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MOLECULAR AND EVOLUTIONARY INSIGHTS INTO *YERSINIA PESTIS*; HARBINGER OF PLAGUE

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ABSTRACT

Plague has been the scourge of mankind for millennia; yet it was not until the late 18th Century that its causative agent was identified. Prokaryotic *Y. pestis* is responsible for plague; bacilli are consumed through arthropod feeding on infected rodential reservoirs. Arthropod uptake is essential for transmission as the bacilli proliferate within their gut before being refluxed into new mammalian hosts. Genomic analysis has elucidated the mechanisms facilitating this cycle along with the means by which bacilli acquire their characteristic virulence. Increasing our understanding of the evolution of *Y. pestis* provides putative avenues for future research. Whilst plague is considered a disease of the past by many, it interaction with humanity continues across various geographic foci. The rise of antibiotic resistant bacteria threatens to bring this ancient foe once again to the fore through the acquisition of drug resistance. This review will detail notable advances of the past decade enabling the elusive possibility of a universal vaccine for the three manifestations of plague. Development of suitable vaccines before drug resistant strains emerge is paramount. Researchers are pitted in an on-going race against bacterial evolution.

Keywords: Evolution, Virulence, Plague, *Yersinia Pestis, Yersinia enterocolitica, Yersinia pseudotuberculosis*

INTRODUCTION

The Black Death is ubiquitous with the modern notion of plague; it decimated Europe in the 14th Century. Chronicles detail chaos, religious paranoia and xenophobia which flourished in its wake. The pestilence of 1348-1350 is thought to have eradicated 30%–60% of Europe's populace; a staggering achievement for any pathogen and one from which Europe would not recover for 150 years **(Haensch *et al*., 2010).**
The source of plague remained elusive until 1894, when Alexandre Yersin identified the bacilli responsible for plague; an achievement for which the genus was later named '*Yersinia'* in his honour. Yersin trained under Robert Koch, and successfully applied his postulates to study plague; finding the same bacilli present in both rat and human instances of the disease **(Rollins *et al*., 2003).**
This review sets out to deliniate the evolutionary divergance of *Y. Pestis* from its comparativley less pathogenic ancestors and designates the numerous instances of molecular loss, mutation and acquisition which heralded the widespread mortality synonomous with *Y. Pestis* infection.

GENUS CHARACTERISTICS

Yersiria bacilli are coccobacillus, gram negative facultative anaerobes of the *enterobacteriacea* kingdom. The genus encompasses 11 species; eight non-pathogenic and three pathogens: *Yersinia enterocolitica, Yersinia pseudotuberculosis* and *Yersinia pestis*. *Y. pestis* is the etiological agent of; bubonic, septicemic, and pneumonic plague, respectively increasing in severity **(Yang *et al*., 2012)**.

EPIDEMIOLOGY

Rodent populations provide the standard host reservoir for *Y. pestis*. Bacilli consumed with rodential blood, by arthropods facilitate necessary changes for their proliferation and transmission forming dense aggregates within the flea gut. Biofilm formation prevents digestion leading to aggressive feeding by their arthropod hosts; increasing *Y. pestis* transmission. Bacilli are refluxed through intradermal bites to their mammalian hosts from where they disseminate to lymph nodes **(Vadyvaloo *et al*., 2010)**.
India, China and North America maintain stable rodential epidemics; whilst transmission to humans remains sporadic. The World Health Organisation (WHO); monitors global outbreaks, dealing with <2,000 per annum largely from of rural areas **(WHO, 2013)**.

MANIFESTATION

Plague symptoms differ according to the particular affliction; Bubonic plague presents itself with similar symptoms to flu or pneumonia ranging from chest pain, fever, weakness and often buboes. Septicemic plague mirrors Bubonic however there is a tendancy for extremeties to turn black and become necrotic. Pneumonic plague stems from the inhalation of infectious droplets, hence its symptoms centre around the respiratory system and commonly lead to respiratory faliure **(Wong *et al*., 2009)**.

INFECTION

Y. pestis infections may be either enzootic or epizootic; enzootic infections occur within the natural arthropod feeding cycle: feeding, transmission and infection occur within a limited mammalian reservoir. Two distinct rodential populations maintain endemic infection; the first exhibiting resistance act as hosts whilst the second, susceptible population, are prone to high mortality. Loss of a healthy rodent population prompts arthropods to seek new hosts proximal to their existing reservoir. Transfer of *Y. pestis* outside of its normal feeding cycle results in epizootic infection. Subsequent infections result in mass mortality due to increased host susceptibility **(Bos *et al*., 2011; Williamson & Oyston., 2012a)**. *Y. pestis* infections amongst humans are characterised by sporadic epizootic outbreaks followed by its regression towards an enzootic infection cycle.
This paper will review a decade of research, advancing of our molecular understanding of the evolutionary mechanisms by which of *Y. pestis* gained its characteristic phenotype and how this may be applied to future work.

GENOMIC EVOLUTION

Prokaryote evolution is not linear like that of mammals it is rapid and in sometimes erratic. Gene acquisition, inactivation and eventual loss create a multitude of phylogenicaly differing strains. Their ability to procure virulence associated mobile genetic elements (VA-MGE) such as; plasmids and pathogenicity islands through horizontal gene transfer (HGT) facilitates such evolutionary advances **(Gogarten *et al*., 2002)**.
Pathogenic *Yersinia* exhibit highly maintained genomes associated with successful pathogens; only genes bestowing advantage to the organism are retained **(Ochman & Davalos., 2006; Thomson *et al*., 2006)**. Phylogenic variations between both species and strains, dictated by microbial lifestyles; such dissimilarities are visible between the genome characteristics of *Yersinia* species (Table. 1) **(Auerbach *et al*., 2007)**. Genomic diversity between the genus

accounts for differing epidemiology. *Y. pseudotuberculosis and Y. enterocolitica* are water-borne pathogens causing gastroenteritis whilst *Y. pestis* is transmitted by an arthropod vector and the etiological agent of plague **(Zhou & Yang., 2009a)**. *Y. pseudotuberculosis and Y. enterocolitica* rarely cause fatalities however *Y. pestis* is associated with mass mortality.

Evolving in response to environmental stresses and change in lifestyle; *Y. pestis* has undergone mass genomic change **(Thomson *et al*., 2006)**. *Y. enterocolitica* is considered the oldest *Yersinia* pathogen, exhibiting extensive genetic diversity *Y. pseudotuberculosis*; whilst *Y. pseudotuberculosis* exhibits >75% homology with *Y. pestis,* indicative of a recent divergence **(Zhou & Yang., 2009a)**.

Similarities in GC content (Figure 1.); between *Y. pseudotuberculosis* and *Y. pestis* is indicative of a recent divergence. Likewise diversity in GC content between *Y. enterocolitica* and *Y. pseudotuberculosis* indicates a greater period since the divergence of *Y. pseudotuberculosis* from its patriarch **(Fuchs *et al*., 2011; Hershberg & Petrov., 2010)**.

Homology diminishes between patriarchal and progeny species over time, consequential to genomic changes conferring phylogenic differences. The divergence of *Y. pestis* from patriarchal *Y. pseudotuberculosis* within the last 20,000 years displays the organism's rapid adaptation to a changing lifestyle **(Auerbach *et al*., 2007)**. Over 75% h*omogony between Y. pestis* and *Y. pseudotuberculosis* infers that small differences in genetic composition confer change in virulence **(Shen et al., 2010)**.

Y. pestis differs from its enteropathogenic forebears *in the frequency at which its genome is punctuated by pseudogenes (Table. 1).* Gene loss, despite retention of virulence is indicative of early stage genomic decay **(Monot *et al*., 2009)**. Following divergence from ancestral *Y. pseudotuberculosis* excess genes become inactivated but reside within the genome as pseudogenes until removal through genomic rearrangement. *Yersinia* adhesion pathogenicity island (YAPI) is a prime example of gene loss within *Y. pestis.* Possession of YAPI facilitates adhesion to host cells permitting enteric infection **(Zhou & Yang., 2009a).** The changing lifestyle of *Y. pestis* rendered YAPI obsolete thus; its eventual removal was inevitable due to changes in bacterial repertoire **(Radnedge *et al*., 2002)**.

Possession of over 140 chromosomal pseudogenes is indicative of a massive shift in lifestyle; adhering to Darwinian evolution, whereby *Y. pestis* is enhanced by genomic change **(Zhou & Yang., 2009a; Zinser *et al*., 2003)**. Negative selection refined *Y. pestis* through removal of less virulent or resilient strains **(Thomson *et al*., 2006).** Conversely; *Y. enterocolitica* and *Y. pseudotuberculosis* poses orthologous pseudogene numbers (Figure 1) indicating little change in lifestyles **(Ochman & Davalos., 2006)**.

Table 1 Genomic overview of *Yersinia* pathogens drawing from genomic studies

Genus Member	Chromosome & Size (Mb)	G+C (%)	Pseudo-genes	VA-MGE	VA-MGE Function	VA-MGE Size (Kb)
Φ	Single circular 4.64 Chromosome	43.92	67	pYV	T3SS and YOPs effectors	70.3
				HPI	yersiniabactin siderophore	103
				YAPI	pili encoding - enables adhesion to host cells	98
¥	Single circular 4.85 Chromosome	47.5	62	pYV	T3SS and YOPs effectors	70.3
				HPI	yersiniabactin siderophore	103
				YAPI	pili encoding - enables adhesion to host cells	98
ǂ	Single circular 4.62 Chromosome	47.7	>140	pYV	T3SS and YOPs effectors	70.3
				HPI	yersiniabactin siderophore	103
				pFRA	Enables biofilm formation	96.2
				pPLA	Plasminogen activator	9.6

Ledgend: *Yesinia spp*. are denoted by ¥, Φ and ǂ; *Y. pseudotuberculosis, Y. enterocolitica* and *Y. pestis* respectively

MOLECULAR BIOLOGY

Y. pestis was classically subdivided into three biovars; *Antiqua*, *Mediaevalis* and *Orientalis,* corresponding to historical pandemics **(Auerbach *et al*., 2007).** Classification was dependent upon reduction of nitrate and fermentation of arabinose & glycerol **(Yang *et al*., 2012). Zhou *et al* (2009)** proposed a fourth biovr; *Microtus;* exhibiting high virulence to small mammals yet none in larger species. DNA microarray-based comparative genomic hybridization identified mass gene loss; a form of reductive evolution through which genes enabling zoonotic transmission were lost, limiting *Microtus* to small niches within isolated foci **(Zhou *et al*., 2004).** Since this discovery many including **(Auerbach *et al*., 2007)** have questioned the suitability of the biovar system.

Bos *et al*., (2012) published the genome of medieval *Y. pestis* (1348-1350), elucidating extant strains evolution from an ancient precursor. Whole genome comparison identified the ancient isolate as the ancestor of extant strains associated with human pathogenicity; thus rendering biovar classification unfit. The pandemic of 1348-1350 is responsible for dissemination of this ancestral strain, allowing extant strains to develop within epidemiological niches **(Drancourt & Raoult., 2002).**

Medieval and extant isolates exhibit extraordinary homology, indicating no major rearrangements over the last 600 years. Absence of significant stress necessitating its genomic adaptation highlights the organism's resilience & plasticity.

Genomic comparisons illuminate the mechanisms bestowing virulence upon *Y. pestis*; three key components enable; transmission, subcutaneous infection and intracellular proliferation.

Type III Secretion Systems (T3SS), are common secretory pathways of pathogens. The pYV plasmid of pathogenic *Yersinia* is homologous to the pWR100 plasmid bestowing T3SS upon S*almonella* and *Shigella* (Figure 1) **(Singer *et al*., 2008)**. T3SS are not limited by genus, host or environmental foci; their rapid acquisition through HGT is sufficient to confer pathogenicity **(Coburn *et al*., 2007).**

Plasmid pYV also encodes secreted *Yersinia* outer proteins (YOP's); *Low calcium response V* antigen (LcrV) is essential for the production these virulence proteins; LcrV knockout renders *Y. pestis* avirulent. Isolated LcrV elicits humoral immune response within murine models **(Smiley, 2008)**, hence its significance in on-going vaccine research. LcrV homologues isolated from *Pseudomonas aeruginosa,* bestowed reliance within an animal model **(Lynch *et al*., 2010).**

Unique virulence determinants of *Y. pestis* are encoded by pFRA and pPLA. The ability of *Y. pestis* to colonise the flea gut and be delivered subcutaneously is endowed by the pFRA plasmid (Figure1.) which encodes 'murine toxin' facilitating biofilm formation within arthropod vectors along with fraction 1 (F1) antigen and phospholipase D capsular components. pPLA encodes plasminogen activators which enable systematic spread within a mammalian host **(Caulfield & Lathem., 2012).**

T3SS, 'murine toxin' and plasminogen activators have been proven to bestow virulence upon *Y. pestis;* individual knockout of each feature was followed by massive virulence attenuation within an animal model **(Caulfield & Lathem., 2012 & Zhou & Yang., 2009b).** Subsequent reactivation of genes corresponded to restoration of virulence within the isogenic mutant **(Prentice *et al*., 2001).**

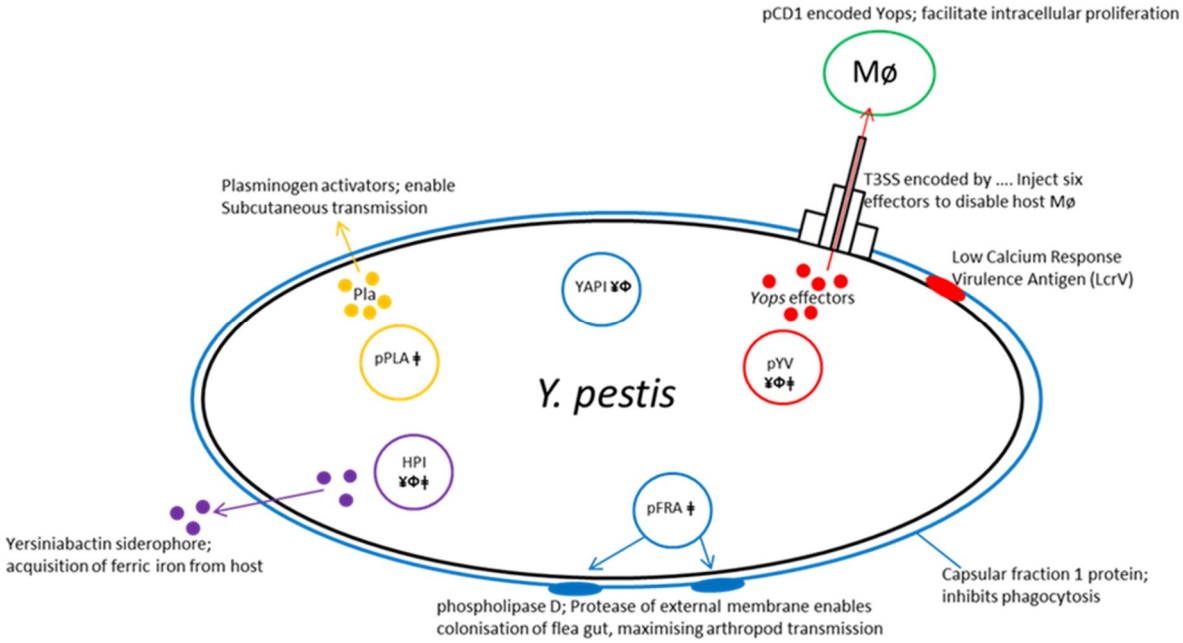

Figure 1 Schematic representation of *Y. pestis*. VA-MGE's distribution throughout pathogenic *Yesinia spp*. is denoted by ¥, Φ and ǂ; *Y. pseudotuberculosis, Y. enterocolitica and Y. pestis* respectively

THERAPIES

Early treatment by antibiotics reduces mortality (<20%); streptomycin is preferable. Panic associated with a modern plague is consdered disproportionate to its mortality **(Patterson., 2010).**

A recombinant F1/ LcrV based vaccine recently gained approval for stage two clinical trials; this vaccine is the most promising development to date. Due to the developmental nature, little information is available however early signs indicate that the vaccine effective against all manifestations of plague **(Williamson & Oyston., 2012)**. This vaccine in particular is the the culmination of over a decade of research into the molecular mechanisms of *Y. pestis* pathogenicity (Figure 2) **(Qiu *et al*., 2010).**

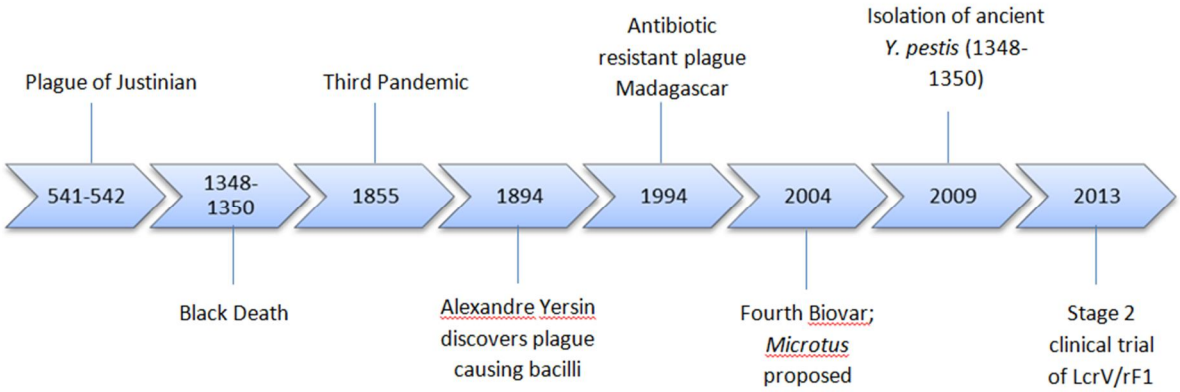

Figure 2 Significant chronological events in the epidemiology of Y. Pestis infections

DISCUSSION

Little has changed in the association of *Y. pestis* with mammalian hosts in over half a millennia. Mediaeval *Y. pestis* bacilli isolated by **Bos *et al*., (2011)** are no less virulent today than during the Middle Ages. Environment, hygiene, high host susceptibility and poor medical understanding likely accounted for its historic mortality rates.

The nefarious use of *Y. pestis* in bioterrorism has tightened control of *Y. pestis* stocks leading to its Tier One designation **(Auerbach *et al*., 2007)**, despite its well characterised genome no long lasting vaccine is yet available.

The rise in antibiotic resistant bacteria is cause for concern; like T3SS, antibiotic resistance may be acquired rapidly through HGT. Isolated cases of plasmid mediated multiple drug resistance (MDR) have occurred notably in Madagascar during 1995 **(Galimand *et al*., 1997,Guiyoule, 2001).**

MDR Madagascar isolates acquired plasmid pIP1202 which confers resistance to streptomycin; the preferential treatment **(Galimand & Courvalin., 2012; Schofield *et al*., 2012)**. The IncA/C plasmid family to which pIP1202 belongs is known to bestow MDR to *Salmonella, Vibrio cholerae* and *Escherichia coli* **(Welch *et al*., 2007)**. Conjugative acquisition of MGE's likely occurs within the gut of arthropod vectors **(Urich *et al*., 2012)**.

Despite its age the classical treatise of Zinser **(1934)** exemplifies the many facets of *Y. pestis* interaction with mankind:

"The struggle for existence between different forms of life…..incessantly, the pitiless war goes on, without quarter or armistice"

No commercial vaccine currently exists; evolution through HGT threatens to outstrip mankind's ability to produce new means of treatment; mirroring an evolutionary arms race. Acquisition and dissemination of MDR on a global scale would negate mankind's current medical advantage **(Bos *et al*., 2011 and Bos, 2012)**; as such the significance of on-going research cannot be underestimated.

Plague may be considered a disease of antiquity however it has survived for millennia; as we enter what some consider the twilight of the antibiotic age need for an all-encompassing plague vaccine is greater than ever.

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