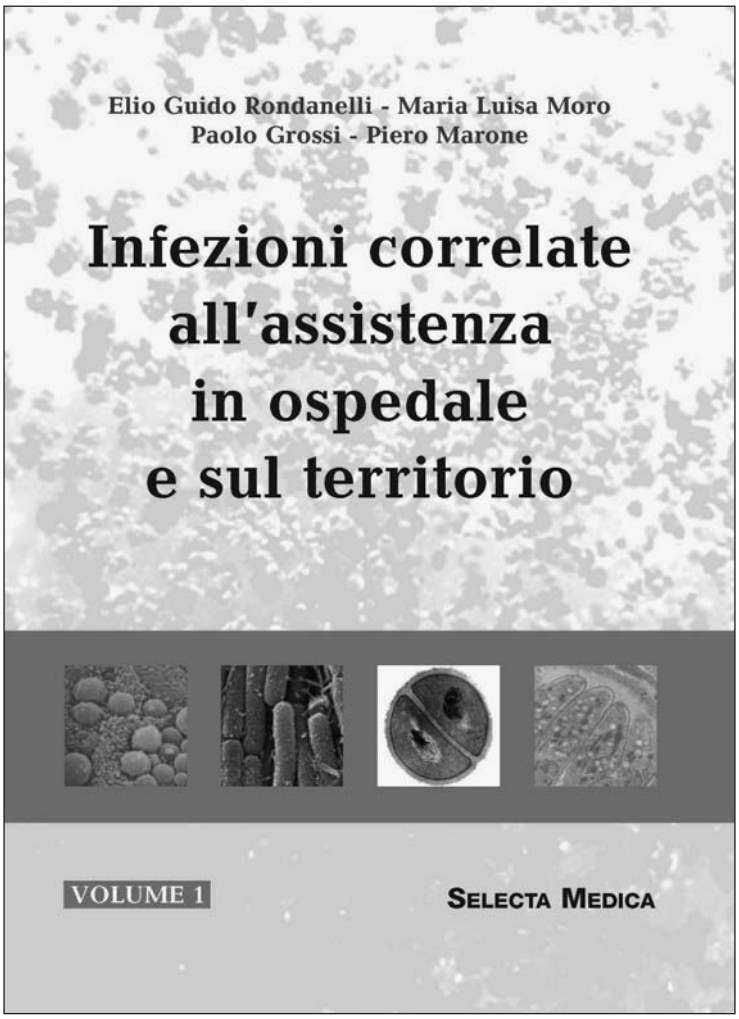
RECENSIONE

"Infezioni correlate all'assistenza in ospedale e sul territorio"

di Rondanelli, Moro, Grossi e Marone, edito da Selecta Medica



L'opera "Infezioni correlate all'assistenza in ospedale e sul territorio" di Rondanelli, Moro, Grossi e Marone, edito da Selecta Medica, affronta un tema di grande attualità: le infezioni contratte da persone assistite in ospedale o in ambito territoriale (in ambulatorio, in strutture residenziali, ecc.). Il panorama della sanità è profondamente mutato negli ultimi anni, l'invecchiamento della popolazione, l'incremento di pazienti con deficit immunitario, la riorganizzazione delle strutture di ricovero e cura che comprendono non solo l'ospedale per acuti ma anche il centro di riabilitazione e la residenza sanitaria assistita ed infine il ricorso sempre più frequente all'assistenza domiciliare e ambulatoriale hanno determinato un nuovo scenario caratterizzato da complicanze infettive correlate ad atti medici e infermieristici.

Queste patologie vedono coinvolti numerosi specialisti. Non a caso il libro di Rondanelli è frutto del contributo di diverse figure professionali quali specialisti di malattie infettive, igienisti, microbiologi, internisti, chirurghi e professionisti della sanità non medici.

Nella prima parte viene delineata la storia delle infezioni ospedaliere e vengono trattati gli aspetti legislativi e relativi ai costi e costo-beneficio dei programmi di controllo.

La sezione relativa all'organizzazione dei servizi di controllo offre un completo ed esaustivo panorama della realtà nazionale e regionale, affronta inoltre le problematiche relative al ruolo dei CIO e del laboratorio di microbiologia, il ruolo della Direzione Medica nel controllo del rischio infettivo, quello della Farmacia e dell'infermiera addetta al controllo delle infezioni. Una particolare menzione meritano i due capitoli sull'epidemiologia, la sorveglianza attiva e la gestione degli eventi epidemici.

I vari capitoli di quest'ultima sezione dedicata all'epidemiologia analizzano i nuovi scenari determinati dai mutamenti del servizio sanitario.

Non vengono poi trascurati gli aspetti legati all'impressionante progresso delle scienze mediche, al ruolo di internet e del sistema informativo sanitario. Un'ampia parte dell'opera è dedicata alle infezioni negli operatori sanitari.

A seguire vengono trattate le principali infezioni correlate all'assistenza e i microrganismi più frequentemente in causa, con particolare riferimento ai patogeni multiresistenti.

Si deve per correttezza segnalare che su talune tematiche alcuni autori presentano punti di vista differenti, ma la vastità dell'opera e la differente estrazione degli autori rendono inevitabili queste disomogeneità.

L'opera è arricchita da una iconografia di elevata qualità e presenta una curata veste editoriale.

Il libro di Rondanelli, Moro, Grossi e Marone può essere considerato un compendio pratico, aggiornato ed utile, anche a scopo didattico e rappresenta un ottimo punto di riferimento per le diverse figure che si occupano di infezioni correlate all'assistenza quali igienisti, infettivologi, microbiologi e anche, più in generale, chirurghi, internisti e operatori sanitari non medici.

INDICE DEGLI AUTORI

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Italian Society of Clinical Microbiology **(AMCLI)**
working group on the
"Sexually Transmitted Diseases" GLIST

INTERNATIONAL *Chlamydia trachomatis* Infections CONFERENCE

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ABSTRACT BOOK

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UNDER THE AUSPICES OF

FISM, Federazione delle Società Medico-Scientifiche Italiane
FNOMCeO, Federazione Nazionale Ordine dei Medici Chirurghi e Odontoiatri
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IN COOPERATION WITH

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Chlamydia trachomatis infections - International Conference Como, Italy, 2009

The Biology of *Chlamydia trachomatis*: latest developments

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Chlamydia trachomatis is an obligate intracellular pathogen that grows with in a specialised cellular vacuole known as an inclusion. Each inclusion represents a micro colony of *C. trachomatis*. The infectious form of the microorganism or elementary body (EB) attaches to cells and after entry differentiates into a reticulate body (RB). RBs are metabolically active and replicate by binary fission: studies on both DNA and recovery of infection show a typical sigmoidal curve. The time to completion of the developmental cycle is dependent on strain and cell type and thusvaries from 36 to 72 hrs. The growth phase typically involves eight to ten bacterial divisions/generations. In the final phase, the developmental cycle becomes asynchronous and RBs differentiate back to EBs. Most studies on the chlamydial developmental cycle have been performed in vitro using continuous cell culture (usually Hela cells) with LGV strains of *C. trachomatis*: these are artificial systems that do not necessarily reflect what occurs *in vivo*. The "normal" lytic developmental cycle described above can be affected by a large number of factors including IFN - ?, antibiotic treatments, nutrient depletion, heat shock and bacteriophage infection. In these circumstances *Chlamydiae* lose their infectivity as RB to EB transition is retarded and the infection becomes "persistent". The detailed study of the biology of chlamydial infection has been severely hampered by the absence of a cell free system for culture. Research advances have also been hindered by the difficulty of making mutants and the lack of a simple routine method for transforming and manipulating the chlamydial genome. As an alternative approach chlamydial research has benefitted from genomics analysis and complete genome sequencing has revealed new insights into the biology of this microorganism. A significant recent milestone was the determination of the genome sequence for a cell culture adapted genital tract strain. The genome sequence of *C. trachomatis* is small relative to other genomes (~1.0 Mb) and it encodes the genes for some 900 proteins. In addition, most strains of *C. trachomatis* possess a 7.5 kb plasmid. All *Chlamydiae* share a core of conserved genes and interesting discoveries included the presence of a complete set of genes for peptidoglycan synthesis; the presence of genes for energy generating system (previously *Chlamydia* were thought to be energy parasites that imported ATP from their host) and the absence of the highly conserved prokaryotic gene for the key septation protein FtsZ. A major challenge is to unravel the molecular mechanism by which the developmental cycle is regulated. With the advent of high resolution time lapse microscopy it is now becoming clear that there is a large amount of signalling "traffic" between the chlamydial inclusion and the host cell. The genome sequence has been used to study gene expression by microarray and proteomic analyses. The development of new rapid sequencing protocols has meant that more genome sequences have become available. We have investigated the nature and extent of plasmid and genome variation in *C. trachomatis* by analysing the sequences of several new plasmids including the new variant Swedish *C. trachomatis* and its parental strain. The plasmid is of interest because it has been linked to virulence and it is also a very useful target for diagnostic testing because it is relatively small and because it is present at ten copies per genome. A detailed knowledge of the sequence conservation and the stability of chlamydial plasmids and genomes will be informative and critical in designing future diagnostics as well as the development of high resolution typing systems for detailed epidemiological studies.

Infections of the male genital tract

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Chlamydia trachomatis (Ct) serovars D to K (predominantly D, E and F), but not A to C, can undoubtedly infect the male genital tract. In so doing they may cause up to 50% of acute, and occasionally chronic, non-gonococcal urethritis (NGU). This is based on the significantly more frequent detection of the organisms in urethral samples and/or urine than in such samples from age-matched controls. LGV serovars 1 to 3 may cause a primary urethral ulcer and subsequent discharge, but this is an unusual presentation of LGV. Ct is also known to enter the ejaculatory duct(s) and ascend to the epididymis to cause acute inflammation. Thus, it is responsible for about 40-70% of cases of acute epididymitis, mainly in young men. This assertion is based largely on detection of the organisms in epididymal aspirates with or without serological support. Ct has also been implicated in reactive arthritis (Reiter's syndrome) occurring concomitantly with but mainly soon after acute NGU. Apart from this temporal relationship, the detection of Ct organisms/DNA in synovial fluids and enhanced antibody responses have supported the relationship. Despite the prostatic duct entering the prostatic urethra close to the ejaculatory ducts, evidence that Ct organisms gain access to the prostate during an episode of acute chlamydial NGU does not exist. This does not mean that they never do so and, although Ct involvement in chronic abacterial prostatitis is dubious, chronic disease sustained by an immunological response to Ct infection cannot be discounted completely. Also contentious is the role of Ct in male infertility. The latter can occur after bilateral epididymitis which is seen in some developing countries but rarely in developed ones. Men without a history of epididymitis but with Ct antibodies have a greater chance of developing anti-sperm antibodies than men without Ct antibodies. However, Ct antibody and HSP 60 antibody in seminal fluid have not been associated with the standard parameters of altered semen quality in male infertility. Indeed, whether unilateral or subclinical Ct epididymitis ever results in infertility remains unknown.

Lymphogranuloma venereum, where we stand in 2009

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Lymphogranuloma venereum (LGV) was formerly known as an STI confined to equatorial regions but also as an "imported" sexually transmissible infection in the Western world. However since 2003, with the first cases of LGV proctitis among men who have sex with men reported in the Netherlands, an ongoing epidemic has been revealed in Western society, including Italy1, dating back to at least 1981. In the beginning of this MSM associated LGV epidemic the far majority of cases were confined to

a "core group" of HIV positive individuals with multiple co-infections like hepatitis C, engaging in high risk sexual behaviour like unprotective anal intercourse, multiple anonymous partners, group sex, and use of anal enema's. Of recent date are the first reports from Spain and Portugal of heterosexual spread of LGV infections caused by the L2 serovar which causes most LGV infections among MSM in Europe.2 It is thus feasible that the LGV epidemic spreads from the initial "core group" via a bridging population to the community at large. For clinicians it is of importance to consider LGV as differential diagnosis in MSM presenting with persistent proctitis complaints. LGV proctitis can mimic inflammatory bowel diseases. Associated complaints, clinical findings and even histopathological finings of the 2 diseases overlap. Cases of LGV proctitis treated for prolonged periods as Crohns'disease with anti-inflammatory medication have been reported.3 The gold standard diagnostic assays for LGV are "inhouse" developed NAAT's. These tests require a specialised laboratory environment, and are often laborious which makes them expensive and not easily disseminated among microbiological laboratories. Moreover, optimal samples for the diagnosis of LGV require specialized examination procedures like anoscopy in case of LGV proctitis or bubo aspiration in case of inguinal LGV. Hence, there is a need for new easy to perform and cheaper diagnostic assays, to screen larger groups of individuals at risk, to prevent complications and to protect the community of more expansive transmission. LGV serovar specific *C. trachomatis* serological tests might fulfil this need but require thorough evaluation. A new improvement in *C. trachomatis* diagnostics is the development of NAAT's that can detect multiple infections with different serovars in one sample. These multiple serovar *Chlamydia* infections could have important implications for the diagnosis and treatment, specifically in LGV serovar co-infections. We have recently shown that proctitis caused by LGV serovars persist up to 16 days under doxycycline treatment whereas trachoma serovars are eliminated within 7 days. These findings have implications for the development of shorter antibiotic treatment courses for LGV. For future research, the microbial and immunological background of LGV infection in relation to hiv should be studied in detail and could help to explain the considerable number of asymptomatic LGV cases.

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Infections of the female genital tract

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Chlamydia trachomatis is the ultimate intracellular pathogen. C. *trachomatis* infections cause substantial morbidity including PID, tubal infertility, chronic pelvic pain, and ectopic pregnancy. *C. trachomatis* has also been linked to cervical cancer. *C. trachomatis* infection increases susceptibility to and transmission of HIV. Most women and men with chlamydial infection are asymptomatic. Recent major breakthroughs in the diagnosis and management of *C. trachomatis* infection include the development of accurate amplification tests, first void urine testing, vaginal or vulvar sampling, self-sampling, single dose therapy, and patient delivered partner therapy, just to name a few. The disease burden is still enormous and increasing. There is major frustration with opportunistic screening programs, implementation of management guidelines, contact tracing efforts, and school health education programs. This suggests that recommendations for screening may be inadequate, inefficient, and poorly implemented. Also, there is lack of connection between young adults and health care systems in general. Young adults do not acknowledge that chlamydial infection remains asymptomatic. Chlamydial infection of the cervix leads to mucopurulent cervicits. Characteristic features are mucopurulent endocervical discharge, edema and induced mucosal bleeding. Colposcopically, immature squamous metaplasia and hypertrophic ectopy are characteristic features of chlamydial cervicitis. Cytologic manifestations include increased number of PMN's, metaplastic atypia and endocervical cell atypia. One can suspect cervicitis based on increased number of PMN's on vaginal wet mount or gram stained cervical smears. Unfortunately these clinical findings are not specific to *C. trachomatis* and majority of women with chlamydial infection show no abnormal signs. Therefore, clinical recognition of chlamydial cervicitis very much depends on a high index of suspicion and a careful clinical examination. At least 50% of women with chlamydial cervicitis also have chlamydial urethral infection with *Chlamydia* isolated from both sites. *C. trachomatis* has been linked to acute urethral syndrome with pyuria but no bacteriuria. Again often times chlamydial urethritis is asymptomatic or minimally symptomatic. Pelvic inflammatory disease (PID) comprises a spectrum of upper genital tract inflammatory disorders in women which includes any combination of endometritis, salpingitis, tubo-ovarian abscess, pelvic peritonitis, and perihepatitis. Salpingitis is the most important feature of PID because salpingitis increases the risk for permanent tubal damage resulting in ectopic pregnancy or subfertility. Most PID in developed countries is sexually transmitted often caused by *C. trachomatis*. Infections following delivery or induced abortion are categorised separately as puerperal or postabortion infections. Clinical spectrum of PID manifestations varies from subclinical to severe. Sometimes chlamydial PID can cause upper abdominal infection which manifests as perihepatitis, i.e. right upper quadrant pain. Perihepatitis should be suspected in young sexually active women who develop right upper quadrant pain associated with general symptoms such as fever, nausea and increased C-reactive protein. Recently, severe inpatient PID has been decreasing in many countries. Most PID today is managed in outpatient clinics. Although *Chlamydia* rates in young adults have been increasing, PID rates have been declining. At the same time tubal pregnancy rates and the proportion of tubal factor infertility of all infertility have been decreasing. Recent population based studies suggest that the risk for PID associated with chlamydial infection has been over-estimated. This is not surprising since historical studies have been focusing on selected high risk populations which easily leads to overestimation of the risks and rates of complications. Thus, old studies have included several biases including selection bias, performance bias, ascertainment bias, detection bias, and perhaps also exclusion bias. Truly population based data may differ from data reported in case-control studies. This also means that the cost-effectiveness and health economical analyses of screening programs should be revised. Implementation of PID management guidelines has improved treatment of PID which may decrease risk for long-term complications. It appears that good news regarding PID are emerging. Inpatient PID has become a rare disease, ectopic pregnancy rates are decreasing and the proportion of tubal factor infertility of all infertility is decreasing suggesting that the primary objective of prevention of PID, i.e. to improve reproductive health in women can be reached. However, this is only true for developed countries and only countries with organised health care systems.

Epidemiology and surveillance of sexually transmitted diseases

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Sexually transmitted diseases (STD's) are among the most common infectious diseases today. More than 20 STD's have now been identified, and they affect several million men and women in Western Countries (where we have relatively reliable estimates) each year. The annual comprehensive cost of STD's, only in the United States, is estimated to be well in excess of $10 billion. To try to understand the epidemiology of STD and evaluate the real possibilities of their surveillance, it is important to take into consideration at least five key points: 1. STD's affect men and women of all backgrounds and economic levels. They are most prevalent among teenagers and young adults. Nearly two-thirds of all STD's occur in people younger than 25 years of age. 2. The incidence of STD's is rising, in part because in the last few decades, young people have become sexually active earlier yet are marrying later. In addition, divorce is more common. The net result is that sexually active people are more likely to have multiple sex partners and are more likely to acquire STD's. 3. Often STD's cause no symptoms, at least in the initial stages of the disease. This is especially true in women. If symptoms develop, they may be confused with those of other diseases not transmitted through sexual contact. Even when an STD causes no symptoms a person who is infected may be able to pass the disease on to a sex partner (ex. genital herpes, HIV). 4. Health problems caused by STD's tend to be more severe and more frequent for women than for men. This is because of the increased frequency of asymptomatic infections. As a result many women do not seek care until serious problems develop. Some STD's can spread into the uterus and fallopian tubes to cause pelvic inflammatory disease (PID), which in turn is the major cause of both involuntary infertility and ectopic (tubal) pregnancy. STD's in women also may be associated with cervical cancer (e.g. *Papillomavirus* infections). STD's can be passed from a mother to her baby before, during, or immediately after birth. Some of these infections of the newborn can be cured easily (ex. *Opthalmia neonatorium*), but others may cause a baby to be permanently disabled (ex. congenital syphilis or CMV) or even die (e.g. Herpes Simplex virus, HIV). 5. When diagnosed and treated early, many STDs can be treated effectively. Some infections have become resistant to the drugs used to treat them and now require different types of antibiotics. Some can not be cured and can be terminal (ex. HIV, chronic HBV). Experts believe that having STD's other than AIDS increases one's risk for becoming infected with the HIV.

The epidemiology of *Chlamydia trachomatis*

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Genital infection with *Chlamydia trachomatis* is the most common bacterial sexually transmitted infection in many European countries. (3) The reasons for the evolutionary success of the organism are complex: although it is easy to treat it frequently evades detection as it produces no symptoms. However, the long term consequences can be severe in a proportion of cases and therefore it is considered a serious public health problem. (2) In women these consequences include pelvic inflammatory disease (PID), tubal infertility, ectopic pregnancy and in pregnancy is associated with poor reproductive outcomes. In the UK it has been estimated that 64000 cases of PID and 3000 ectopic pregnancies each year are attributable to Chlamydial infection, although the evidence for these statements is weak. These complications cause considerable distress to the individuals and, in the case of infertility, have major cost implications for health services. (1) The number of cases of *Chlamydia* being diagnosed in Europe continues to rise. This increase is in part due to more widespread testing and greater sensitivity of the tests used, both of which make it difficult to interpret time trends. Variations in screening and reporting also result in a wide range of reported rates of *Chlamydia* across different countries. The highest rates of *Chlamydia* occur in young people aged <25 years, and risk increases with the number of sexual partners (4, 7) but there are no other clear risk factors. Household surveys in seven European countries showed a *Chlamydia* prevalence of 1.4 to 3.0% in people aged 18 to 44 years. The National *Chlamydia* Screening Programme in England offers screening to young men and women (aged under 25); around 10% of tests have been positive (6) and the programme with estimates of up to 450,000 young people infected. In France a national study estimated that around 160 000 young people aged 18-24 years were asymptomatic carriers of *Chlamydia*. In the USA, there are an estimated 2.8 million new cases of *Chlamydia* each year. (5) The lack of specific risk factors, combined with the wide distribution of disease, are key challenges for the control of *Chlamydia* infection. Strategies based on case finding and partner notification in core groups are likely to be ineffective, and therefore widespread systematic screening is likely to be required.

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Chlamydia trachomatis infections in Italy

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In Italy, infection with *Chlamydia trachomatis* (Ct) is not subject to mandatory notification. There do not exist national guidelines regarding who should be tested for Ct, nor do there exist screening programmes. The Ct test is not mandatory even for pregnant women. Since 1991, a Sexually Transmitted Infection (STI) Sentinel Surveillance System collects, among other STIs, information on patients with a laboratory confirmed diagnosis of *Chlamydia* infection. Socio-demographic and behavioural

information are recorded, as is the person's HIV serostatus. According to the STI Sentinel Surveillance System, in the period from 2000 to 2005 (most recent data available), Ct infection represented 5.1% of all of the STIs reported, and it was the second most commonly reported bacterial STI, following syphilis. In January 2008, a laboratory based surveillance of new cases of Ct infection has been launched. The data are provided by 14 large public laboratories in Italy; socio-demographic and behavioural information are also collected. According to the data collected by this network of laboratories, 3.0% of all persons tested in the period from 1 April to 31 July 2008 were positive for Ct infection. The highest proportion of Ct-positive cases (7.2%) was found among young persons (i.e., 14-24 years of age).

Screening programmes for Chlamydial infection

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There are many beliefs, perceptions and interpretations about the existence, types, effectiveness and sustainability of screening programmes for sexually transmitted *C. trachomatis* infections. This talk will explore the history of, evidence for, and current status of *Chlamydia* screening in Europe, North America and Australia. The talk aims to provide clear definitions of terminology, explain the public health objectives of screening programmes, and evaluate the scientific evidence for *Chlamydia* screening using systematic reviews, critical appraisal, and results of current research. The needs for future research will be discussed.

The evolution of *Chlamydia trachomatis* diagnostics

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More than 30 years ago, diagnostic laboratories were abandoning the inoculation of yolk sacs in embryonated hen's eggs to propagate *Chlamydia trachomatis*. This was being replaced by inoculation of cervical and urethral samples onto cell cultures, then staining intracellular inclusions to diagnose infections. This improvement in laboratory technology enabled discovery of organism transmission and disease etiology in a more accurate way than the measurement of chlamydial antibodies. Serology has always been a difficult laboratory approach to diagnosis because of uncertainty in assigning levels of significance to the different immunoglobulin classes observed after infection. Fast on the heels of cell culture the discovery of monoclonal antibodies enabled the design of immunoassays for the detection of chlamydial antigens in clinical specimens. New enzyme immunoassays and fluorescent antibody-based assays enabled many diagnostic laboratories, without cell culture capacity, to begin diagnosing chlamydial infections. Many published comparisons appeared using cell culture as the gold standard and less invasive samples such as urine were shown to be effective in identifying infected men, who were often asymptomatic. Commercialization of diagnostic assays and effective drug therapy significantly moved the field forward. Antigen detection assays were soon joined in the market place by nucleic acid hybridization methods, which were equally sensitive and specific. Soon diagnostic companies were introducing Nucleic Acid Amplification Tests (NAATs) such as polymerase chain reaction, ligase chain reaction, strand displacement amplification, nucleic acid sequence based amplification and transcription mediated amplification. *Chlamydia* diagnosticians thought they were in a candy store; however, not all the "candy" was equally sweet. During the last 20 years thousands of comparisons were performed and showed that, although the NAATs are much superior for diagnosing infections in both symptomatic and asymptomatic men and women, they were not all created equal. These differences became apparent from studies which tested a multitude of different sample types from individual patients in more than one test. This showed the ability of a test performed on a specimen type to identify an infected patient. The approach also showed the real benefit of testing less invasive samples [urine, vaginal, meatal, etcl] and samples routinely collected in Pap screening programmes. Thus the ability to screen patients with highly accurate laboratory assays, now provides opportunities to treat infections, which may often be asymptomatic. This can provide interruption of transmission to uninfected individuals, and prevent ascending infection and the consequences of pelvic pain, ectopic pregnancy and infertility. This four-decade success story has been possible because of co-operation between clinical researchers and industry.

Self collected or non invasive samples

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Diagnosis of *Chlamydia trachomatis* (Ct) as one of the most common and important bacterial sexually transmitted pathogens has improved by the introduction of nucleic acid amplification tests (NAATs). There is clear evidence that the performance pattern of molecular biological test systems is high for invasive as well as for noninvasive genital specimen types in symptomatic and asymptomatic individuals. The advantage of the high sensitivity of NAATs is their ability to detect organisms with a low target concentration, which often occurs in genital samples of asymptomatic individuals and their contact persons without signs of inflammation. Urine, vulval, and vaginal samples have been compared with invasive specimen types and can be recommended for screening programmes in asymptomatic young individuals, who are expected to have a low load of organisms, but belong to a risk group for acquiring *C. trachomatis*. Several comparison studies have indicated that vulval or vaginal specimens and even patient-obtained vaginal swabs serve as suitable alternative non-invasive testing to urine collection for the detection of Ct. Screening abilities are even increased by using self administered vulvovaginal samples for the diagnosis of both, Ct and GC which permit testing without a speculum examination within few hours and even without clinical inspection. Also data on the performance of penile swabs are available. A low number of organisms may also be observed at atypical infection sites such as rectal or pharyngeal regions, where amplification tests are recommended as the preferable diagnostic techniques to all other methods. Although it is well known that NAATs are nowadays regarded as the gold standard method for diagnosis of genital *Chlamydia* infections these technologies are still not well established in many parts of the world for routine diagnosis. Focusing on NAATs, is there still room for further improvement? Have we already reached the endpoint of *Chlamydia* diagnosis? There are still open questions which will be discussed and solved in the next several years.

The Swedish variant

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Sweden experienced an increase in reported chlamydial infections from the mid 90ies to 2005. In some areas of the country there was however a decrease in + 2006 up to 25%, a decrease that could not be explained by changes in general sexual behaviour or health care giving behaviour. Laboratory diagnose of Ct in the country was almost exclusively done by NAATs, 80% by kits provided by Abbott and Roche. The unexplained decrease was found to be caused by a big deletion in the plasmid DNA, covering the target areas for these two tests. The spread of the variant in Sweden was studied and 25-60 % of all chlamydial infections were found to be variant infections in 2006. Few known cases have been detected in other countries. New tests were developed by the named producers that targeted the variant as well as the wild types. The fate of a chlamydial clone escaping specific diagnose for an unknown time period when it suddenly can be detected by routinely used tests will be described.

The role of serology

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Diagnosis of acute uncomplicated urogenital *C. trachomatis* infection is most reliably based on amplification and detection of bacterial nucleic acid in first-void urine or in urogenital swab. However, serology has a role in the diagnosis of complicated *C. trachomatis* infections (reactive arthritis, PID, tubal factor infertility), infant pneumonitis and LGV infections as well as in research and seroepidemiological studies. Chlamydial antibodies can persist after infection and on the other hand, after mucosal infection, systemic antibody response may not be measurable, which limit the value of existing serological tests in the diagnosis. Moreover, testing of a single serum specimen cannot usually provide conclusive evidence of current infection. Also, prompt antimicrobial treatment can "arrest immunity" and result in delayed or diminished humoral immunity after infection. The first widely used serological test in was complement fixation (CF) test that detects antibodies against the chlamydial genus-specific antigen. The frequent occurrence of *C. pneumoniae* IgG antibodies in population potentially complicates use of genus-specific serological tests in diagnosis. Moreover, the CF test is relatively insensitive in genital and ocular *C. trachomatis* infections and of little value in their diagnosis. The golden standard for *Chlamydia trachomatis* serology has been the micro-immunofluorescence (MIF) test. The MIF test, initially developed for serotyping of chlamydial isolates, measures antibodies against chlamydial surface antigen, the major outer membrane protein (MOMP). This test is able to differentiate genus-, species- and even serotype-specific antibodies. Moreover, IgG, IgM and IgA antibodies can be measured separately and therefore MIF may distinguish recent infections from past infections, if optimal (sequential) samples are available. The MIF test offers high sensitivity and specificity, but it is labor intensive, and requires special skills and experience. Efforts to simplify the MIF test have resulted in loss of sensitivity and specificity. A number of supposedly *C. trachomatis* antibody specific enzyme immunoassays (EIA) using synthetic peptides as antigen have recently been developed. Also, recently identified surface proteins (putative outer membrane proteins, pmps) might prove to be useful antigens in serology. Sensitivity and specificity of the novel assays need to be rigorously evaluated. Despite of the limitations serology can aid in the diagnosis of *C. trachomatis* infection, but the serological results should not be interpreted out of context.

Antimicrobial susceptibility in *Chlamydia trachomatis*

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In vitro susceptibility testing for *Chlamydia trachomatis* is not currently performed because a standardized method is lacking. *In vitro*, the most active drugs are rifampin and the tetracyclines, followed by macrolides and some fluoroquinolones (ofloxacin and the newer fluoroquinolones). No apparent emergence of antimicrobial resistance has been observed in *C. trachomatis* human isolates although some case reports suggested resistance as a cause of treatment failure. Mutants resistant to fluoroquinolones and rifampin have been selected *in vitro* and four clinical isolates, resistant to macrolides, were shown to carry 23S rRNA mutations. Heterotypic resistance can be observed when cells are inoculated with a large number of organisms. It affects a small proportion of organisms that are difficult to propagate and may not survive in cell culture. Supplemental studies would be necessary to explain the treatment failures.

Syndromic approach and *Chlamydia* infection in women

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INTRODUCTION. Syndromic Approach (SA) has been promoted for the management of Sexually Transmitted Infections (STI) but its performance in identifying cervical infections has been poor. Our objective was to evaluate the performance of SA for Chlamydial infection in women in a STI clinic run by trained medical staff.
MATERIALS AND METHODS. Women attending the STI clinic placed in the Infectious Diseases Department of the University of Torino and ASL TO2, Torino, Italy, between 1997 and 2008 were classified according to WHO syndromic approach. All women received a medical examination by trained medical staff and were tested for Chlamydial infection by PCR on cervical swabs (Roche Amplicore).
RESULTS. Results were available for a total of 4106 examinations on 2878 women. 245 cases of *Chlamydia trachomatis* (Ct) were detected (5.9%). Women classified as having a vaginal discharge (n=990) had a prevalence of *Chlamydia* of 7.3%, with no statistically significant difference with the prevalence rate in asymptomatic women (5.8%). Women with cervical discharge,

observed on examination with speculum, had a rate of infection of 18.4% (p<0.001): however, only 39 out of 245 Ct cases (15.9%) presented with this sign. Cervical motion tenderness was associated with Ct infection but this sign also was unfrequently detected (12.2% of women with Ct infection, p<0.001). Risk factors predicting a Ct infection were analysed: the only association found was with age less than 25 years. No association was found with age at sexual debout, number of partners/life or number of partners in the last 6 months, prostitution, drug use, HIV infection and presence of symptoms. Only 84 out of 245 women with Ct (34.2%) were appropriately treated on the first visit; 78% of women who received a presumptive treatment for Ct were negative at PCR.
CONCLUSION. Syndromic approach does not perform well in Ct infection even if applied by trained medical staff. Overtreatment is an important problem but even more important is the possible loss of more than 65% of cases who are not treated upon the first examination. Risk assessment can add very little to the sensitivity of this approach for *Chlamydia* infection in women.

Chlamydial Infection in Males: a prevalence study in Turin

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INTRODUCTION. *Chlamydia trachomatis* (Ct) is the most common sexually transmitted agent, causing millions of new infections each year. The majority of acute infections in males and females remain undetected and untreated because of few or no specific symptoms. If untreated, Ct infections may progress to severe chronic complications such as pelvic inflammatory disease in females, urethritis and epididymitis in males. Aim of the study was to determine the prevalence of *Chlamydia trachomatis* infection in males attending the infectious disease hospital Amedeo di Savoia in Turin, Italy, over a one-year period (from January to December 2008).
METHODS. A retrospective analysis was conducted on 893 male patients, aged 14-88 (median age 35). Ct DNA was tested on urethral and anal swabs, first void urines and seminal fluids, using a real time qualitative PCR (COBAS TaqMan Ct Test v2.0, Roche Diagnostics). The PCR mix contains primers pairs and probes specific for the Ct cryptic plasmid DNA and the Ct Chromosomal ompA gene DNA.
RESULTS. Among the 893 patients, 695 (78%) were from the Sexually Transmitted Infections (STI) clinic and 198 (22%) were outpatients. The overall prevalence of Ct infection was 7% (62/893): 53/62 (85,5%) patients were from the STI clinic and 8 (13%) were outpatients. The highest percentage (13.3%) of positive patients were less than 20 years old. In the other age categories, the prevalence of Ct infection was as follow: 7.7% in patients between 20-30 years old, 7% in those between 30-40 and 6% in those > 40 years old. The most representative specimens were urethral and anal swabs (19% and 18,4% of positive samples respectively) vs urine specimens (only 4% of positive samples). No seminal fluid resulted positive. In a detailed analysis of the STI patients, the majority of them (41/53, 77.4%) were of Italian origin, 26 (49%) were homosexual, 27 (51%) heterosexual; the average number of the partners in the previous 6 months was 7 (range 1-50 partners). The 17% (9/53) were HIV positive. Asymptomatic infection was detected in 41,5% of patients (22/53) (7 with other concomitant STI infections: 4 syphilis and 3 condylomata), while symptomatic infection were present in 31 patients (urethritis n=22, proctitis n=8 and HSV ulcers n=1). Other STI infections were found in 19/31 symptomatic patients: 5 patients had syphilis, 2 HSV, 8 gonococcal infection and 4 condylomata. Concomitant gonococcal infections were found only in symptomatic patients.
CONCLUSIONS. WHO guidelines recommend that screening strategies for Ct infection should be aimed at women. In our experience, however, infection in males is as prevalent as in females, therefore screening for Ct should also be performed at least on high risk male patients. The importance of Ct infection in male is often underrated. Even if first void urine is the more amenable way of collecting samples, urethral and anal swabs are more representative in high risk patients for Ct detection.

Prevalenza e fattori di rischio di infezione da *Chlamydia trachomatis* e *Neisseria gonorrhoeae* in detenuti di età compresa tra 18 e 40 anni

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OBIETTIVI. Poiché in Italia non esistono studi epidemiologici sistematici sulla prevalenza dell'infezione da *Chlamydia trachomatis* (Ct) e da *Neisseria gonorrhoeae* (Ng) tra la popolazione carceraria, ci si è proposti di fornire una prima valutazione dell'infezione urogenitale prevalente da Ct e Ng in una popolazione relativamente omogenea, di età compresa tra i 18 e i 40 anni, rappresentata da soggetti maschi detenuti presso la Casa Circondariale di Quarto d'Asti (AT).
METODI. Studio sistematico di prevalenza tra soggetti maschi, asintomatici, per infezione da Ct e Ng, previa somministrazione di un questionario conoscitivo anonimo sull'esposizione ai principali fattori di rischio riportati dalla letteratura (età del primo rapporto, numero di partners negli ultimi 5 anni, orientamento sessuale, uso di metodi contraccettivi di barriera, tossicodipendenza, alcolismo e sesso a pagamento). Ogni soggetto ha contestualmente fornito un campione di urina (primo getto), raccolto in provette da trasporto (UPT, Becton Dickinson) su cui è stata effettuata RT-PCR con tecnologia SDA per *Chlamydia trachomatis* (BD ProbetecTM). Risultati. Sono stati analizzati sino ad ora 60 campioni. Non sono state riscontrate positività per infezione da Ng mentre sono stati evidenziati tre campioni positivi per infezione da Ct (prevalenza: 5%) appartenenti a soggetti eterosessuali, di età compresa tra i 23 e 28 aa. Tutti hanno dichiarato un precoce inizio dell'attività sessuale (15-17 aa), un numero di partners negli ultimi 5 anni superiore a 5 ed un utilizzo non costante dei metodi contraccettivi di barriera. Nessuno dei tre soggetti ha riferito l'assunzione di sostanze d'abuso ed uno solo di essi ha dichiarato sesso a pagamento nell'arco dei 5 anni precedenti l'esecuzione del test.
CONCLUSIONI. La prevalenza per infezione da Ct sino ad ora riscontrata risulta in accordo con quella riportata dalla letteratura internazionale in studi condotti su popolazioni di detenuti. Si segnala altresì l'assenza di casi di positività per *Neisseria gonorrhoeae*, a conferma della ridotta prevalenza rilevata dalla letteratura internazionale. Tuttavia la numerosità non elevata del campione non permette al momento di poter condurre un'analisi approfondita dei dati raccolti, che andranno completati nel prosieguo dell'indagine.

Ruolo di *Chlamydia trachomatis* nella patogenesi della prostatite cronica batterica

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INTRODUZIONE. Nell'ultimo decennio, numerosi gruppi di ricerca hanno concordemente affermato che, sulla base di evidenze cliniche, microorganismi diversi dagli uropatogeni tradizionali possono essere implicati nella genesi della Prostatite Cronica Batterica (PCB di classe II, Classificazione NIH). Tra essi, microorganismi responsabili di patologie sessualmente trasmesse quali *Chlamydia trachomatis* (Ct) sono indicati quali potenziali agenti causativi delle prostatiti batteriche. Allo scopo di valutare questa ipotesi e di porre in relazione l'andamento della sintomatologia clinica con l'eradicazione di Ct dai distretti interessati (uretra, prostata, vescicole seminali), abbiamo valutato in uno studio retrospettivo 50 pazienti con positività per Ct in campioni microbiologici diversi.
METODI. I pazienti sono stati stratificati in tre categorie in base all'identificazione unicamente prostatica (gruppo P: 17 pazienti), unicamente uretrale (gruppo U: 19 pazienti) o prostatica e uretrale (P+U: 14 pazienti) del patogeno. I pazienti P, sono stati trattati con terapia medica di associazione comprendente levofloxacina (500 mg/die) e azitromicina (1500 mg/settimana) per quattro settimane. I pazienti U, sono stati sottoposti a trattamento a breve termine con azitromicina (500 mg/die per tre giorni consecutivi), e i pazienti P+U, hanno subito in sequenza due trattamenti: azitromicina per tre giorni seguita, dopo valutazione microbiologica, da 4 settimane di terapia di associazione (levofloxacina+azitromicina). Segni e sintomi di PCB sono stati analizzati mediante questionario NIH-CPSI e indagini microbiologiche (tampone uretrale, Test di Meares-Stamey, eiaculato totale) e strumentali (uroflussimetria). Risultati. Nei gruppi P e U+P, è stata osservata un'associazione significativa tra la regressione clinicamente apprezzabile (6 punti) dello score NIH-CPSI e l'eradicazione del patogeno (Odds Ratio: 12; 95% CI: 1.28- 111.77). Stratificando le risposte relative al dominio "dolore" del questionario NIH-CPSI, i pazienti del gruppo U, hanno mostrato una riduzione marcata e significativa dei punteggi relativi alla sintomatologia dolorosa al termine del breve ciclo di trattamento con azitromicina. Al contrario, i soggetti U+P hanno mostrato persistenza della sintomatologia e risoluzione della stessa solo dopo terapia a lungo termine, mirata all'infezione profonda. Tale comportamento è sovrapponibile a quello dei pazienti che mostravano solo l'infezione a livello prostatico.
CONCLUSIONI. Nei limiti di un'analisi di tipo osservazionale, il presente studio suggerisce come, in presenza di positività per Ct in campioni uretrali, l'indagine microbiologica delle secrezioni prostato-vescicolari può rivelare la presenza del patogeno a livello profondo. Queste osservazioni presentano importanti implicazioni diagnostico-terapeutiche e possono contribuire a spiegare i numerosi casi di mancata risoluzione di patologie inizialmente diagnosticate quali uretriti aspecifiche.

Le infezioni oculari da *Chlamydia trachomatis*. Studio su 756 casi presso l'ospedale oftalmico di Torino

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INTRODUZIONE. Scopo del lavoro è stato valutare la frequenza delle infezioni oculari da *Chlamydia trachomatis* nella popolazione generale affluente ad un ospedale oftalmico, in base all'età, al sesso, a durata della sintomatologia, fattori di rischio quali: frequentazione di piscine, uso di lenti a contatto, presenza di concomitanti infezioni genitali, abitazione rurale o urbana.
METODI. Sono stati valutati 756 pazienti giunti alla nostra osservazione presso il servizio di Patologia Clinica dell'Ospedale Oftalmico di Torino con diagnosi di congiuntivite o cheratocongiuntivite. Ad ogni paziente oltre il prelievo per la ricerca della *Chlamydia trachomatis* in immunofluorescenza diretta (IF) con tampone di dacron sfregato sul fornice congiuntivale inferiore per prelevare un numero sufficiente di cellule, veniva anche eseguito uno striscio congiuntivale per la ricerca batterica. La positività per le *Chlamydia trachomatis* era data dalla presenza di tre o più corpi elementari. Per ogni paziente era compilata una scheda riportante: dati anagrafici e fattori di rischio.
RISULTATI. Dei 756 casi esaminati (479 femmine e 277 maschi), 159 (21.03%)sono risultati positivi. Tra i maschi, i positivi sono stati 52 (18.8%) e le femmine 107 (22.3%). I pazienti sono poi stati ragruppati in 4 fasce di età: 16-30, 31-45, 46-60, 61-75 con le seguenti percentuali di positività: 25.8%, 21.7%, 18.4% e 12.8% con una differenza statisticamente significativa per $p<0.05$ tra la fascia 16-25 e 61-75. In base alla durata della sintomatologia (giorni, mesi, anni) si è visto che il 24% dei positivi aveva sintomatologia datante giorni, il 23% mesi, il 18.7% anni. Per i singoli fattori di rischio si è osservato che il 25.5% dei positivi era frequentatore di piscine, il 25.6% portatore di lenti a contatto, il 21.7% di infezioni genitali note, il 20.3% abitava in zone rurali. Dei 756 pazienti, 82 erano poi anche portatori di infezioni batteriche di cui 9 (1.2%) in associazione con le *Chlamydiae* e 73 (9.6%) senza associazione.
CONCLUSIONI. Si è osservata la massima frequenza di infezioni nella fascia di età 16-20 anni (36.6%) con picco per le femmine pari al 42.8% in corrispondenza del periodo di massima attività sessuale e nel periodo in cui si ha la maggior tendenza a cambiare partner. Per i singoli fattori di rischio, considerando i valori di X quadro, che tiene conto anche della numerosità della popolazione, solo chi è portatore di lenti a contatto ha un $p<0.25$. Inoltre secondo i nostri dati non sempre la patologia è cronica; infatti la percentuale riscontrata nei pazienti giunti con sintomatologia datante pochi giorni è pressoché simile a quella datante mesi o anni, così come circa il 10% di congiuntiviti follicolari erano sostenute da batteri. Possiamo concludere che non sono sufficienti all'oculista, le osservazioni cliniche e una buona anamnesi, ma spesso è necessario ricorrere alla conferma microbiologica.

Dry flocked swabs for the collection and transportation of clinical specimens for the diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* with the ProbeTec Assay

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OBJECTIVES. Self collection of genital samples is less invasive and well accepted by patients as a screening tool for the diagnosis of sexual transmitted diseases. It has been reported that Flocked Swabs (FS) have been used for professional or self collection of vaginal specimens for the detection of Sexual Transmitted Diseases (STD) using Nucleic Acids Amplifications Tests (NAATs). The objective was to compare the Copan FS to the ProbeTec Swabs (PTS) for the ability to preserve nucleic acid for collecting and transporting dry samples for the diagnosis of sexual transmitted infections with the BD ProbeTec (PT) assay.
METHODS. Cervical FS and PTS were tested to determine the positivity rate after Room Temperature (RT) storage. Cervical samples from a gynaecologist practice (n=151) and from a hospital clinic (n=168), transported dry, were used in this clinical study. ATTC strains of *Chlamydia trachomatis* (Ct) and *Neisseria gonorrhoeae* (Ng) were titrated to find the detection limit with the PT. 2 dilutions above and one at the limit of detection were used. FS and PTS, contrived specimens, were prepared by dipping each swab in the Ct and Ng suspensions and stored dry in its own plastic tube. Triplicate testing was done at 0 times, and after 1, 2, 3, 4, and 5 weeks at RT. Two FS and one PTS were used, in random order, to collect cervical samples from 151 patients. A FS and a PTS samples were tested after collection, the other FS sample, stored dry at RT, was tested after 2 weeks. Ct and Ng detection for the contrived and 151 clinical samples was done with the PT assay. Two cervical samples were collected with FS from 168 patients, one was tested at collection for Ct using the Nanogen Ct real time PCR, the other was stored in its own plastic tube and tested after 2 to 3 weeks for Ct and Ng with the PT.
RESULTS. The positivity of the dry FS contrived samples, tested after 1 or 5 weeks at RT, was higher than the PTS samples: After one week, Ng 77% versus 66%, Ct 66% versus 22%. After 5 weeks Ng 66% versus 22% Ct 22% versus 0%. In the 151 clinical specimens 9/151 (6%) were positive for Ct and 1/151 for Ng when tested at collection time with FS and PTS and after 2 weeks, stored dry, with the flocked swabs. In the 168 clinical specimens, collected with FS and tested for Ct at collection by real time PCR, 21/168 (12.5%) were positive; the FS stored dry and tested with the PT after 2 or 3 weeks detected 22/168 Ct (13%) positive and 2/168 (1.19%) Ng.
CONCLUSION. The positivity of the dry FS contrived specimens was better than PT for both Ng and Ct after one or 5 weeks storage at RT. The Copan flocked swabs, stored and transported dry in its own plastic tube demonstrated the ability to preserve Ct and Ng nucleic acids in clinical specimens and can be used for professional or self collection of genital samples for STD's screening with NAATs.

Chlamydia surveillance in a country where it is not a reportable disease: Data from the German STD-S Sentinel

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BACKGROUND. *Chlamydia trachomatis* (Ct) infections are often asymptomatic, especially in women, and can lead to infertility. In Germany, the new Infection Protection Act was implemented in 2001 and only syphilis and HIV were stated reportable STIs in the stricter sense. Within the STD-Sentinel a subset of health care providers voluntarily report the incidence of STIs.
METHODS. Since the end of 2002 local health authorities, specialized outpatient clinics and practitioners continually report examination and infection data, demographic characteristics and possible risk factors. Patients are asked to return completed questionnaires which provide information about patients' social state, sexual behaviour and possible source of infection.
RESULTS. Between Jan. 2003 and Dec. 2008, 80960 Ct-examinations were performed in sentinel- institutions, 6% (4845/80960) of these were positive. The number of positive *Chlamydia*-results was higher than any other lab-confirmed STI, namely *gonorrhoeae* 3.6% (2946/80873), HIV 1.1% (2996/264085), syphilis 3% (2751/91627) and *Trichomonas* 2.6% (1961/76581). For 2640 of these Ct infections further data were provided: 65% were women and the overall mean age was 29 years, men being significantly older.61% of women were not of German origin. When Ct-patients were asked for the reason of attending, 54% of women said that they wanted to have a "health check" whereas 24% reported "health problems". In men the rates were 19% and 57%. As a possible source of infection doctors constituted in 76% heterosexual contacts (46% prostitution) and in 14% MSM contacts.12% of all Ct-patients had already had a Ct-infection in the past. 10% were co-infected with *gonorrhoeae*. Of the 908 Ct-patients who filled in the questionnaire 61% were women. 62% of men reported casual partners as their perceived source of infection. In women, 34% believed their regular partner to be the source of infections, 33% named customers. The median number of sexual partners within the last 6 months was 3. 13% of men reported consistent condom use with their regular partner, and 17% of women. 22% of men and 52% of women reported consistent condom use with casual partners, 31% of men and 13% of women reported never using condoms.
CONCLUSION. Due to high rates of Ct-infected women who only attended for a "health check" i.e. were asymptomatic, Ct might be an under diagnosed but frequent infection in Germany. Continuous surveillance through a sentinel system is labour-intense and disease-trends over time have to be interpreted cautiously as selection bias is always an issue. Patients seen in STD-Sentinel institutions have high (re-)infection rates, are frequently working in the sex industry and are migrants or MSM. Therefore data from the sentinel cannot be interpreted as infection rates in the general population. The recently implemented Ct screening programme for all women under the age of 25 will hopefully soon provide more reliable public health data on the epidemiology of STIs in Germany.

La Sorveglianza delle Infezioni da *Chlamydia trachomatis*, da *Neisseria gonorrhoeae* e da *Trichomonas vaginalis* mediante una Rete Nazionale di Laboratori

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INTRODUZIONE. In Italia, nonostante l'apporto conoscitivo del Sistema di Sorveglianza delle Malattie Sessualmente Trasmesse, i dati sulla diffusione soprattutto delle infezioni da *Chlamydia trachomatis* (Ct), da *Neisseria gonorrhoeae* (Ng) e da *Trichomonas vaginalis* (Tv) sono ancora molto scarsi.
OBIETTIVI. Per migliorare le conoscenze sulla diffusione di queste infezioni, l'Istituto Superiore di Sanità con il Gruppo di Lavoro "Infezioni Sessualmente Trasmesse" (GLIST) dell'Associazione Microbiologi Clinici Italiani (AMCLI) ha avviato un Programma di Sorveglianza Epidemiologica basato sulla segnalazione dei nuovi casi di infezione da una Rete Sentinella di Laboratori pubblici.
METODI. Sono stati inclusi 14 laboratori di microbiologia clinica operanti sul territorio nazionale, selezionati tra quelli partecipanti all'Indagine policentrica sulle infezioni da Ct, da Ng e da Tv, condotta nel 2007 dal GLIST. I laboratori dovevano segnalare le determinazioni riferite a individui sottoposti a diagnosi per una o più delle tre infezioni (sia positive che negative all'identificazione del patogeno), utilizzando metodi di amplificazione dell'acido nucleico (NAATs) per la ricerca di Ct, Ng e Tv e di identificazione microscopica o colturale per la ricerca di Ng e Tv . Inoltre per ogni individuo si poteva segnalare l'eventuale identificazione di più di un patogeno.
RISULTATI. Il programma ha previsto uno studio pilota dal 1 aprile al 30 giugno 2008. In questo periodo i laboratori hanno riportato dati relativi a 6.403 individui testati; l'88.6% di questi era costituito da donne e l'11.8% da stranieri. L'età mediana del campione era di 34 anni (range interquartile = 29-39 anni) per le donne e di 37 anni (range interquartile = 32-43 anni) per gli uomini. Il 46.4% degli individui era sintomatico al momento del prelievo. L'80.2% riferiva di non aver utilizzato nessun metodo contraccettivo negli ultimi 6 mesi e il 25.8% delle donne era in gravidanza. Il 95.4% dei soggetti dichiarava di aver avuto un solo partner sessuale negli ultimi 6 mesi e nell' 82.7% dei casi questo era un partner stabile. Le prevalenze di Ct, di Ng e di Tv erano rispettivamente pari a: 2.8%, 0.2% e 0.8%. Le prevalenze più elevate sono state osservate tra i maschi sintomatici sia per l'infezione da Ct (13.2%) che da Ng (1.6%), tra le donne con sintomatologia non determinata per l'infezione da Ct (4.6%), e tra le donne sintomatiche per l'infezione da Tv (1.4%). Non c'era una differenza significativa tra gli italiani e gli stranieri per le prevalenze di infezione da Ct e da Ng; mentre la prevalenza dell'infezione da Tv era significativamente più elevata tra gli stranieri rispetto agli italiani (2.0% vs. 0.7%, OR= 3.13, IC95%: 1.58-6.12).
CONCLUSIONI. La generale applicabilità del protocollo per la quasi totalità dei laboratori partecipanti allo studio pilota, ha consentito di fissare quale data di attivazione ufficiale della sorveglianza quella del 1 aprile 2009.

Prevalence and risk factors of *Chlamydia trachomatis* infection in young women attending family planning clinics in Torino

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INTRODUCTION. *Chlamydia trachomatis* (Ct) infection accounts for one of the most widespread Sexually Transmitted Infections (STIs) in industrialized countries. The infection is most common among young women less than 25 years old. Unlike other infections, the higher costs of Ct infection are associated with undiagnosed cases and management of the long-term sequelae associated with untreated infection, including PID, ectopic pregnancy, or infertility. Therefore, timely diagnosis and care of Ct infection are among the biggest priorities in Public Health.
OBJECTIVES. The aim of the study is to determine the prevalence of Ct infection in people less than 25 years of age attending family planning clinics in Torino and to characterize risk groups in order to develop a screening policy.
METHODS. Women were tested for Ct by a vaginal sample performed during routine gynecological examination. Participating clinics received a kit for collecting the sample, a structured patient questionnaire, and an illustrated brochure about Ct infection. Tests were performed with Real Time PCR (Real-Time Alert-Nanogen), upon DNA extraction with the system EasyMag (bioMérieux). Samples were grouped in pools of 5 so as to keep costs low. The positive pools were tested a second time as single samples.
RESULTS. During 2008, 741 women were enrolled. Among the study cohort, 30% were 19 years of age or younger. More than 84% of the cases were Italian women and 16% were women born abroad. 56% of the women reported leucorrhoea as the most frequent symptom. The median age for first sexual intercourse was 16 years. Over 84% of women had a steady partner, 27% did not use any form of contraception, and only 17% regularly used condoms. 3% had at least another IST in the

past, and 30% were tested for HIV at least once. Over 25% have had 4 or more sexual partners in their lifetime. The prevalence of Ct infection was 5.8%. The prevalence rate in the symptomatic and asymptomatic patients was 4.6% versus 6.7%; in Italian and born abroad patients was 5.8% versus 6.0%. 11.7% of tested population with symptomatic partner was positive versus 4.7% with asymptomatic partner. With regard to numbers of sexual partners lifetime, the prevalence rate for patients who had between 1 and 3 partners was 3.75% while in women with more than 4 partners was 12.23%. Only 4.5% of women with regular partner was positive versus 13.5% of women with more than one partner in the last six months. Among the variables considered, statistically significant risk factors for Ct infection include numerous sexual partners over a lifetime and lack of a long-term sexual relationship. Age, nationality, and symptoms do not appear to be statistically significant.
CONCLUSIONS. The value and risk factors highlighted can be compared with other studies. The high number of asymptomatic cases confirms the need to consider selective screening in order to reduce the spread of this STI and other secondary complications.

Prevalence and determinants of *Chlamydia trachomatis* infection among non- national women in Turin, Italy

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OBJECTIVES. To estimate the prevalence of *Chlamydia trachomatis* (Ct) infection among non-national women and to determine risk factors associated with infection.
METHODS. The cross-sectional study was performed among non-national women attending a public gynaecology clinic in the city of Turin (northern Italy) for the first time between January 2002 and December 2007. The study population consisted of sexually active women (14-60 years of age). All women were tested for Ct infection using the Polimerase Chain Reaction-Real Time on endocervical sample. The prevalence of Ct infection was calculated as the proportion of Ct-positive women out of the total study population. The risk factors of Ct infection were calculated through the construction of a binary logistic regression model.
RESULTS. A total of 3,483 non-national women were included in the study; 1.397 (40.1%) originated from Eastern Europe, 1188 (34.1%) from Africa, and 691 (19.8%) from South America. The mean age was 31.2 years [Standard deviation (SD) ± 6.6 years]; 47.2% had a high school diploma and 14.3% had a degree; 77.9% were married or in a common law marriage. The median age at first sexual intercourse was 19.5 years (SD ± 4.1 years). Of the women, 66.9% reported having had two or more lifetime partners, 2.4% reported having had two or more partners in the previous six months, and 35.8% were pregnant. Ct was detected in 3.3% of the women. High prevalence rates were observed among women aged 14-24 years (9.4%), reporting more than one partner in the previous six months (11.6%), having first sexual intercourse before 15 years of age (6.5%), having a partner with symptoms of urethritis (6.7%), being pregnant (3.8%). At the multivariate analysis, the positivity for Ct was significantly associated with younger age [14-24 years vs. >24 years of age, OR 4.5; 95% Confidence Interval (CI) 2.8-7.4], having had more than one partner in the previous six months (OR 3.2; 95% CI 1.2-8.6) and being pregnant (OR 1.8; 95% CI 1.1-3.0).
CONCLUSION. In Italy there do not exist national guidelines regarding who should be tested for Ct, nor do there exist screening programmes for Ct. The prevalence found was higher compared to that found among Italian women (1.05%) (Grio R. et al., Minerva Ginecol 2004). The present study suggests that non-national women represent a population at higher risk for Ct infection compared to national women. According to our results, Ct testing should be offered to non-national women, especially to young women, to those with multiple partners, and to pregnant women. In Italy, specific initiatives should be directed to this population for controlling and preventing Ct infection.

Diagnosis of *Chlamydia trachomatis* using dual swabs comparing novel flocked swabs with conventional rayon swabs sent to the laboratory in dry tubes

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OBJECTIVES. Novel flocked swabs promise higher sensitivity compared to conventional swabs. We compared the detection rate of *Chlamydia trachomatis* (Ct) from flocked and conventional swabs by using a special dual swab device.
METHODS. 494 asymptomatic patients were tested. A vaginal dual swab was collected by the gynecologist during routine visits. In 119 of the patients an endocervical dual swab was also taken. The dual swab collection kit consisted of a rayon swab and a regular flocked nylon swab (Copan Italia) attached to the same cap and stored in a plastic tube and a 100x12 mm sterile tube. After a specimen collection the flocked swab was placed into the sterile tube, the swab shaft was broken at the marked breaking point and the tube was securely closed. The rayon swab, left still attached to the cap, was placed back into the original swab transport tube. Both swab tubes were placed inside an envelope and sent as dry swabs to the laboratory by courier. There 1 ml of 0.9% normal saline was added to each tube, tubes were vortexed for 30 seconds before removing and discarding the swabs. A volume of 0.2 ml was used for DNA extraction using Qiagen columns, 0.1 ml was transferred to a ProbeTec sample buffer tube, and the remainder of the sample was stored frozen for additional testing. Detection of CT was performed by an in-house real-time PCR. 5 µl of extracted nucleic acid was added to 15 µl of master mix and tested on the Roche Light-cycler. Confirmatory testing of samples was done using the BD ProbeTec CT testing procedure.
RESULTS. In the 494 vaginal specimens tested by real time PCR and PT, the flocked swabs detected 13 positives while the rayon swabs detected 9. All specimens positive by rayon swabs were also positive by flocked swabs. The positivity rate from vaginal swabs was 2.6% with an average of 198,618 DNA-Copies/ml for the flocked swabs and 1.8% with an average of

13,072 DNA-Copies/ml for the rayon swabs. The 119 paired cervical/endocervical specimens yielded 8 positives. The flocked swabs detected 7 and the rayon detected 5 of them.
CONCLUSIONS. Flocked swabs shipped in a dry state, provided a higher positivity rate for *C. trachomatis*. Higher DNA-copy numbers were seen with the flocked swabs. Flocked swabs may be used to collect vaginal or cervical samples for PCR or ProbeTec for the diagnosis of *C. trachomatis* from health care professional-collected samples or from patient self collected samples. This opens a wide range of self-testing applications (see also www.self-testing.com) that can be offered for prophylaxis and diagnosis of diseases.

Anticorpi anti *Chlamydia trachomatis* nella popolazione afferente al laboratorio analisi di Collegno (Torino, Italia)

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INTRODUZIONE. Gli anticorpi anti *Chlamydia trachomatis* possono servire quali marker per il rilevamento di infezione attiva e possono essere particolarmente elevati in infezioni croniche o sistemiche. Con questo lavoro si è voluto valutare la siero epidemiologia nella nostra popolazione paragonando i risultati ottenuti nell'anno 1998 e quelli dell'anno 2008.
METODO. Nei sieri pervenuti è stata saggiata la presenza di anticorpi anti *C. trachomatis* mediante una metodica di immunoperossidasi indiretta proposta dalla ditta Savyon Diagnostic. I sieri sono messi a contatto con cellule infettate da *C. trachomatis*: gli anticorpi eventualmente presenti formano un complesso antigene-anticorpo rilevabile mediante una reazione cromogena evidenziata da una perossidasi.
DESCRIZIONE. Sono giunti alla nostra osservazioni 627 campioni di donne nel 1998 e 718 nel 2008. I campioni dei maschi sono stati 240 nel 1998 e 288 nel 2008. Valutando gli anticorpi nella popolazione suddivisa per classi di età, si osserva che, nel 1998, le IgG erano presenti con percentuali variabili tra un minimo del 41.7% nelle donne con età < 25 anni e un massimo del 48.2% in quelle > di 44 anni. Dieci anni dopo le percentuali variano, tra il minimo del 38.1% nelle donne con età 25-34 anni e il massimo del 63.6% in età > di 44 anni. Nei maschi, nel 1998, le IgG variano tra un minimo del 39.7% in età 35-44 anni ed un massimo del 52.7% in età > di 44 anni. Nel 2008 tra il 30.0% dei maschi con età 25-34 anni e il 51.4% con età > di 44 anni. Considerando le IgA con titolo uguale o maggiore 1:16 (possibile indice di infezione attiva), si evidenzia che, nel 1998, nelle donne erano comprese un minimo del 6.7% (< ai 25 anni) ed un massimo del 9.3% (35-44 anni); nel 2008 tra l'8.8% (25-34 anni) e il 19.1% (> 44 ann)i. Per i maschi nel 1998, tra un minimo del 4.1% (età 35-44 anni) e il 13.9% (> 44 anni); dieci anni dopo tra il 9.8% (età 25-34 anni) e il 33.8% (> di 44 anni).
CONCLUSIONI. Dopo dieci anni rileviamo che mentre non vi sono sostanziali differenze nella quantità di Ig rilevate, riscontriamo un aumento di prevalenza dell'antigene attribuibile al mutato comportamento sessuale e alla maggior attenzione dei medici del nostro territorio per le IST. Riteniamo che la determinazione degli anticorpi possa costituire un valido approccio diagnostico da associare alla ricerca dell'antigene per migliorare la prevenzione delle complicazioni.

Diagnosis of *Chlamydia trachomatis* and sexual behaviour in the population frequenting a health clinic in Collegno (Turin, Italy)

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INTRODUCTION. *Chlamydia trachomatis* infection has long been recognized as the most common sexually transmitted bacterial disease. A study has been carried out to see if the prevalence of *Chlamydia trachomatis* in the population frequenting the consulting room in our clinic, apparently without risk of sexually transmitted diseases, is conform to data obtained in literature and also to see if sexual behavior in different age groups has changed over the years.
METHODS. All patients were subjected to a detailed history taking into account their sexual behaviour. *Chlamydia trachomatis* was diagnosed in molecular biology with BD ProbeTec ET from Becton Dickinson (Strand Displacement Amplification technology).
CASE STUDY DESCRIPTION. It was noted that in our population the average age of first sexual intercourse has progressively decreased from 19.26 years of age in the 45-50 year group to 15.98 in the 15-20 year age group. Comparing data obtained from our patients under 25 years of age that came to our consulting room in 1998 (181) to those that came in 2008 (196) we noticed an increase in the total number of sexual partners in young women. In 1998 17.7% of young women had had at least 4 partners and in 2008 this percentage rose to 31.01. In female patients positive for the *Chlamydia trachomatis* test 14.4% had had more then 7 partners. 7% of these had also had more than 2 partners within the last 6 months prior to examination. There has been a progressive increase of positive tests rising from 2.84% in 2004 to 10.89% in 2008. 24% of the positive patients said to use condoms as contraception. 28.2% of these were non symptomatic and 29.1% declared to have had at least 1 occasional partner but only 19.7% of the patients regarded an HIV test as important.
CONCLUSIONS. From this study it can be confirmed that the relative risk of *Chlamydia trachomatis* infection in our population has increased because of earlier sexual activity and an increase in the total number of partners. Seeing that *Chlamydia trachomatis* has been found in women who consider themselves at low risk (use of condoms and a low number of HIV-test requests) we think it is beneficial to rely more on a detailed history for the choice of patients at risk.

Chlamydia trachomatis proctitis in italian men who have sex with men (MSM): report from an STD unit

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INTRODUCTION. The burden of sexually transmitted infection (STIs) is increasing all around the world; in the United States reported cases of *Chlamydia* and Gonorrhea exceeded 1.4 million in 2007 and primary and secondary syphilis have been diagnosed in more than 11.000 cases. In our experience we observed, especially in high risk populations (MSM or HIV pos subjects), increasing rates of Syphilis, Gonorrhea, sexually transmitted HAV, HSV and *Chlamydia trachomatis* infections including proctitis (CTP).
OBJECTIVE. to describe the CTP epidemic among MSM attending an STD unit and to evaluate the correlation with other STIs.
METHODS AND PATIENTS. Patients attending the STD unit - L Sacco University Hospital, Milan (Italy) between October 2007 and February 2009 with proctitis were included in this analysis. The diagnosis of CTP has been achieved through a real time PCR (*C. trachomatis* Q PCR Alert - Nanogen) on anal swab. All included patients underwent anoscopy, anal swab for bacteria, HSV nucleic acid amplification (HSV2 Q-PCR Alert AmpliMix), TPPA and RPR for syphilis, HIV test and anal HPV genotyping on liquid based cytology with InnoLipa test.
RESULTS. 13 MSM aged 36.5y (range 26-45) with symptomatic proctitis/proctocolitis associated to tenesmus, rectal pain, bleeding, discharge and ulceration or anal fissure showed during ano/colonscopy, have been observed during a 17 months period. Mean time to CTP diagnosis was 23 days (IQR 15-43); in 3 patients the diagnostic delay (3-6 months) was due to an initial misdiagnosis of Crohn's disease on histology from colonscopic biopsies. Co-infection with HIV was observed in 12 patients (92.3%) most of whom (75%) off-HAART; co-infection with primary syphilis in 4 patients (33.3%), with anal *Mycoplasma hominis* in 1 patient (8.33%). Anal HPV infections, found in 10/10 patients tested, were due to High Risk genotypes (HPV-HR). HPV genotyping, performed in 9/10 patients showed multiple HPV infections (from 3 to 6) in 5 cases (55.5%). Among high risk the HPV-52 (55.5%), HPV-51 (44.4%), HPV-16, HPV-35 and HPV-66 (33.3% each) were the most frequently observed genotypes. Anal cytology, performed in 8 patients, showed anal LSIL in 7 (87.5%) and HSIL in 1 (12.5%). Histology on anoscopic guided biopsies showed AIN1 in 3 patients and AIN2 in 1 patient. Overall a mono-infection with CT was observed only in 1 pt (8,33%) while in all the other cases multiple infections with =2 pathogens have been diagnosed (mean infections/pt = 3.25) All patients with anal CT were treated with doxycycline 100 mg twice daily for 7 days or azitromycine 1 g daily for 3 days with clinical and microbiological resolution.
CONCLUSION. CTP, firstly observed in our STD unit in sept 2007, continue to be observed with a steady trend since now. Results of this study, which underlines the high rate of multiple STI infections in high risk patients, suggest the need for a complete evaluation for STI and for specific counselling programs.

Prevalenza e fattori di rischio di infezione da *Chlamydia trachomatis* in studentesse di medicina e donne afferenti ai consultori di Asti e provincia

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INTRODUZIONE. L'infezione da *Chlamydia trachomatis* (Ct) è la più comune e prevenibile infezione sessualmente trasmessa (89 milioni di casi/anno nel mondo); è asintomatica nel 75% delle femmine e nel 90% dei maschi, con alto rischio di trasmissione e di complicanze. La prevalenza in Occidente varia tra il 2 e il 6% con picco tra i 16-19 anni nelle femmine e i 20-24 nei maschi; i dati italiani sono scarsamente confrontabili per l'eterogeneità delle popolazioni studiate e dei metodi diagnostici. Ci si è proposti di individuare i comportamenti maggiormente predittivi per l'infezione, ricercata con metodo ad elevata sensibilità, confrontando due popolazioni femminili a rischio per età (18 - 26 anni) ma di diversa estrazione socioculturale.
MATERIALI E METODI. Alle studentesse dei 6 anni del corso di laurea in Medicina di Torino, a.a. 2007/08, e a donne di età 18-26 anni afferenti ai consultori dell'ASL AT dal gennaio 2008, è stato proposto un identico questionario su comportamenti a rischio per IST ed è stata effettuata una RT-PCR per Ct (Becton Dickinson) su primo getto di urine.
RISULTATI. Su 492 studentesse 6 sono risultate positive (1.2%), a fronte di 11 positive (4.2%) su 262 pazienti dei consultori (X2=6.9, p=0.008). Sono emersi i seguenti fattori di rischio, rispettivamente tra le studentesse e le pazienti dei consultori: uso di contraccettivi non di barriera nel 34% vs. 59% (X2< 0.0001); età < 18 aa. all'epoca del primo rapporto nel 40% vs. 60% (X2 <0.0001); più di 1 partner nell'ultimo anno nel 16% vs. 20% e, negli ultimi 5 anni, nel 52% vs. 56% (n.s.). Il rischio di infezione era correlato a >4 partners negli ultimi 5 anni in entrambi i gruppi (rispett. R.R.=10.7 con I.C. 2-49; R.R.=3.7, I.C. 1.2-12) e a un'età < 18 aa al momento del primo rapporto nelle pazienti dei consultori (R.R.= 7.6, I.C. 1.1-30), ma non nelle studentesse (R.R.= 1.5, I.C. 0.3-8). La regressione logistica ha confermato come unici fattori predittivi di infezione: un numero di partners > 4 negli ultimi 5 anni in ambedue i gruppi (studentesse: Wald= 7.3; p< 0.01; pazienti dei consultori: Wald= 3.7; p< 0.05) e un'età < 17 anni all'epoca del primo rapporto solo nelle pazienti dei consultori (Wald= 3.5; p=0.06).
CONCLUSIONI. Uno screening selettivo su una popolazione femminile a rischio di infezione da Ct per età (studentesse di medicina), ha evidenziato una prevalenza inferiore sia ai dati europei/USA, sia ad una popolazione di controllo di diversa estrazione socio-economica afferente ai consultori; tale dato è da ricondurre all' uso prevalente del profilattico quale contraccettivo e a minor precocità sessuale tra le studentesse, indipendentemente dal numero dei partners. La consapevolezza del rischio di trasmissione e l'utilizzo corretto di metodi di barriera sembra ridurre nettamente la prevalenza di Ct; si sottolinea l'importanza di limitare i programmi di screening a popolazioni a rischio promuovendo una prevenzione fondata su informazione e consapevolezza.

Comparing flocked swabs to kit swab for the collection of clinical specimens for the Diagnosis of *Chlamydia trachomatis* infections with the APTIMA Combo 2 assay

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Chlamydia trachomatis (Ct) infections are one of the most common sexually transmitted diseases. It has been reported that, contrived specimens, prepared with flocked swabs (FS) inoculated with an ATCC strain of *Chlamydia trachomatis*, placed in the Gen-Probe APTIMA Combo 2 assay transport media and tested with the same assay. No interference was noted and was shown to enhanced the analytical of the assay.
OBJECTIVES. a) to compare the detection of Ct and *Neisseria gonorrhoeae* (Ng) nucleic acid in the Gen- Probe Aptima Combo 2 (AC2) assay from clinical specimens collected with Copan flocked swabs to clinical specimens collected with AC2 swabs and transport medium. b) To determine the Ct prevalence in a low and a high risk patient population.
METHODS. 400 patients were tested in this clinical study, 253 from a clinic with low risk patients and 147 from a clinic with high risk patients. 3 randomized cervical specimens were collected from each patient, one with the system in use at the collection centers, one with an AC2 swab and the other with a flocked swab. After collection, both the AC2 swab and the flocked swab were placed in separate tubes of AC2 specimen transport medium and sent to the testing laboratory. All samples in AC2 medium were tested with the Gen-Probe AC2 assay as per manufacturer procedure. Specimens collected from low risk patients were tested with the BD ProbeTec assay, the specimens collected from high risk patients were tested with the Nonogen Ct real time PCR.
RESULTS. In the low risk population 6 patients were found to be Ct infected using the AC2 swabs and 6 with the flocked swabs. In the high risk population 22 were found to be Ct infected using the AC2 swabs and 23 with the flocked swabs. None of the patients were infected with Ng. All the Gen-Probe AC2 CT positives were confirmed with the Gen-Probe APTIMA Ct assay. The flocked swabs detected 1 additional Ct positive. The Ct positivity rates were 2.37% (6/253) in the population of the low risk clinic, and 15.67% (23/147) in the population of the high risk clinic.
CONCLUSIONS. The Copan flocked swabs and AC2 swabs transported in the AC2 transport medium and tested with the Gen-Probe AC2 Assay detected almost the same number of Ct positive patients; one more Ct positive patient was detected with the flocked swabs. Flocked swabs placed into AC2 medium can be used for the diagnosis of Ct or Ng using the AC2 assay. The *Chlamydia trachomatis* prevalence rates were 2.3% in the low risk population and 15.67% in the high risk population.

Prevalence of *Chlamydia trachomatis* infection among younger women attending STI centres in Turin, Italy

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INTRODUCTION. *Chlamydia trachomatis* (Ct) is one of the most common sexually transmitted infection (STI) in Europe. WHO estimates a prevalence rate between 3 and 6% in the European adult population. The infection affects mostly women under the age of 25 years. The evidence for effectiveness of Ct screening for younger age groups it is now well recognized. However, in Italy data on STI prevalence are still very limited and only a few, scattered epidemiological studies are available. No national Ct screening programmes have been implemented so far.
OBJECTIVES. To determine Ct infection rate and risk factors among younger women attending two major STI centres in Turin, a 1.000.000 inhabitants metropolitan area in Northwest Italy.
SUBJECTS AND METHODS. All female patients at risk for STI, aged 14-24, seen in 2007-2008 at two major STI centres in Turin were considered. Demographic, sexual exposure and laboratory data were collected from clinic files, tabulated and analyzed using Epi-Info ver 3.4.3. A commercially available TaqMan real-time PCR assay was used (*C. trachomatis* Q-PCR Alert Kit, Nanogen Advanced Diagnostics; COBAS® CT Test 2.0, Roche Diagnostics)
RESULTS. A total of 997 girls aged 14-24 have been examined: they represent 23.4% of females examined at the two STI centres. Mean age was 21 yr, mostly were Italians (59%). Among foreigners, East European women were prevalent 18.5%), Africans being the second largest ethnic group (12.5%), whereas Asian women was the group less represented (0.5%). Only 18% were married, 54.2% had a higher education. Ct overall prevalence rate was 10.9% compared to 5.3% among older age class (14-24 vs 25-66 yrs: OR 2.32 -95% CL 1.65-3.26 $p< 0.001$). Ct infection was not associated to: STI centre, country of origin, marital status, education, drug abuse, having a steady partner, having had casual partners, condom use, presence of symptoms, pregnancy, previous STI, HIV serostatus. Ct infection was statistically associated with younger age classes (OR 1.94 - 95%CL 1.25-3.00 $p< 0.01$), early sexual debut (< 16 years: OR 2.09 - 95% CL 1.32-3.31 $p<0.001$), higher lifetime median number of partners (7 vs 3 $p< 0.01$), previous Ct infection ($p<0.001$).
CONCLUSION. Ct Prevalence among younger women attending STI centres in Turin is almost double than the European general population rate. As expected, risk factors associated with Ct are age <25, early sexual debut, a higher number of partners, to have had a previous infection with Ct. Young women are a minor proportion of people seeking sexual health care. Two reasons could be assumed: low awareness of Ct infection associated risks and a lack of health education programmes. These data suggest the opportunity of planning interventions on health education and on the need for screening of adolescents in Northwest of Italy. A coordinated action for an Italian national screening program is urgently needed.

Molecular diagnosis of *Chlamydia trachomatis* conjuntivitis is improved by Polymerase Chain Reaction

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INTRODUCTION. Conjuntivitis can be related to a variety of viruses, bacteria and parasites, few studies reported the prevalence of *Chlamydia trachomatis* (Ct). Diagnosis is based on clinical history and the objective conjunctival sign but patients with epithelial conjuntival inflammation can have similar clinical features, despite different clinical etiologies, such as immune-mediated disease or an underlying infective proces; it is important to distinguish between viral or bacterial involve-

ment. Bacterial conjuntivitis can be due to several pathogens and is often characterized by mucopurulent discharge, hyperemia and lid oedema. Acute follicular conjuntivitis is a clinical entity due to multiple etiologies, out of these chlamydial infection requires specific antibiotic treatment. This prospective study was designed to evaluate the prevalence of Ct as the agent of acute, sub clinical or chronic follicular conjuntivitis in the Central Italy area.
MATERIALS AND METHODS. We enrolled 171 patients affected with follicular conjuntivitis, subdivided into three subgroups; the first group (A Gr. = 92 patients) is performed by immuno fluorescent direct (IFD) and enzyme linked fluorescent assay (ELFA) to evaluate the Ct presence. The second group (B Gr. = 79 patients) evaluates Ct by molecular technique, using the procedure described by S.A. Morrè (1). A third group (C Gr. = 15 patients) serologically negative to antibodies anti *Chlamydia* at IFD and ELFA tecniques is reviewed by molecular analysis in PCR. Immuno fluorescent direct (IFD) and enzyme linked fluorescent assay (ELFA) were commercial products purchased from bioMérieux (Vidas instrument); the blood was collected to determine serological antibodies against Ct by common enzyme immuno assay (EIA) test (Abbott Laboratories-chlamydiazyme).
RESULTS. Our results show that A group as significantly lower number of positive answers than B group ($p< 0.001$). In C group, both IFD and ELFA were found negative in all cases, while analysing the same patients by nucleic acid analysis all samples were found positive ($p<0.001$).
CONCLUSIONS. In this preliminary study the analysis performed by IFA and ELFA techniques was found less sensitive to identify Ct infection than nucleic acid analysis. The higher incidence of patient with positive response to PCR analysis (greater than 90%) when correlated with IFA and ELFA techniques suggests that molecular analysis is the best technique to identify ocular infection due to *Chlamydia trachomatis*.

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Undervalued ocular clinical pictures are disclosed by molecular diagnosis of *Chlamydia trachomatis* infection

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INTRODUCTION. Acute, sub clinic and chronic conjuntivitis is a clinical entity due to multiple etiologies, but chlamydial infection portrays different clinical features and is often characterized by mucopurulent discharge, hyperemia and lid oedema. Several studies reported the prevalence of *Chlamydia trachomatis* infection (Ct) (1). This prospective study is designed to evaluate the different ocular clinical appearances of Ct infection as the agent of acute, sub clinical or chronic follicular conjuntivitis in our region.
MATERIALS AND METHODS. The patients enrolled for this study were subjected to scraping on upper tarsal conjunctive, while blood was collected to determine serological IgA and IgG against Ct by EIA test (Abbott Laboratories-chlamydiazyme). 48 patients that presented more or less clinical evidence of conjunctivitis were screened for cultural and PCR analysis, using the procedure described by Morrè S.A. (2).
RESULTS. All the patients analyzed for IgA and IgG antibodies against Ct resulted negative. *C. xerosis*, *C. ulcerans* and *C. haemolyticum* represented the major coltural bacterial pollutants of conjuntival ulcered areas and they were were always associated Ct infection variously. Of the 48 patients analyzed for Ct-DNA, 44 (44/48 about 92%) were positive for Ct analysis and affected by acute, sub clinical and chronic follicular conjuntivitis. On the contrary, some pictures of upper and lower tarsal conjunctival inflammation showed negative molecular analysis for Ct infection.
CONCLUSIONS. In this preliminary study, the higher incidence of patients with positive response to PCR analysis (greater than 90%) suggests that molecular analysis is the best technique to identify undervalued ocular infection due to *Chlamydia trachomatis*.
If on one hand, some patients show the pathognomonic Arlt's sign of the tarsal conjunctiva that it's caused by chlamydial infection, more difficult it's hypothesized in cases with ligth inflammation signs. Only the use of PCR analysis unveils the pathological appearance in cases that otherwise couldn't remain disclosed and the missing diagnosis could perpetuate, in susceptible persons, the chlamydial infection until trachoma.

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Comparison between (UTM-R RT) and M4RT kits for the collection of clinical specimens for the detection of *Chlamydia trachomatis* with COBAS® TaqMan® 48 CT Test

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BACKGROUND. *Chlamydia trachomatis* (Ct) is the most common agent associated with sexual transmitted diseases that can occur asymptomatically. The diagnosis of Ct infections is done with nucleic acid amplification assays; quality and stability of the clinical specimen are essential for the performance of the molecular assays.
OBIECTIVE. To compare the flocked swabs and Universal Transport Medium (FS+UTM-RT) (Copan Italia spa) to the

M4RT® (MicroTest Culture Transport System di Remel) for the collection of clinical specimens for the detection of *Chlamydia trachomatis* with the Roche COBAS® TaqMan® 48 CT Test, v2.0.
METHODS. For this study, 2 clinical specimens were collected in random order from patients attending a sexual transmitted diseases clinic. One specimen was collected with a flocked swab stored in a tube of UTM- RT medium, and another specimen was collected with a rayon swab and stored in a tube of M4RT medium. All specimens were analysed with the COBAS® TaqMan® 48 Ct assay as per manufacturer testing procedure. The kit positive control (LowCP) was tested in duplicate, one with the kit recommended M4RT medium and the other with the UTM-RT medium. For each specimen test and control the qualitative result (pos/neg) and the Ct (threshold Cycle) of the internal Quality Standard (QS) and of the target were recorded. To date 55 patients were tested with double specimens (110 specimens) and the study is still in progress.
RESULTS. In the 55 specimens, collected in the SF+UTM-RT, 10 CT positive e 45 negative were found, while 8 CT positive and 47 negative were found in the specimens collected in the M4RT. 53 patients (96%) had concordant results in both collection systems. The 2 discordant samples were repeated and they confirmed positive in FS+UTM-RT and negative in M4RT. 23 LowCP were tested in duplicate, one in M4RT and the other in UTM-RT. The Ct average of the target is 0.7 lower in the LowCP-UTM-RT (Ct = 37.3) compared to the LowCP- M4RT (Ct = 38). In the CT positive specimens, the target Ct average is 2.23 lower for the specimens in UTM-RT (Ct = 28.77) compared to the specimens in M4RT (Ct= 31). No inhibitory results were found in both collection systems. In the Ct negative specimens, the Ct average of the QS was equal (M4RT Ct =36, UTM-RT Ct =36.1).
CONCLUSIONS. The Copan FS+UTM-RT collection system detected 2 Ct infected patients that were missed by the M4RT collection system; there are not inhibitors, confirmed by the Internal Quality Standard that had similar Ct in both collection systems. For the FS+UTM-RT collection system, the fluorescent signal has a lower Ct average, demonstrating a better amplification efficiency due to less interference found in the medium used. The Ct-Target analysis of the positive specimens in FS+UTM-RT system looks like is increasing the Roche COBAS® TaqMan® 48 Ct assay sensitivity.

Diagnostic value of a peptide based enzyme immunoassay test in diagnosis of *Chlamydia trachomatis* triggered reactive arthritis

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Reiter's syndrome is a reactive arthritis (ReA), where the arthritis occurs as a "reaction" to an infection starting elsewhere in the body. In many patients, the infection begins in the genitourinary tract. The diagnosis of ReA is difficult because there is no specific test to confirm. However, about 80% of people with Reiter's syndrome are HLA-B27 positive. Patients are generally tested for *Chlamydia* infection because increasing evidence demonstrates that early treatment in *Chlamydia*-induced Reiter's syndrome may improve the course of the disease. Cross-reaction between chlamydial species has been reported. This study was conducted to assess the presence of specie-specific circulating IgA and IgG antibodies to *C. trachomatis* in the sera of patients with ReA.
METHODS. Species-specific enzyme immunoassay incorporating synthetic peptide (IgA and IgG, Ct- Eurospital) was used to study 61 patients: 42 patients (24 men and 18 women) with *C. trachomatis* triggered ReA (tested for urogenital swab and HLA-B27 positive) and 19 patients with inflammatory arthropaties without evidence of urogenital *C. trachomatis* infection as controls.
RESULTS. An increased prevalence of *C. trachomatis* antibodies was observed in patients with ReA triggered by *C. trachomatis*; 31/42(73%) had IgA and 34/42(80%) had IgG antibodies. In patients with other inflammatory arthropaties, *C. trachomatis* IgA/IgG antibodies were detected in 3% and 7% of patients, respectively. Based on positivity of both isotypes the sensitivity of the assay was 83% and specificity 91%.
CONCLUSION. *C. trachomatis* species-specific peptide EIA highly correlates well with diagnosis of *C. trachomatis* infection in patients with ReA. Species-specific tests have high performance for rheumatic diagnostic purposes.

Valutazione della presenza di *C. trachomatis* mediante real-time PCR: 18 mesi di sorveglianza

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INTRODUZONE. *C. trachomatis* rappresenta, nei paesi industrializzati, la più frequente causa di infezione batterica nelle malattie a trasmissione sessuale. L'infezione si manifesta comunemente con uretriti e/o cerviciti, ma può dare sequele quali la malattia infiammatoria pelvica, sterilità tubarica e gravidanza ectopica nella donna ed epididimite e prostatite nell'uomo.
METODI. Nel periodo 1/7/2007 - 31/12/2008 sono stati analizzati i campioni di 608 pazienti (161 uomini, pari al 26,4% e 447 donne, pari al 73,6%) suddivisi in 3 fasce d'età (20-30aa: 152; 31-40aa: 289; = 41aa :167) afferenti all'Azienda Ospedaliera - Polo Universitario L. Sacco di Milano. La ricerca di *C. trachomatis* è stata eseguita con metodica real-time PCR (*Chlamydia* Tr Q. PCR Alert - Nanogen) su campioni di urine, tamponi cervicali, tamponi uretrali e tamponi vaginali. Su tutti i campioni è stata inoltre effettuata la ricerca colturale di batteri, miceti e micoplasmi.
RISULTATI. La presenza di *C. trachomatis* è stata riscontrata in 25 pazienti (4.1%) di cui 14 donne (2.3%) e 11 uomini (1.8%). Le donne risultate positive sono state 11/120 (9.1%) nella fascia d'età compresa tra 20 e 30aa; 3/219 (1.4%) di età compresa tra 31 e 40 aa; nessuna positività è stata riscontrata nelle 108 donne con più di 41 aa Nella popolazione maschile si è riscontrata positività in tutte e tre le fasce d'età considerate con questa distribuzione: 20-30aa positivi 4/26 pazienti (15.4%); 31-40aa positivi 4/69 pazienti (5.8%) e= 41aa positivi 3/66 pazienti (4.5%). Tra i pazienti positivi per *C. trachomatis* in due casi è stata riscontrata la concomitante presenza di *Ureaplasma urealitycum* nel tampone vaginale e in un terzo caso la presenza di *C. albicans*.
CONCLUSIONI. I risultati ottenuti confermano che l'infezione da *C. trachomatis* è diffusa sia nella popolazione femminile sia in quella maschile, in particolare nelle fasce d'età più giovani, mentre nella fascia d'età superiore ai 41 anni la presenza di *C. trachomatis* è stata riscontrata solo in tamponi uretrali maschili Dei 25 pazienti con infezione da *C. trachomatis*, 10 hanno ripetuto il test dopo aver effettuato la terapia antibiotica, in tutti questi pazienti il test di amplificazione genica ha dato esito negativo.

The PCR analysis to improve the diagnosis of the *Chlamydia trachomatis* infection in pharyngeal scraping samples from patients attending an oro-pharyngeal chronic inflammation

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INTRODUCTION. From several times, the *Chlamydia trachomatis* (Ct) is considered an important human pathogen for respiratory tracts and it's was suggested as one of the causes of pharyngitis in adult (1). Chronic pharyngitis in adult is a wide and frequent health problem; each year we observed about a thousand of people to ambulatory and at the same time, several thousand work days are lost on account of the sore throat. Actually, there is no routine molecular study that highlights the presence of atypical bacteria like *Chlamydia trachomatis* during chronic pharyngeal inflammation episodes. Our goal was to detect the presence or absence of "atypical bacteria" like *Chlamydia trachomatis* in throat specimens collected from patients whose primary complaint chronic pharyngitis with more recurrent episodes.
MATERIALS AND METHODS. Our study was conducted at the consulting room of the ENT clinic of University of Chieti, Italy, between September and November 2008. During this period, we enrolled 30 patients that presented more or less clinical evidence of chronic pharyngeal inflammation; this population were subjected to pharyngeal scraping and it must present at least three of our following criteria to screen by PCR analysis, using the procedure and primers reported by Morrè S.A. (2): - atrophy or hyperplasia of pharyngeal mucous membrane.
- least three worsening episodes of pharyngitis in the last year at least from three years.
- remote positive pathological anamnesis for infections of higher oral tract.
- sore throat or dysphasia, without any signs or symptoms of lower respiratory tract infection.
- presence of atypical associated symptoms like peripheral arthralgia with or without diffused floating itchy, intermittent slight fever (37°-37.5°C) or episode of high fever (38°-39,5°C).
RESULTS. Of the 32 patients analyzed for Ct-DNA, 29 (29/32 greater than 90%) were positive for Ct analysis and affected by chronic pharyngeal inflammation.
CONCLUSIONS. In this preliminary study, the higher frequency of patient with positive response to PCR analysis (greater than 90%) suggests that molecular analysis is the best technique to identify undervalued pharyngeal infection due to *Chlamydia trachomatis*. Only the use of PCR analysis unveils the pathological appearances in these cases that otherwise couldn't remain disclosed and the missing diagnosis could perpetuate, in susceptible person, the chlamydial infection until trachoma.

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Chlamydia trachomatis screening among men attending STI center of dermatological division of University In Turin, Italy

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INTRODUCTION. *Chlamydia trachomatis* (Ct) is the most frequently reported bacterial sexually transmitted infection in sexually active young adults in Europe. Since over 2/3 of chlamydial infection in women are asymptomatic, several screening programmes have been established to detect and treat women with undiagnosed infections and their partners and to minimalize severe complications (pelvic inflammatory disease, extrauterine pregnancy, and infertility). In the male population the symptoms can be mild, non- specific, or absent, therefore recently more attention was focused on *Chlamydia* men infection. The goal of this study was to estimate the prevalence of Ct among men who attending STI Center of Dermatological Division in Turin, Italy, to evaluate the most efficient screening programme for a STI Clinic, and to determine the correlation between some risk factors and Ct infection.
METHODS. From May 2005 to May 2006, 1000 sexually active men between the age of 15 and 50 were screened for Ct at STI Clinic of San Lazzaro Hospital in Turin. Male urethral swabs or urine samples were tested for Ct using Polymerase Chain Reaction (PCR). All the patients answered a specific questionnaire which included demographic data and informations concerning sexual behaviour. Statistical analysis was performed using the X2 test; a p value of less than 0.05 was considered significant.
RESULTS AND DISCUSSION. In our analysis the prevalence of Ct infection was found to be 7% with an average age of 32 years. A statistically significant correlation ($p<0.05$) was found between positive Ct test and people from East Europe, age <34 years, urethritis symptoms, >1 partner during past 6 months, number of lifetime sexual partner > 5. Forty seven out of 70 infected individuals (68%) presented urethritis symptoms whereas 23/70 infected individuals (32%) attended STI Center for other raisons (11/23: other STI, 10/23: STI risk behaviors, 2/23: partner affected).
CONCLUSIONS. High prevalence (7%) of Ct infection found in our study (with 2.6% of asymptomatic cases) confirmed the utility of screening programme adopted in STI Centers of Piedmont Network.

Prevalence of *Chlamydia trachomatis* infection among women and men in Bari, Italy

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INTRODUCTION. *Chlamydia trachomatis* is one of the most frequent causes of sexually transmitted diseases. A substantial

number of chlamydial infections has an asymptomatic course, and the published prevalence varies between 1.6 and 19%, depending on the population studied and the techniques used. I is assumed that asymptomatic *C. trachomatis* infection can also spread to the upper genital tract, causin PID and long-term sequelae such as tubal factor infertility and increased risk of ectopic pregnancy Therefore, the detection of asymptomatic *C. trachomatis* infections is essential. In the present study, the prevalence rate of *C. trachomatis* infections was investigated in a asymptomatic group of young women and men.

METHODS. From January 2008 to the end of December 2008, 2098 unselected sexually active women (1764) and men (334), aged 18 - 40 year, were tested for *C. trachomatis* using the Real-Time PCR. The specimens tested were: cervical swabs (1515), vaginal swabs (249),urethral swabs (76), urine (24) and sperm (234).

RESULTS. In our analysis the prevalence of *C. trachomatis* infection was found to be 2.5%, with an average age of 23 years among the infected patients. Among 1764 women, the prevalence was 2.4%, whereas among 334 men was 3.3%. *Chlamydia* was detected in 2.4% of the cervical swabs, in 2% of the vagina swabs, in 2.6% of the urethral swabs, in 3% of the sperm specimens and in 8% of the urine. *Chlamydia* prevalence in the group aged 18-22 years and in the group aged 23-27 was similar, with prevalence rates of 3.8% and 3.3%, respectively, in women, and 4.1% and 3.9%, respectively, in men. The *C. trachomatis* prevalence rate declines in women and men older than 30 years.

CONCLUSION. The prevalence (2.5%) found in this study was in range with published Italy prevalence. Molecular techniques are valuable tools for the detection of asymptomatic genital chlamydial infection. The increased sensitivity and specificity of the DNA amplification assays and the ability to test urine sample have moved chlamydia testing into a new dimension. Such advance not only increases the predicative values of the test but also makes community-based screening a realistic possibility.