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Epidemiology and diagnosis of genital chlamydial infection

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Introduction

Chlamydia trachomatis is a pathogen of global public health significance. It is associated with trachoma (serovars A, B, B1 and C), lymphogranuloma venereum (LGV, serovars L_1, L_2 and L_3) and genital infection (serovars D to K).

Genital *C. trachomatis* infection can cause pelvic inflammatory disease (PID) and is a threat to the reproductive health of women in both developed and developing countries: the World Health Organisation (WHO) estimates that 89 million new cases of genital *C. trachomatis* infection occur each year[1]. *C. trachomatis* eye infection is easily treated, and the WHO aims to eliminate trachoma blindness by 2020. In contrast, genital *C. trachomatis* infection and LGV will not be eliminated in the foreseeable future, as both are sexually transmitted infections (STI) with complex epidemiologies.

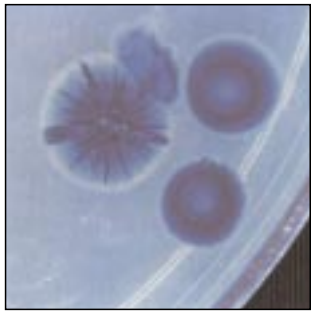
Sequelae of genital chlamydial infection

Genital *C. trachomatis* infection is of public health importance because it is considered to be the dominant cause of PID in developed countries. It is estimated that 10% of women infected with *C. trachomatis* develop PID[2]. In turn, PID can cause ectopic pregnancy, tubal factor infertility (TFI) and chronic pelvic pain[3]. Risk of developing sequelae is dependent on the number of PID episodes: the risk of ectopic pregnancy and TFI increased after one PID episode (odds ratio=6) and again after two episodes of (OR=17)[4].

Diagnosis

Diagnosis of chlamydial infection relies entirely on laboratory techniques and consequently chlamydial research has been guided and determined by developments in diagnostic technology. Microscopy was used from 1905 and, in 1957, *C. trachomatis* was cultured in the laboratory using egg yolk sac inoculations[5].

Culture of *C. trachomatis* in McCoy cells grown on cover slips established the importance of *C. trachomatis* and its role in the development of ectopic pregnancy and TFI in the 1970s. However, although culture has high specificity (100%), sensitivity is lower (around 70%). The technique was also found to be time consuming to perform because of the need to stain each coverslip using Giemsa stain or iodine. A multi-well plate culture was introduced in the 1980s to reduce processing times, and staining techniques, such as Periodic Acid-Schiff stain, were used to visualise chlamydial inclusions (**Figure 1**). However, other limitations of the culture technique, such as reliance on carefully transported fresh samples, the need for skilled technical expertise and high labour costs made it unsuitable for processing the large numbers of samples generated by diagnostic services and epidemiological studies. This reconciliation of high sensitivity and specificity against the need for cheap, simple techniques that can process large numbers of samples quickly is a paradox that is central to the development of chlamydial diagnostic tests that continues to this day.



Blue colonies of *Escherichia coli* mutated by the bacteriophage *Mu* to produce β-galactosidase on Xgal medium.

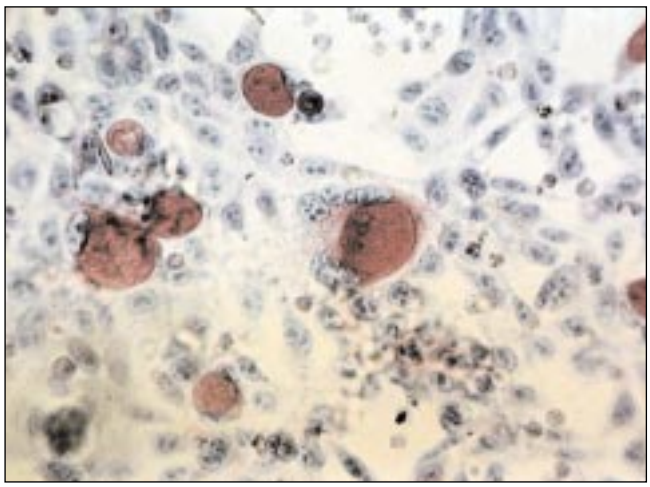


Figure 1. *Chlamydia trachomatis* inclusion in McCoy cell culture stained magenta using Periodic Acid Schiff (PAS) stain. Reproduced by kind permission of Dr H Mallinson, Liverpool Public Health Laboratory, UK.

Antigen detection tests, such as enzyme-linked immunoassay (EIA) and direct immunofluorescent assay (DFA), use monoclonal or polyclonal antibodies against either the *C. trachomatis* major outer membrane protein (MOMP) or lipopolysaccharide (LPS).

A variety of fluorescence-labelled monoclonal antibody DFA tests that are directed against MOMP and LPS antigens are commercially available. Antibodies to LPS are genus-specific and exhibit less intense staining than antibodies to MOMP because LPS is unevenly distributed on the surface of elementary bodies. However, the DFA technique is time consuming and relies on experienced operators as each specimen has to be examined individually.

The EIA test uses LPS, which is more abundant and soluble than MOMP, as the target antigen. LPS is extracted from the elementary bodies by heating with detergent and detected using an anti-LPS antibody. Unfortunately cross-reaction with LPS from other Gram-negative bacteria may occur and result in false-positive readings. The EIA technique allows large numbers of samples to be processed quickly but has limited specificity and sensitivity: all reactive EIA samples should thus be confirmed using alternative technology.

A limitation of the antigen detection tests is that they require invasive specimens, such as cervical and urethral swabs, taken as part of a genital examination in a specialist clinical setting. Epidemiological studies undertaken in the 1980s and '90s showed that genital chlamydial infection could only be controlled through screening for asymptomatic infection. Such intervention and surveillance programmes require low cost, simple tests with high sensitivity and specificity that use non-invasive samples such as urine.

Over the past decade molecular techniques have been applied to chlamydia diagnosis. Nucleic acid amplification tests (NAAT) can detect *C. trachomatis* DNA at a level of a few organisms per sample and the use of these techniques has led to the development of tests that combine ease of collection and transport with high sensitivity and specificity. Self-obtained vaginal and vulval swabs or urine can be used and collected in non-clinical settings and mailed to laboratories for testing. This has increased patient acceptability and allowed increased flexibility in study design, which in turn has allowed the closer integration of microbiological and behavioural research within screening and epidemiological studies. Molecular techniques allow large numbers of samples to be processed quickly. Nevertheless there is an ever increasing demand for diagnostic services, and satisfying this demand inevitably means automation but automation of NAAT presents considerable difficulties.

The main problems are the space required for sample preparation to prevent cross-contamination and the processing capacity of the techniques. Polymerase chain reaction (PCR) techniques use 3 rooms and the ligase chain reaction (LCR) test takes 2 rooms. In contrast, the Becton Dickinson strand displacement amplification assay (Probetec ET), which has a microwell plate format, and employs closed detection of amplicon with a fluorescent signal, uses one preparation room and has an improved specimen throughput.

The sensitivity of both the PCR and LCR techniques is limited by the amount of test target: there are 4 to 10 copies of the *C. trachomatis* cryptic plasmid per copy of genomic DNA. Transcript-mediated amplification, an isothermal (42ºC) technique that targets rRNA, is potentially more sensitive because of the higher concentration of the target rRNA and automated systems are being developed. However, rRNA is more labile than DNA and is sensitive to heat and enzyme degradation, so the technique may be limited in terms of its application to epidemiological studies.

Incidence, prevalence & burden of disease

The majority of genital *C. trachomatis* infections are found in the developing world, reflecting provision and access to healthcare, health-seeking behaviour, and the distribution of the global population. The highest prevalence is in sub-Saharan Africa, the lowest in East Asia and the Pacific (**Table**)[1]. However, these estimates are biased due to the quality of available surveillance data. Many countries do not have national surveillance data and, where prevalence studies have been undertaken, these have generally been derived from high risk groups, such as attendees at sexually transmitted disease (STD) clinics. A further source of bias is the high level of asymptomatic infection: up to 70% of infections in women and 50% of infections in men are asymptomatic and are unlikely to be represented in surveillance datasets. However, although biased, these estimates illustrate the global importance of genital *C. trachomatis* infection.

Even though most countries undertake national surveillance, few have collected data for more than a decade and, where available, it is usually restricted to laboratory reports or attendences at STD clinics. Trends in surveillance data should be interpreted with caution as they can be influenced by factors such as changes in testing policy, clinical practice, the availability and accuracy of diagnostic tests, access to healthcare and the prevalence of asymptomatic infection. A surveillance system also needs to evaluate the complex mixture of behavioural, micro-biological, social and demographic factors that drive sexually transmitted infections (STI) epidemiology. In addition, surveillance needs to include an assessment of sexual causes of ill-health, as well as the provision and uptake of clinical services, as these factors play a key role in establishing an

evidence base which can be used to plan, monitor, audit and evaluate programmes intended to improve sexual health.

Who is at risk?

Risk factor studies can identify population subgroups at increased risk of genital chlamydial infection, and used to initiate timely, effective intervention strategies. A large number of studies have been published from various countries but invariably these studies have been undertaken in specific clinical settings and are insufficient to allow detailed analyses.

Incidence of infection is influenced by a complex interaction of demographic and behavioural factors. Aspects of sexual behaviour, such as age at first sexual intercourse, number of lifetime sexual partners, frequency of partner change, concurrent partners and unsafe sex, are key determinants of STI transmission. Young people are behaviourally more vulnerable to STI acquisition as they generally have higher numbers of sexual partners, and change partners more often than older age groups. In the UK, young people are more likely to report concurrent and serial partnerships than monogamous relationships[7]. Those who initiate sex early are more likely to report higher numbers of sexual partners, acquire an STI or become pregnant. Early onset of sexual behaviour is associated with low socio-economic status, poverty, poor educational opportunities, being born to a teenage mother and high rates of unemployment. In addition, low socio-economic status is associated with reduced knowledge of contraception. The consistent, effective use of condoms reduces the risk of STI transmission, but young people may not have the skills and confidence to negotiate safer sex. Irregular use of barrier contraception will not prevent STIs.

Young women are also biologically more susceptibility to genital chlamydial infection than older women. High rates of PID in women aged 16 to 24 years may reflect longer duration of infection or reduced clearance of *C. trachomatis* . This could be due to increased susceptibility, lower concentration of protective chlamydial antibodies, higher cervical ectopia, and greater permeability of cervical mucus than in older age groups. Young women continue to place themselves at risk of infection and its sequelae. A study based in Genito-Urinary Medicine (GUM) clinics showed that between 20% and 30% of teenage females diagnosed with an STI were diagnosed with another STI within 18 months of the first attendance with an STI[8]. Similarly, 22% of males aged 12 to 15 years old re-attended within one year. These factors are reflected in the high chlamydial incidence in the 16 to 24 year age group, that peaks at a younger age in females than males (**Figure 2**)[6].

The relationship between sexual behaviour and STI prevalence has been little studied in general population samples. Sexual behaviour varies between societies, individuals and during an individual's lifetime. The interaction between the 'core' group (people who have large numbers of sexual partners, concurrent partners and frequent partner change) and the rest of the population, is important to STI transmission. The investigation of sexual networks, that is interactions between the core and the rest of the population, is a key area of research. Behavioural, demographic and morbidity surveillance at the individual level provides more precise estimates of risk factors associated with genital chlamydial infection.

Table: Estimated number of new cases, prevalence and incidence of genital *C. trachomatis* infection between ages 15 and 49, by sex and United Nation global region: 1995[1]

Region	New cases (million)		Prevalence (%)		Incidence (per 1000)	
	Male	Female	Male	Female	Male	Female
North America	1.64	2.34	0.8	2.7	21.46	30.73
Western Europe	2.30	3.20	0.8	2.7	21.46	30.73
Australasia	0.12	0.17	0.8	2.7	21.46	30.73
Latin America & Caribbean	5.01	5.12	2.5	4.0	40.03	40.77
Sub-Saharan Africa	6.96	8.44	4.8	7.1	55.04	65.95
North Africa & Middle East	1.67	1.28	1.2	1.7	19.93	16.29
Eastern Europe & Central Asia	2.15	2.92	1.7	3.7	27.29	37.09
East Asia & Pacific	2.70	2.63	0.4	0.7	6.53	6.75
South & South East Asia	20.20	20.28	3.7	4.9	41.65	44.32
Overall	**42.75**	**46.38**	-	-	-	-

Control & intervention

Primary prevention, based on education and behavioural change, is fundamental to disease control. Behavioural change, such as increased use of barrier contraception and delayed sexual debut, has been documented in European countries in response to HIV and STI health campaigns[9,10].

In the UK and many other countries, the major obstacle to primary prevention is a low awareness of genital chlamydial infection and its sequelae, among healthcare

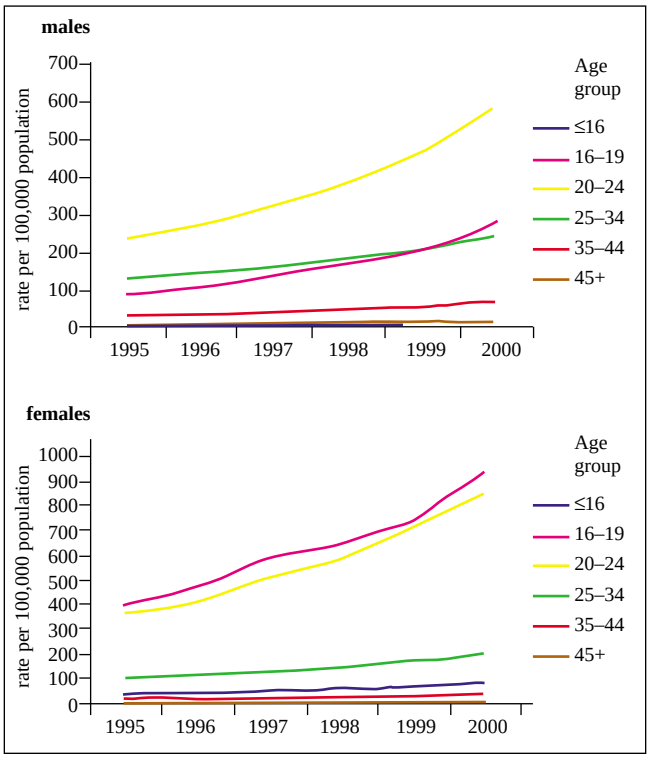


Figure 2. Rate of diagnosis of uncomplicated genital *C. trachomatis* infection by sex and age group, United Kingdom: 1995 to 2000.

professionals and the public. Secondary prevention, the diagnosis and treatment of asymptomatic infection, has successfully reduced the prevalence of both genital *C. trachomatis* infection and PID. Only one randomised controlled trial studied the effectiveness of screening for genital *C. trachomatis* infection and showed it brought about reductions in PID prevalence[11]. In the USA, surveillance data indicated that intervention based on screening for genital *C. trachomatis* infection has reduced the incidence of PID and ectopic pregnancy by more than 50% and 20% respectively[12]. In Sweden, screening for genital *C. trachomatis* infection rapidly reduced the incidence of ectopic pregnancy amongst 20 to 24 year olds[13]. No study has demonstrated that screening for genital *C. trachomatis* infection can reduce the incidence of TFI.

Conclusions

Genital *C. trachomatis* infection is a key issue facing women's reproductive health. This rapidly expanding area of research requires the routine collection of behavioural, demographic and laboratory data tailored to the public health needs and priorities of individual countries. Nevertheless, despite substantial developments of the past two decades, we are only just beginning to comprehend the impact of this important infection.

References

1. Gerbase A, Rowley J, Heymann D, Berkley S, Piot P. Global prevalence and incidence estimates of selected curable STDs. *Sex Transm Inf* 1998; **74:** S12–4.
2. Stamm W, Guinan M, Johnson C, Starcher T, Holmes KK, McCormack W. Effect of treatment regimens for *Neisseria gonorrhoeae* on simultaneous infection with *Chlamydia trachomatis*. *N Engl J Med* 1984; **310:** 545–9.
3. Buchan H, Vessey M, Goldacre M, Fairweather J. Morbidity following pelvic inflammatory disease. *Br J Obstet Gynaecol* 1993; **100:** 558–62.
4. Weström L. Sexually Transmitted Diseases and Infertility. *Sex Transm Dis* 1994; **21:** S32–7.
5. T'ang F-F, Chang H-L, Huang Y-T, Wang K-C. Trachoma virus in chick embryo. *Nat Med J China* 1957; **43:** 81–6.
6. PHLS, DHSS&PS and the Scottish ISD(D)5 Collaborative Group. Sexually Transmitted Infections in the UK: New cases seen at Genitourinary Medicine Clinics, 1995 to 2000. London: Public Health Laboratory Service, 2001.
7. Johnson A, Wadsworth J, Wellings K, Field J, Bradshaw S. Sexual Attitudes and Lifestyles. Oxford: Blackwell Scientific Publications, 1994.
8. Hughes G, Brady A, Catchpole MA, *et al.* Characteristics of those who repeatedly acquire sexually transmitted infections: a retrospective cohort study of attendees at three urban sexually transmitted disease clinics in England. *Sex Transm Dis* 2001; **28:** 379–86.
9. Weström L. Decrease in incidence of women treated in hospital for acute salpingitis in Sweden. *Genitourin Med* 1988; **64:** 59–63.
10. Coutinho R, Rijsdijk A, van den Hoek J, Leentvaar-Kuijpers A. Decreasing incidence of PID in Amsterdam. *Genitourin Med* 1992; **68:** 353–5.
11. Scholes D, Stergachis A, Heidrich F, Andrilla H, Holmes KK, Stamm W. Prevention of pelvic inflammatory disease by screening for cervical chlamydial infection. *N Engl J Med* 1996; **334:** 1362–6.
12. Hillis S, Nakashima A, Amsterdam L, *et al.* The impact of a comprehensive chlamydia prevention program in Wisconsin. *Fam Plan Perspect* 1995; **27:** 108–11.
13. Egger M, Low N, Smith G, Lindblom B, Herrmann B. Screening for chlamydia infections and the risk of ectopic pregnancy in a county in Sweden:ecological analysis. *BMJ* 1998; **316:** 1776–80.

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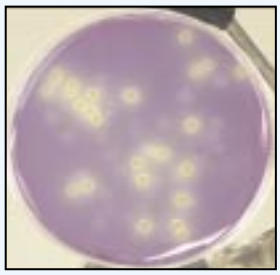

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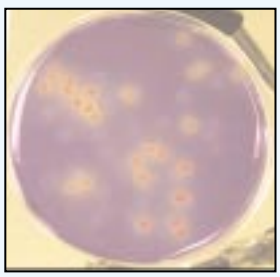

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Quantal Microbiology: a stressed cell phenomenon

Dr Eric Bridson MPhil, PhD, FIBMS, FIBiol

Introduction

Quantal microbiology can be defined as the study of individual stressed cells rather than the more usual studies with microbial populations, whether stressed or unstressed. The adjective quantal, which is more commonly associated with quantum mechanics, was chosen because it emphasises the unpredictability and complexity of resuscitation of a single cell. There is a similarity of association between the contrast of macro-Newtonian physics and subatomic Quantum physics with the contrast between Classical microbiology and Quantal microbiology.

Newtonian physics and Quantum physics:

Isaac Newton published his Laws of Motion and Gravity in 1686 (**Figure 1**) which were based on observations and measurements of movement by large bodies, from the flight of cannon balls to the stately procession of the Moon and planets. These Laws established a mathematical basis for physics which seemed to be universal and immutable. Up to the end of the 19th century, the science of physics was based on absolute certainty about six rules:

1. The Universe was like a giant machine set in a framework of absolute time and space.
2. Newtonian rules stated that all motion had a cause and motion was the effect. Cause and effect were never questioned.
3. If the state of motion was known at any one point, it could be determined at any other point in the future or in the past. This was determinism, nothing was uncertain.
4. Maxwell completely described the properties of light with the electro-magnetic wave theory.
5. There are two physical models to represent energy in motion: a hard sphere (particle) or a wave. Energy must be one or the other, they are mutually exclusive.
6. The properties of any system can be measured to any degree of accuracy.

By the early 20th century, these absolute truths were revealed to be uncertain assumptions when they were applied to investigations into subatomic particles. These particles did not obey Newtonian rules. A series of high power conferences of top physicists was held in the early 20th century and at the 1927 Solvay Conference, the science of Quantum Mechanics was formulated to explain subatomic phenomena. In quantum mechanics, subatomic particles can have their velocity or position determined but not both parameters simultaneously. Light can be in wave-form or particles (photons) and in both forms (wavicles). Predictability was out and probability was in, uncertainty and chaos took over. Albert Einstein never accepted quantum mechanics.

The situation today is that large bodies appear to obey Newton's Laws (macro-physics) but subatomic particles obey Quantum rules (micro-physics).

It must not be thought that Newtonian Laws have been put to one side; in macro-physics they still rule. A striking example is when NASA staff using Newtonian physics steered the Voyager 2 space craft approximately 5 billion km, on a 12 year journey from Earth to the planet Neptune, landing it on schedule and within one km of its target.

Quantum mechanics showed that large bodies, averaging the characteristics of the individual structural atoms, operated under Newton's Laws. However, when testing the individual atoms of structure, the uncertainty principles of quantum mechanics displaced deterministic principles[1]. How does microbiology compare with this physical model?

Classical microbiology and Quantal microbiology:

Classical microbiology is the basic technology of propagating organisms, developed by Robert Koch[2] in the 1880s. The apparently simple procedure of spreading organisms on the surface of a sterile nutrient agar surface, remains unaltered 120 years later. Its success lies in its efficiency, versatility and low-cost technology. A disadvantage is that it calls for considerable human skill to

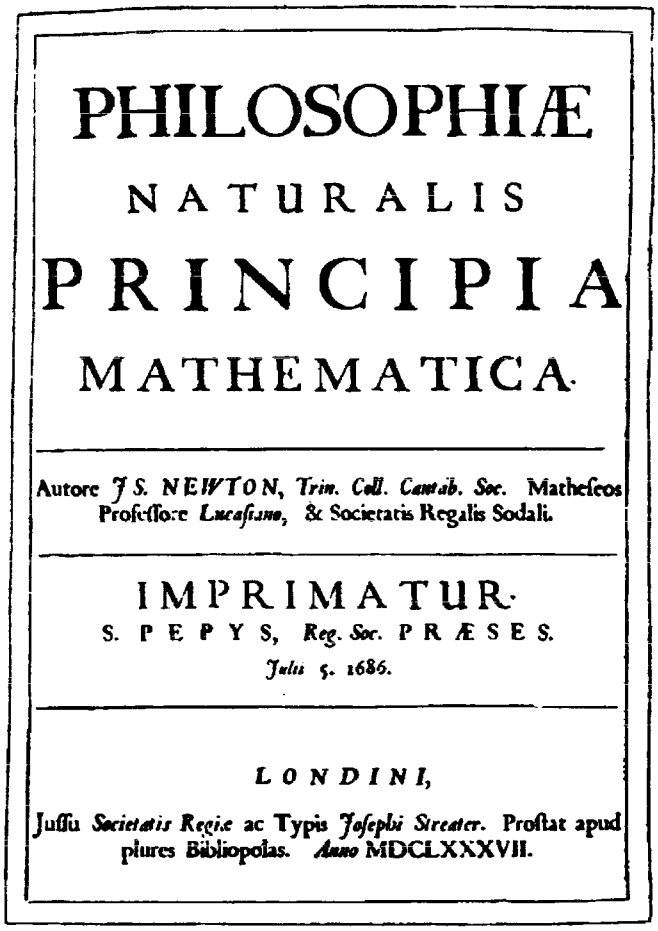
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Figure 1. The title page of Isaac Newton's Mathematical Principles of Natural Philosophy.

draw accurate conclusions from the plethora of mixed colonies that may appear after incubation. The use of agar media containing selective and/or indicator agents does help differentiate the colonies in mixed cultures. This human skill requirement of analysing images, has so far defied computer-aided mechanization. The identification of micro-organisms at this stage of isolation, is a largely qualitative process which is not compatible with quantitative computer algorithms.

The success of Koch's technique is that by widely separating the organisms in the inoculum- spreading process, it can be postulated that each separate colony arising after incubation, represents a clone of identical cells. This simple technique is really a complex interaction of culture media ingredients and the various growth attributes of the microbial cells. The most important attribute being whether the cell was stressed or unstressed at the time of inoculation. Primary cultures from clinical material or from processed food samples may be highly stressed and a separate process of resuscitation may be required before subculture[3-6]. Prior to the discovery that stressed organisms required resuscitation and enrichment of culture media, the principle of "What does not grow, does not exist." applied. Later the concept of organisms being viable but not culturable on conventional culture media (VBNC) was accepted[7,8] More recently, Barer & Harwood[9] have rejected the VBNC concept and have suggested that better resuscitation/enrichment steps will always recover viable organisms.

Where are single stressed organisms most likely to occur?

Quantal microbiology is most apparent either at the beginning of the growth curve or at the terminal death phase (**Figure 2**) It is important to bear in mind that growth curves reflect population characteristics, not single cells but it can be accepted that a population can start from a single cell and prior to total destruction a single cell may be the sole survivor. The organism at the commencement of growth may be stressed but the organism at the end of the death phase will most certainly be highly stressed. If more than one cell is present at the commencement of growth, then uncertainty will arise about which particular cell replicated first to form the new population. Equal uncertainty will arise if, following resuscitation and enrichment, growth re-commenced from organisms left at the end of the death phase. Barer & Harwood[9] questioned whether bacterial viability could be assessed at the individual cell level or at the community level. The duration of the lag phase, for example, has been shown to be inversely related to the number of cells present at the time of inoculation. This has been demonstrated by Stephens *et al.*[10] using heat-stressed salmonella showing the very prolonged lag phase of single stressed cell inocula. A community of organisms can provide protective factors eg., 'shareware' antibiotic- resistance plasmids or powerful anti-oxidation chemicals. However, it is accepted that there are circumstances in which the survival of a single cell is of considerable biological and practical significance. For example, sterilization processes that require a total absence of any viable organism must be prolonged until the probability that a single cell can survive

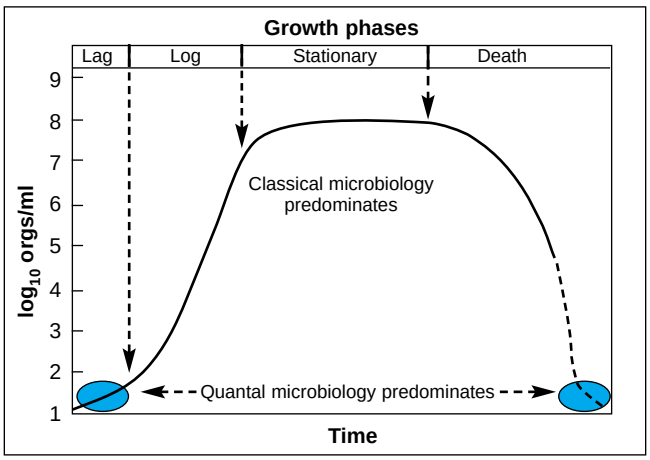

Figure 2. A typical bacterial growth curve illustrating the predominance of 'Classical' and 'Quantal' microbiology.

is exceedingly small. The establishment of infection is also numerically controlled. Highly pathogenic organisms may require a single cell only to initiate infection but most infectious organisms have a minimal infectious dose of organisms, unless the host is severely immuno-compromised.

Are all microbial cells of any specific species identical?

For most of the history of microbiology, the answer to this question would be an obvious 'Yes'. The very definition of a species required every cell within it to conform to defined phenotypic and sometimes genotypic characteristics. Robert Koch had no doubt that by the time he was able to raise separated colonies from individual cells, he could regard each colony as composed of identical cells, perfectly cloned. Such perfect replication could be tested by looking for identical DNA in each cell. This alone, however, would not be sufficient because similarity of the genome may not guarantee functional identity. A variation of function between individual cells could improve the chances of population survival, if changes occurred in the eco-system. Simple observation shows that microbial populations can survive and flourish under changing conditions. Mutant variations of genes (alleles) provide opportunities for 'genetic sports' that may function better in the same or changed environments and could ultimately replace the original population. It would be essential that the favoured mutation was heritable and produced phenotypic changes in the cell. Although spontaneous mutation in microbes may be small (10^{-5}) in a colony population of 10^{10} cells with 10^{13} genes per cell, some 10^{8} mutated genes could arise.

Conservation of species in eukaryote cells is rigorously controlled with less than 1% exchange of DNA between species. Prokaryote cells are more promiscuous and up to 25% of DNA can be transferred. This free exchange of DNA enables highly complex homologous recombination to take place in recipient microbial cells.

Genetic exchange in prokaryotes can be vertical through cell division transmission or horizontal by lateral transmission of plasmids or transposons etc., the latter would be a form of DNA shareware. This apparent free exchange of DNA does not breach species-specific DNA

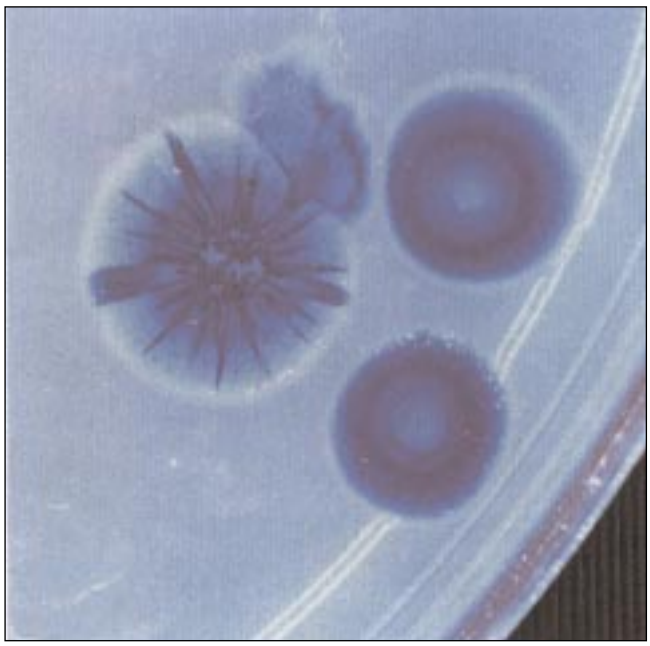

Figure 4. Blue colonies of *Escherichia coli* mutated by the bacteriophage *Mu* to produce β-galactosidase on Xgal medium. The secotred colony contains cells where *Mu* has been incorrectly orientated and they cannot produce β-galactosidase.[18]

exchange rules because there is no evidence that staphylococci ever become streptococci, or that any other species can take on another species identity. It does, however, provide opportunity for individual cells to display disparate growth characteristics in the cell population which could offer advantages, if and when the local eco-system changed. It is in these circumstances that cells in a 'cloned' population can be described as non-identical.

Although species conservation still operates, some individual cells may be more successful than others. In microbiology, the rapid rate of reproduction means that the smallest advantage of an individual cell eg., shorter lag and/or mean generation time will give it sufficient advantage to eventually dominate the microbial population until the next change of environment.

What is the evidence that sub-species variations in organisms are significant?

Chemostats can provide variable eco-systems in which to cultivate organisms and measure the effect of deliberate environmental changes. Dykhuisen & Hartl[11] reviewed the use of chemostats to study the effects on naturally occurring or genetically engineered mutations, using plasmids, transposons and temperate phages. They considered that the success of the chemostat in detecting subpopulations of competing mutants is its sensitivity to very small differences in growth rates[12].

A mutation which conferred a selective advantage could purge 99% or more of a population's variation and replace all competing cells[13]. This was described as a periodic selection event.

Perhaps the most striking form of mutant selection is the effect of antibiotics added to the culture medium and revealing antibiotic-resistant organisms. This very self-evident test inhibits all susceptible organisms and will prominently display, on agar plates, resistant mutants that may be present only in minuscule numbers. So valuable are the advantages of these resistant plasmids that they can be exchanged across genera eg., *Shigella species* and *Escherichia coli*[14,15].

It should not be thought that antibiotic resistance is unique. Many other metabolic mutant effects could confer equal advantages. The problem is to devise *in vitro* tests that will reveal the mutants as effectively as antibiotics.

Some pathogenic organisms are naturally toxigenic, other pathogens can become toxigenic by acquiring virulence plasmids. Virulence can be detected *in vitro* by immunological tests and *in vivo* by animal toxicity studies[16]. The ability of organisms to exchange virulence genes is another example of cell variation which can have immediate impact on human, animal and plant populations. Gillet[17] stated *"In any population or species, the individuals that survive when the environment changes … are those lucky few already fitted with an appropriate assortment of genes"*.

Mutant variations of cells in colonies have been closely studied. Perhaps the most frequently cited work was carried out by Shapiro[18] who used a Mu prophage (Mu*dlac*) and Xgal indicator to reveal highly complex, colourful patterns of mutant galactose-fermenting forms of *Escherichia coli* arising in the colonies.(**Figure 4**)

The application of fractal geometry to study the development of bacterial colonies has shown how minute alterations of instructions in fractal modelling can have striking effects on the final distributions of the 'mutant' organisms in the colony.(**Figure 5**) Several standard formulae can be used and an artificial mutant can be simulated by a small variation in the formula for one cell. Highly complex, multiple variant colony forms can be 'grown' on computer screens[19]. However, Wimpenny[20] cautioned that bacterial colonies are far more complex than fractal models.

Conclusions

Physicists have had to come to terms with the fact that Newtonian deterministic physics seems to work only with large bodies. Subatomic particles appear to require the probability functions and uncertainty of Quantum mechanics. However, macro-physics and micro-physics seem to be co-existing with some understanding that macro-physics represents the averaging of micro- physical phenomena. Newtonian physics still rules in macro-physics.

Classical microbiology is the averaging of millions of 'identical' cells which gives it great stability and it will continue to be used for many years to come. Quantal microbiology deals with single stressed cells and all the difficulties of working from such a minute base with all its uncertainties of outcome. More needs to learned about the single cell, whether a spontaneous mutant or an acquired genetic variation and this will be to the benefit of microbiology generally. There is no intention to replace Classical microbiology with Quantal microbiology, only to distinguish important differences between these two concepts.

It remains an intriguing situation that two apparently widely disparate scientific fields share a macro- and micro-function in which the macro-function is more deterministic and the micro- function is more probabilistic.

There is more to Quantal microbiology than this short paper allows. The applications of the theories of Chaos and Complexity, fractals and computer generated models of colonies are relevant. Even more interesting is the serendipity of Koch's nutrient agar medium plus Loeffler's addition of

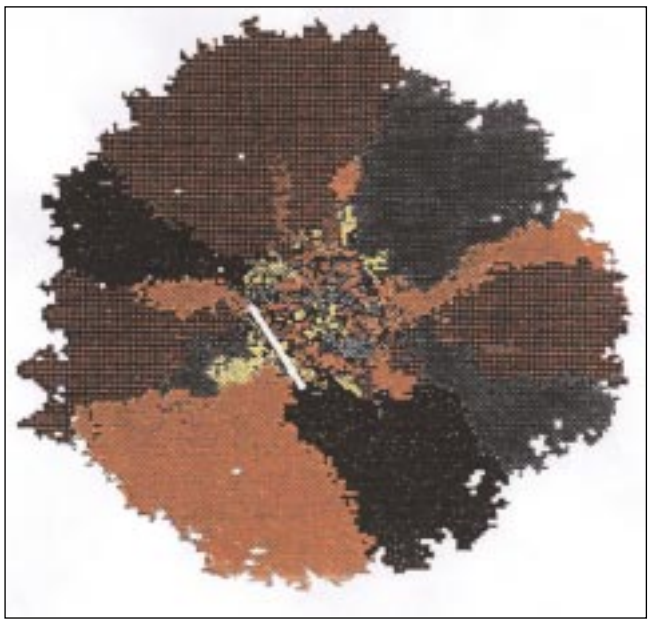

Figure 5. A fractal 'Natural Model' of bacterial colonial growth showing 'mutants' (different colours) in a 'mature' colony.[19]

hydrolysed protein, which appears to be the best culture medium recipe to recover stressed cells. The fact that stressed cells cannot be recovered on synthetic or defined culture media, indicates that large and small polypeptides play a key role in the recovery of these organisms.

Those readers who wish to obtain more information on Quantal microbiology are referred to my thesis[21] and a recent paper[22].

References

1. Feynman RP. The Character of Physical Law. 1992; London. Penguin Books. pp. 33 and 35.
2. Brock TD. Robert Koch: a life in medicine and bacteriology. 1988; Madison. USA. Science Tech. Publishers. pp.94–104.
3. Gould GW. Heat-induced injury and inactivation. In: Mechanisms of Action of Food Preservation Procedures. Ed. Gould G W.1989; Elsevier Science Publishers. UK. pp. 11–42.
4. Mossel DAA, Corry JEL. Detection and enumeration of sublethally injured pathogenic and index bacteria in foods and water processed for safety. 1977; *Alimenta*. pp. 19–34.
5. McDonald LC, Hackney CR, RayB. Enhanced recovery of injured *Escherichia coli* by compounds that degrade hydrogen peroxide or block its formation. *Appl. Environ. Microbiol.* 1983; **45:** 360–365.
6. Andrew MHE, Russell AD. The revival of injured microbes. 1984; Academic Press. London.
7. Colwell RR, Brayton BR, Grimes DJ, Roszak DB, Huq SA, Palmer LM.Viable but non-culturable *Vibrio cholerae* and related pathogens in the environment: implications for the release of genetically engineered micro-organisms. *Bio/Technology* 1985; **3:** 817–820.
8. Roszak DB, Colwell RR. Metabolic activity of bacterial cells enumerated by direct viable count. *Appl. Environ. Microbiol.* 1987; **53:** 2889–2983.
9. Barer MR, Harwood CR. Bacterial Viability and Culturability. *Adv. Microbiol. Physiol.* 1999; **41:** 93–137.
10. Stephens PJ, Joynson JA, Davies KW, Holbrook R, Lappin-Scott HM, Humphrey TJ. The use of an automated growth analyser to measure recovery times of single heat-injured Salmonella cells. *J Appl. Microbiol.* 1997; **83:** 445–455.
11. Dykhuizen DE, Hartl DI. Selections in Chemostats. *Microbiol. Rev.* 1983; **47:** 150–168.
12. Dykhuizen DE, Hartl DI. Selective neutrality of 6 PGD allozymes in *Escherichia coli* and the effects of genetic background. *Genetics.* 1980; **96:** 801–817.
13. Cohen FM. Genetic exchange and evolutionary divergence in prokaryotes. *Trends Ecol. Evol.* 1994; **9:** 175–180.
14. Maas WK. Mutations to Antibiotic Resistance. In: Antibiotics in Laboratory Medicine. 2nd Edn. 1986; Ed. Lorian V. Williams & Wilkins. Baltimore. pp. 669-682.
15. Elwell LP, Falkow S. The characteristics of R plasmids and the detection of plasmid-specified genes. In: Antibiotics in Laboratory Medicine. 2nd Edn. 1986; Ed. Lorian V. Williams & Wilkins. Baltimore. pp. 683–721.
16. Kamali AM, Goglio AG Eds. Recent advances in verotoxin-producing *Escherichia coli* infections. In: Internal Congress Series. 1072. 1994; Elsevier.
17. Gillet JD. Exons and Introns. *Biologist.* 1992; **39:** 85.
18. Shapiro JA. The use of dlac transposons as tools for vital staining to visualize clonal and non-clonal patterns of organization in bacterial growth on agar surfaces. *J Gen Microbiol* 1984; **130:** 1169–1175.
19. Schindler J, Rata T. Fractal geometry and growth models of a Bacillus colony. *Binary.* 1992; **4:** 66–72.
20. Wimpenny JWT. Microbial systems: patterns in time and space. In: Advances in Microbial Ecology. Vol 12. 1992; Ed. Marshall K C. Plenum Press. New York. pp. 469–522.
21. Bridson EY. Quantal microbiology. The interactive effect of chaos and complexity on stressed cell recovery. PhD thesis. 1999; University of the West of England.
22. Bridson EY, Gould GW. Quantal microbiology. *Letters in Applied Microbiology.* 2000; **30:** 95–98.

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