

Original Research Article

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The Pathogenicity of Cold and Heat Shocked *Pasteurella multocida* for Rabbit

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ABSTRACT

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The purpose of this study was to study the pathogenicity of cold and heat-shocked bacteria using *Pasteurella multocida* and rabbits as a model. *Pasteurella multocida* is an enigmatic pathogen. The capsule and lipopolysaccharide (LPS) of *Pasteurella multocida* constitute the major components of the bacterial cell surface; they play key roles in a range of interactions between the bacteria and the hosts they colonize or infect. *Pasteurella multocida* was inoculated into nutrient broth tubes and incubated at 37°C for 24 hours. The culture was shocked at -15°C for 30 mins and ten serial dilutions were done. The dilutions which did not show visible colonies on the recovery media were centrifuged and inoculated intra peritoneally in four rabbits. The rabbits postmortemed and cultures were made from heart blood by puncturing with a sterile Pasteur pipette. The same test was repeated using treated culture at 50°C and 40°C for 10 mins such heat-treated cells did not lead to death of the inoculated rabbits.

Introduction

Many bacterial species have been found to exist in a viable but non-culturable (VBNC) state since the discovery in 1982. VBNC are characterized but loss of culturability in routine agar, which impairs their detection by conventional plate count techniques. This leads to an underestimation of total viable cells in environmental or clinical samples and thus poses a risk of public health (Laam, *et al.*, 2014). Cold shock as defined by El

Sanousi (1975) comprises the injury, death or both which are caused by sudden chilling of the microorganisms.

The cold shock phenomenon occurs when growing bacteria are exposed to sudden temperature drop of at least 10°C leading to cold shock in susceptible microorganisms (Jones, *et al.*, 1996). Collee, *et al.*, (1961) termed the phenomenon as "phoenix" phenomenon due to disappearance of colonies at recovery medium and attributed this to the

temperature of inoculums, a finding which was disqualified by El Sanousi (1975) who explained the phenomenon to be a mere cold shock one. This is due to cells being shocked when transferred from high temperature to low ones. Low temperature can influence the response of a microorganism either directly or indirectly.

Direct effects include decreased growth rate, enzyme activities, alteration of cell composition and differential nutritional requirements. Indirect effects are usually observed on the solubility of solute molecules, diffusion of nutrients, osmotic effects on membranes and cell density (Shekhar, 2011).

Heat treatment is one of the most widely used methods to control the rates of bacterial growth and death and is considered to be one of the most effective food processing technologies for eradicating food borne pathogens (Huang 2004), (Enache, *et al.*, 2006). Definitions of pathogenicity: A pathogen or pathogenic microorganism is usually defined as a biological agent that can cause damage to its host during, or as a consequence of, the host- microorganism interaction. Damage may be inflicted directly by the microorganism (e.g. by toxins or other so-called virulence factors) or indirectly through the activity of the host immune responses (Casadevall and Pirofski, 1999).

The ability of the pathogen to infect is called its pathogenicity. Microorganisms express their pathogenicity by means of their virulence, a term that refers to the relative, quantitative degree of pathogenicity (Casadevall and Pirofski, 1999).

Moreover, pathogens are distinguished by their virulence from nonpathogen, which are considered to be a virulent (Casadevall and Pirofski, 1999).

Materials and Methods

The ingredients that were used included of blood agar as an enrichment medium, nutrient broth and normal saline. *Pasteurella multocida* was inoculated into nutrient broth tubes and incubated at 37°C for 24 hours. Ten serial dilutions in duplicate were performed. One of the duplicate was put in 37°C; the other was shocked at -15°C for 30min.

After incubation at 37°C for an overnight, the dilutions which did not show visible colonies on recovery media were centrifuged and inoculated i/p in four rabbits. They were postmortemed and cultures were made from heart blood by puncturing with a sterile Pasteur pipette. The same test was repeated with bacteria heated at 50°C and 40°C for 10mins.

Results and Discussion

Only cold-shocked cells showed their effect on the experimentally inoculated rabbits. Rabbits inoculated with VBNC bacteria died within 24 hours. They showed typical lesions of hemorrhagic septicaemia. *Pasteurella multocida* is an enigmatic pathogen. It is remarkable both for the number and range of specific disease syndromes with which it is associated, and the wide range of host species affected.

The pathogenic mechanisms involved in causing the different syndromes are, for the most part, poorly understood or completely unknown. The biochemical and serological properties of some organisms responsible for quite different syndromes appear to be similar.

Thus, the molecular basis for host predilection remains unknown. The recent development of genetic manipulation systems together with availability of multiple genome sequences

should help to explain the association of particular hosts as well as helping to elucidate pathogenic mechanisms (Wilkie. *et al.*, 2012). Cold-shocked *P.multocida* was able to resuscitate and cause death of inoculated rabbits. Such results are similar to those obtained by Nagla (2010) using *P.multocida*, *E. coli*, *Salmonella* spp and *Staphylococcus aureus* bacteria in that cold-shocked. *P.multocida* could retain its pathogenicity for rabbits.

Effective monitoring of microbial pathogen is essential for a successful preventive food safety and hygiene strategy. However, as most monitoring strategies are growth- based, these tests fail to detect pathogenic bacteria that have entered the viable but non-culturable (VBNC) state (Christian, *et al.*, 2018).

Such information necessitates the importance of studies concerning (VBNC) bacteria. The capsule and lipopolysaccharide (LPS) of *Pasteurella multocida* constitute the major components of the bacterial cell surface. As well as forming the basis for the most widely used classification systems, they play key roles in a range of interactions between the bacteria and the hosts they colonize or infect. Both polysaccharides are involved in the avoidance of host innate immune mechanisms, such as resistance to phagocytosis, complement-mediated killing and the bacterial activity of antimicrobial peptides; they are there for essential for virulence.

In addition, LPS is a major antigen in the stimulation of adaptive immune responses to infection (Arumugam. *et al.*, 2011).

It is concluded from this experiment that cold-shocked *Pasteurella multocida* seemed to regain all pathogenic factors when they were resuscitated in the inoculated rabbits.

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