *Brucellae* species sequencing has confirmed 3,200 to 3,500 open reading frames. (*Brucellae* genomes in PATRIC & gold data base) *Brucellae* species does not harbor plasmid but can be manipulated in laboratory to accept plasmid like IncP, IncW and R751. (IncP) [27,28]. If *vir* B operon genes of chromosome II regulation and expression can be regulated through genetically engineering, the infection can be prevented to larger extent.

5. *Brucellae* PATHOGENICITY

Brucellae primarily infects animals as dogs, sheep cattle, swine, camel or desert wood rats and *bacteria* prevalence vary from animal to animal as *B. melitensis* more in goats and sheep , *B. abortus* in cattle, *B. canis* in dogs, *B. suis* in pigs and *B. ovis* in sheep. The *Brucella melitensis* is the most pathogenic species among all. The Human populations get disease Brucellosis directly or indirectly from animals [30]. In animals the bacteria enters through mucous membranes of the reproductive, respiratory, digestive tracts or conjunctiva but human the entry usually occurs through respiratory and digestive tracts [30]. After entering in to the blood, Brucellae attacks phagocytic immune cells and non phagocytic epithelial cells. The phagocytic macrophage is site for bacterial survival and multiplication but bacterial presence in non phagocytic epithelial cell affects reproductive organs and may cause abortion [31,32,33]. Unlike many other bacteria, the *Brucellae* does not secrete toxins and pathogenicity is conferred due to its extended stay and efficient replication in the macrophage [34]. The some of most pathogenic factors of *Brucellae* are described here.

5.1 O Polysaccharide or O Antigen, Lipopolysaccharide (LPS)

The O antigen antibodies provide only partial resistance but does not protect host during chronic brucellosis. The cell mediated immunity play an important role in bacteria survival because *Brucellae* modulate intracellular pathway for survival [35]. They can stay in antigen presentation cell. They also influence phagocytosis, inflammatory cytokinin secretion and intracellular membrane fusion [36].

5.2 Two Component Regulatory System

The other factors contributing to virulence is a two-component regulatory system BvrR/BvrS (TCS BvrRS), which has cell membrane histidine kinase sensor(BvrS) along with cytoplasmic regulator (BvrR). The BvrR/BvrS maintain outer membrane homeostasis (OM), periplasmic proteins (Omp) lipopolysaccharide (LPS) structure and link to O antigen [27,38,39]. The role of Blue light in enhancing macrophage infection by *B. abortus* has confirmed role of flavin-containing histidine kinase as a photoreceptor regulating virulence factor, which was reported earlier also [40]. The histidine sensor kinases work through phosphorylating Phy R protein ,a general stress regulator for activating transcription of required gene [41].

5.3 Type IV Secretion System (T4SS)

Type IV secretion system (T4SS), large proteins complex form channel for protein or DNA translocation and also for virulent factors, which help bacteria to survive by manipulation host immune system [24,42]. The bacteria interact with various regulatory molecules involved in intracellular trafficking like Rab 5, Rab7 (small GTPases molecule), Rb5 effector molecule, early endosome antigen (EEA) and Brucella containing vacuole after entering in to

macrophage [43,44,45,46]. which is followed by phagosomal degradation of 90 percent Brucella cells by host hydrolyzing enzymes. Only a very small percentage (10 percent) of Brucella survives this degradation event [47]. This might be possible because of vir B operon activation for type IV secretary system (T4SS), which release effector molecules in host cytosol [48]. The intracellular pathway for Brucella starts with its entry into macrophage. In macrophage Brucellae is fused with early and late endosome to form Brucella containing Vacuole (BCV), which further interact and fuse with endoplasmic reticulum to form mature replicative phagosomes, where it for survive and proliferate .The T4SS play an important role in endoplasmic reticulum derived replicative mature Brucella vacuoles formation. The formation of autophagic Brucella vesicle (aBCV) is necessary for cell to cell spreading and signature for Bacteria intracellular cycle completion [48]. T4SS is a large macromolecular complex of 12 subunits and containing five different parts as i. Core/outer membrane complex including VirB7, VirB9, and VirB, ii. Linking stalks of VirB5 or VirB10, iii Inner membrane complex of VirB3, VirB4, VirB6, iv. N-terminus containing VirB10 and ATPases units VirB4 & VirB11 **[49,**50]. All of the subunits except B1, B7 and B12 are pathogenic in nature [51,52].

The Vib R and Bab R are the regulator for number of genes ,which code proteins involved in stress response, metabolism, and virulence [53]. These proteins participate in bacteria morphology and physiological adaptation which is required for intracellular replication at various level like Histidine utilizing protein HutC [54], the global regulator ,BvrR/BvrS two component system, [55,56]. RelA/SpoT homolog (Rsh), regulator for alarmone ppGpp [57], Transcription factor sodium deoxycholate-responsive activator (MdrA) [53] and Integrity host factor (IHF) .A bi-functional Rsh enzyme regulate alarmone (p)ppGpp synthesis and breakdown under stringent nutritional stress [58]. The various two component systems in different bacteria are targeted by various antibiotics but these antibiotics have shown many effects 59].

T4SS functions in the translocation of effector proteins across bacterial and host membranes, These effector molecules enter into the host cytosol, mediate bacterial survival and control host immune system for infectious survival. That is the reason to target T4SS for inhibition [60,61]. T4SS is essentially required for prolonged stay in host cell [62,63]. The subunits Vir B 2– 6 and Vir B 8-11, participate in replication of bacteria in host cells. T4SS inhibit host immune system as confirmed from T4SS deficient mutants but mechanism is unclear [64]. T4SS deficient bacteria are killed very soon in host.

6. TYPE IV SECRETARY SYSTEM (T4SS) KNOWN EFFECTORS MOLECULES

Any protein, secreted in to host through T4SS is called effector molecules. Globally by using various filters and criteria “in silico” screening [65,66], total 15 effector molecules are identified, which participate and regulate bacterial survival either through debarring lysosomal markers, accepting endoplasmic reticulum markers, interact with secretary pathways, and modulate host immune system to adopt to cell environment. Some of the effector molecules like BtpA (Btp1/TcpB) and BtpB act as mediator for the signaling cascades of innate immune cells recognition because of conserved Toll/Il-1 Receptor (TIR) domain [67]. The (Btp) A and BtpB contribute to virulence by inhibiting dendritic cells maturation [68]. The Btp A, BtpB and F trio proteins reduces cellular secretion during infection [69]. The BtpA is negative regulator of NF-kB., while BtpB has shown opposite effect on NF-kB. The SepA protein is secreted is through T4SS in very early stages of infection into periplasm stating protein role in bacterial early survival [70]. BspC is found to induce endoplasmic reticulum stress without any role in secretary pathway [68]. BspB participates in rBCV Biogenesis and Bacterial Replication [71]. BPE005, BPE275, BPE123 & BPE043 commonly present in other class of bacteria also [65]. BPE123 is positioned on BCV surface during infection.The effector proteins like BspC, BspE, BPE123, BPE005, BPE275, and BPE 043 functions are not very clear. The Vib R and Bab R, regulator for number of genes and/or proteins involved in stress response, metabolism, and virulence. These effectors molecules synthesis or functions can be targeted for better future medicines.

7. CONCLUSION

As stated earlier, The Brucellosis may have short and long term severe consequences in animals and human population [4]. In this present paper Brucella bacteria genome, structure, intracellular cycle in macrophage /host cell, various proteins role in proliferation, virulent genes and factors are focused like on O antigen linked to lipopolysaccharide, two-component regulatory

system BvrR/BvrS (TCS BvrRS) for photoreceptor kinases and Type IV secretary system with its various effecters molecules for infection and intracellular survival pathway has been summarized. Till now 39 strains of Brucella has been sequences (http://www.broadinstitute.org/annotation/genome/brucella_gro up/GenomeStats.html). The circular chromosome II have virB operon, responsible for virulence. The gene can be focus to manipulate through genetic engineering to for future better vaccines for animal and human both. Vir B2-6, vir B 8-11 genes are essential for prolonged stay of bacteria in host which provide clear site to target . Understanding of brucellae survival and proliferation mechanism provide great understanding about bacterial infection, survival and proliferation. The present live vaccines *B. melitensis* Rev. 1 and *B. abortus* S19 are not found perfect because of causing sometimes abortion in target and non-target animals both. These vaccines are metabolized in short time by immunized animals. The S19 and RB51 are approved *B. abortus* vaccine strains for bovine brucellosis across the world. One of the vaccine RB51 is also resistant to rifampicin drug, which is used in Brucellosis treatment in human. The efforts are on to design DNA vaccine, recombinant vaccines, *B. abortus* recombinant mutants, Subunit vaccines, Vector vaccines without side effects [71]. 'In silico' analysis might be useful to design better vaccines. China recent report of brucellosis suggests requirement for better directed bacterial mutants vaccines to protect our live stocks of animals and human population before it challenge us.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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