DIAGNOSIS OF NON-VIRAL SEXUALLY TRANSMITTED INFECTIONS IN LITHUANIA AND INTERNATIONAL RECOMMENDATIONS

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To evaluate the range, quality and availability of diagnostic services for non-viral sexually transmitted infections (STIs), i.e. *C. trachomatis, N. gonorrhoeae, T. vaginalis* and *T. pallidum*, in Lithuania from September 2002 to December 2003.

Surveillance data describing the organisation and performance characteristics of non-viral STI diagnostic services in Lithuania were collected using a questionnaire and subsequent site-visits. International evidence-based recommendations for non-viral STI diagnosis were used to evaluate the quality of the STI diagnostics.

There were 171 facilities providing non-viral STI diagnostic services for the 3.5 million inhabitants of Lithuania. However, only 6% (n=9) of the respondents (n=153) could provide a confirmatory diagnosis, in accordance with international recommendations, for the full minimum range of relevant non-viral STIs in Lithuania, i.e. *C. trachomatis, N. gonorrhoeae, T. pallidum,* and *T. vaginalis*. In addition, accessibility to STI diagnostic services differed significantly among the different counties in Lithuania. Several of the respondents analysed low numbers of samples each year, and overall the sampling size was extremely low, especially for *C. trachomatis* diagnostics.

In Lithuania, optimisation of non-viral STI diagnostics as well as of epidemiological surveillance and management of STIs is crucial. It may be worth considering a decrease in the number of laboratories, with those remaining having the possibility of performing STI diagnostic services that are optimised, in concordance with international recommendations, standardised, and quality assured using systematic internal and external quality controls and systems. In addition, establishment of national inter-laboratory networks and reference centres for non-viral STIs is recommended.

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Introduction

Sexually transmitted infections (STIs) are recognised as major public health threats worldwide. However, in most of the countries of the former Soviet Union, including Lithuania, STIs and reproductive health have received insufficient attention, contributing to a decrease in the birth rate and an increase in the rate of medical abortions [1]. Lithuania has a population of 3.5 million inhabitants and comprises 10 different counties. In 2002, the population of the counties ranged from 133 000 to 848 000 (in the country which includes the capital city, Vilnius). The estimated incidences of non-viral STIs in Lithuania, especially genital chlamydial infection and gonorrhoea, are comparatively low at present, and have tended to decline during the past decade [2, 3]. However, reliable figures for the incidences of non-viral STIs are lacking, primarily due to suboptimal diagnostics, incomplete epidemiological surveillance, and self treatment.

Since the re-independence of Lithuania in 1991, the national healthcare system has undergone several major changes. For example, state-controlled mandatory hospitalisation has been replaced by a more decentralised system based on an outpatient primary care approach, and there are many new private STI outpatient clinics and laboratories with an anonymous care and treatment approach. Previously, it was mandatory for local dermatovenereological diagnostic facilities to report all diagnosed STI cases to the central dispensary (dermatovenereological out-patient clinic). Cases must now be reported to newly established regional public health centres, which then report to the National Centre for Prevention and Control of Communicable Diseases [4]. Many of these changes have contributed to distortion of the epidemiological data.

International evidence-based recommendations regarding diagnostics [5-7] are still mainly unknown in non-viral STI diagnostic services in Lithuania. In many cases, the choice of diagnostic strategies and assays is consequently based on empirical knowledge or even on the economic status of the particular facility, which significantly affects the quality of the diagnostics. Unfortunately, highly sensitive and specific laboratory-based diagnoses of several non-viral STIs are quite expensive. Issues regarding laboratory quality control have recently emerged in Lithuania but these have not yet attracted sufficient attention on the part of healthcare administrators. Thus, there are still no accredited clinical microbiological laboratories in Lithuania [8].

In the absence of effective vaccines, the mainstay in the prevention of non-viral STIs is based on the availability of adequate healthcare, effective diagnostics and treatment, and epidemiological surveillance. Consequently, the number of physicians specialising in STIs and in counselling afflicted patients, and the number and geographical location of adequate healthcare institutions and laboratory facilities that can provide sensitive and specific STI diagnostics are highly important.

The aim of the present study was to evaluate the range, quality and availability of diagnostic services for non-viral STIs (*Chlamydia trachomatis, Neisseria gonorrhoeae, Treponema pallidum* and *Trichomonas vaginalis*) in Lithuania from September 2002 to December 2003.

Material and methods

Surveyed Lithuanian laboratories and demographic data

All Lithuanian laboratories and other facilities that performed diagnostic tests for *C. trachomatis*, *N. gonorrhoeae*, *T. pallidum*, and *T. vaginalis* from September 2002 to December 2003 were included in the present study. Laboratories were identified from information obtained from the Lithuanian Department of Accreditation (LDA; in Lithuanian, the Lietuvos akreditacijos tarnyba), which is responsible for the certification of facilities performing laboratory diagnostics. In addition, the county-level STI management groups in all 10 Lithuanian counties [9] updated the information received from LDA. The study questionnaire was sent to all laboratories that had confirmed they performed non-viral STI diagnostics. Demographic

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data on each of the counties in Lithuania (n=10) were obtained from the Lithuanian Statistical Department (in Lithuanian, the Lietuvos statistikos departamentas) [10].

Survey questionnaire and data collection procedure

The utilised questionnaire consisted of 150 questions focussing on (a) organisation of the STI diagnostic service (type of laboratory, number of personnel, professional qualifications of the personnel, existence of a laboratory quality assurance system, etc.), and (b) performance characteristics (STI agents diagnosed, number of tests performed per month, diagnostic methods employed for the different STIs, etc.). The laboratories provided surveillance data by email, fax, or telephone interviews. In addition, follow up visits for validation of these data were performed at many of the laboratories.

In the final data analysis, the laboratory diagnostics for the full minimum range of relevant non-viral STI agents in Lithuania underwent more comprehensive evaluation.

Adherence of Lithuanian STI diagnostics to international evidence-based recommendations

For evaluation of the quality of the non-viral STI laboratory diagnostic strategies and assays used at the Lithuanian laboratories, international evidence-based recommendations for non-viral STI diagnostics and definitions of STI surveillance cases [5-7] were used.

Results

STI diagnostic laboratories in Lithuania and response rates

In total, 171 laboratories in Lithuania, of which 28 (16%) were private, were LDA certified for STI diagnostics and currently performed non-viral STI diagnostics. Consequently, the median number of inhabitants served by one STI diagnostic laboratory in Lithuania was 20 470 (range: 10 400-30 600) in the different counties. Of the 171 laboratories identified, 153 (89%) agreed to participate in the assessment study and provided all the surveillance data that were requested. The remaining 18 laboratories gave various reasons for declining to participate, for example only performing STI diagnostics occasionally, testing very few samples or not wishing to be involved in the present study. Minor discrepancies were identified between the data provided in the questionnaires and observations at the site visits; however, these were associated only with incomplete filling in of the questionnaire or occasional misunderstanding of a question.

Laboratory diagnostics for non-viral STIs in the responding Lithuanian laboratories

Microscopy of genital samples

In all, 150/153 (98%) respondents used microscopy of genital samples for non-viral STI diagnostics. Of note 34/153 (22%) respondents reported microscopy of genital samples as the only method used for STI diagnostics. In 49/153 (32%) responding laboratories, only specialists with a master's degree in medicine or biology, and who also specialised in laboratory medicine, were responsible for evaluation of the genital samples. Six respondents (6/153, 4%) reported that practicing, non-specialist physicians performed the microscopy in their laboratories. In 31/153 (20%) of the responding laboratories, laboratory technicians were solely responsible for evaluating the genital samples, and in 67/153 (44%), technicians and/or laboratory physicians performed the microscopy.

Laboratory diagnostics for the main non-viral STIs in Lithuania

No accredited clinical microbiological laboratories exist in Lithuania and all respondents also lacked a complete and thoroughly implemented laboratory quality assurance system; that is, one which included internal and external quality controls, written guidelines describing the entire procedure for processing of samples, interpretation of results, equipment control measures, etc. Only 10% of respondents could provide diagnostics for the full minimum range of relevant non-viral STIs in Lithuania. The main characteristics of the diagnostics for these pathogens are summarised in the table.

Laboratory diagnostics for *C. trachomatis* was available in only six of the 10 counties in Lithuania and, in total, only in 16 (10%) of the responding laboratories [TABLE]. Consequently, the number of samples was much lower than for the other main non-viral STI pathogens [TABLE]. Two thirds of the analyses were performed in the largest county. Most respondents used an enzyme immunoassay (EIA) or a direct immunofluorescence (DIF) assay [TABLE].

For diagnosis of *N. gonorrhoeae*, the number of samples each month varied dramatically among the different counties in Lithuania and ranged from 22 to 1629 for men and from 270 to 8888 for women. Almost 75 per cent of all samples from men were tested in the two largest counties of Lithuania. Most respondents diagnosed *N. gonorrhoeae* exclusively by microscopy of stained (methylene blue or Gram stained) genital samples [TABLE]. In seven of the 10 Lithuanian counties at least one respondent was able to perform culture of *N. gonorrhoeae*, but the number of samples was low.

Regarding the diagnostics of *T. pallidum*, respondents in the larger Lithuanian counties analysed almost equal numbers of samples from men and women, while respondents in most of the smaller counties tested more samples collected from women. For screening purposes, the majority (60%) of respondents performed rapid plasma reagin (RPR) tests of STI samples, units of donated blood and plasma, pregnant women, etc. For RPR positive samples, mainly *T. pallidum* haemagglutination (TPHA) was used for subsequent specific confirmation [TABLE].

For diagnosis of *T. vaginalis*, the number of samples analysed and the women/men ratio of samples tested were very similar to that of *N. gonorrhoeae* [TABLE]. Almost 75% of the men tested were tested in the

Table

Diagnosis of non-viral sexually transmitted infections, monthly average, Lithuania, 2002-2003

	C. trachomatis (n=16)[a]	*N. gonorrhoeae* (n=152)[a]	*T. pallidum* (n=92)[a]	*T. vaginalis* (n=114)[a]
Total number of samples (range)[b]	1 551 (11-1253)	27 247 (465-10 517)	24 829 (405-10 749)	25 704 (405-9735)
Female/male ratio (range)[b]	1.3 (1.2-6.7)	6.8 (1.4-36)	1.5 (0.5-59)	6.1 (1.6-57)
Number of samples per 100 000 inhabitants (range)[b]	44 (5.8-148)	784 (307-1334)	709 (205-1267)	734 (217-1165)
Diagnostic methods[c] (%)[d]	EIA (5.9) DIF (5.2) REIA (3.3) NAATs (2.0) Serology (1.3) Cell culture (0.7)	Microscopy of genital smears (94) Culture (5.0) DIF (0.7) NAATs (0.7)	RPR (60) TPHA (53) DFM (6.0) Serology for newborns (6.5) VDRL (2.6) REIA (2.0) FTA (1.3)	Microscopy of genital smears (75; i.e. 75% used methylene blue, 42% Gram, 14% Giemsa and 23% wet smear) Culture (0.7)

a. Number of respondents that diagnosed the pathogen

b. Range of numbers of samples or of the female/male ratio of samples in different counties in Lithuania

c. EIA, enzyme immuno assay; DIF, direct immunofluorescence; REIA, rapid enzyme immuno assay; NAATs, nucleic acid amplification tests; RPR, rapid plasma reagin; TPHA, *T. pallidum* hemagglutination; DFM, dark field microscopy; VDRL, Venereal Disease Research Laboratory assay; FTA, fluorescent treponemal antibodies

d. Percentage of responding STI diagnostic laboratories in Lithuania (n=153) that perform the method

two largest counties. The vast majority of the respondents diagnosed *T. vaginalis* using microscopy of genital samples [TABLE].

Discussion

The present study highlights several shortcomings in the diagnostics and management of non-viral STIs in Lithuania. A similar situation has previously been described in one of the neighbouring Baltic countries, Estonia [11]. In Lithuania, there is mandatory reporting of syphilis, gonorrhoea and genital chlamydial infections [4]. Before the re-independence of Lithuania in 1991, patients with non-viral STIs were managed exclusively by specialists in dermatovenereology, mainly at dermatovenereological (DV) dispensaries. Each case of syphilis and gonorrhoea was reported by the local DV dispensary to a central dispensary. The chief dermatovenereologist at the central DV dispensary would then be kept up to date with the progress of each case, including partner tracing. Each primary healthcare facility had its own dermatovenereologist who managed all the non-viral STI patients. There were no private practices or laboratories. Following the re-independence of Lithuania, there was no ready concept within healthcare reform for the diagnostics and management of non-viral STIs. Many private clinics and laboratories arose, which introduced and usually used the cheapest available methods for STI diagnostics. There was a lack of expertise and financial resources for controlling the STI diagnostic strategies and the quality of the diagnostic methods used in these laboratory services. As revealed in the present study, the 3.5 million inhabitants in Lithuania during 2002-2003 had 171 facilities providing non-viral STI diagnostic services, but the availability of STI diagnostic services for each inhabitant and the number of inhabitants served by each laboratory varied significantly between counties. Several of the responding laboratories were small and received low number of samples for STI diagnostics. This may not be cost-effective, there may be insufficient experience, inadequate use or even lack of standardised controls, and it may be more difficult to implement systematic internal and external quality assurance controls and systems.

Only 10% of the respondents that diagnosed any STI in Lithuania were able to provide *C. trachomatis* diagnostics and there were only 532 samples per 100 000 inhabitants per year. In contrast, 4726 samples per 100 000 inhabitants are tested each year in Estonia [11]. The low sampling size in Lithuania may partially explain why the estimated incidence of *C. trachomatis* infection in Lithuania (11.08 per 100 000 inhabitants in 2004) is significantly lower than in the neighbouring countries of Estonia [11], Belarus, Poland, Sweden, and Denmark [2]. Although Latvia has also reported a low incidence of *C. trachomatis* infection [2], more information about the reliability of these figures is needed. In Lithuania, older diagnostic assays such as EIA or DIF were most often used, which may not give optimal sensitivity and specificity. However, with the exception of two respondents who used serology only, the diagnostics used by the laboratories that diagnosed *C. trachomatis* corresponded well with international recommendations [5, 7], using at least one antigen detection assay (DIF or EIA), nucleic acid detection method, or culture.

It is alarming that for *N. gonorrhoeae*, most respondents reported using microscopy of stained genital samples as their sole method, because it is cheaper to use, and only 5% of the respondents were able to culture the bacteria. Consequently, most respondents were only able to provide definitive diagnosis of male symptomatic gonococcal urethritis [6, 7, 12]. According to international recommendations, to provide a definitive diagnosis, the following kinds of samples should be cultured: urethral and cervical samples from women, samples from asymptomatic patients of both sexes, and tests of cure, as well as all extra-genital samples [5, 6, 12]. Failing this, antigen or nucleic acid of the bacteria should be identified [5, 7]. Culture allows subsequent identification of antimicrobial resistance in *N. gonorrhoeae* and there is a complete lack of in depth knowledge about the level of antimicrobial resistance in Lithuania.

Concerning diagnosis of syphilis in Lithuania, all respondents that performed syphilis diagnostics could identify probable cases of primary and secondary syphilis according to international guidelines [5, 7]. However, only 6% of the respondents reported using IgM or DFM (or another direct detection method such as PCR) and therefore being able to provide a confirmatory diagnosis of early primary or secondary syphilis [5, 7]. These data are mainly in agreement with the results of our previous study in the neighbouring country of Estonia [11].

As for *N. gonorrhoeae*, the sample size for *T. vaginalis* diagnostics reflected the number of genital samples for microscopy from STI patients and from women attending gynaecology clinics, which is mirrored in the female/male sample ratio. Only 23% of the respondents used wet smear microscopy, which is considered to be the most sensitive method for microscopic diagnosis of the agent [13].

As mentioned previously, only 10% (n=16) of the respondents could provide diagnostics for the full minimum range of relevant non-viral STIs in Lithuania, and only 6% (n=9) of the respondents were able to provide confirmatory diagnoses in accordance with international recommendations for diagnostics [5, 7] for all the non-viral reportable STIs in Lithuania (*C. trachomatis* infection, gonorrhoea, and syphilis).

The comparatively low and declining estimated incidences of non-viral STIs in Lithuania may, in part, reflect incomplete case reporting and epidemiological surveillance, the low number of samples for some of the STIs, the availability of STI diagnostic services, and, in many cases, the suboptimal diagnostics [present study, 14]. The main reason for this situation is the low level of funding in the healthcare budget for each non-viral STI patient, which should cover the cost of clinical investigation and all laboratory analyses. For more thorough and complete laboratory diagnostics the patient may have to pay for each additional assay himself/herself [15].

In conclusion, for optimisation of non-viral STI diagnostics, epidemiological surveillance and management of non-viral STIs in Lithuania, improved adherence to international recommendations for diagnostics, increased accessibility of diagnostic services, and overall improvements of reproductive healthcare are crucial. To achieve this, we propose that: (i) national inter-laboratory networks be established; (ii) the number of STI diagnostic laboratories be decreased; (iii) the diagnostics of some STIs be centralised to larger laboratories in order to ensure diagnostics in accordance with international recommendations and quality assurance; (iv) internal and external quality control (EQC) systems be introduced; (v) reference centres for STIs other than HIV be established, which will be responsible for recommendations of adequate diagnostic methodologies, coordination of EQC systems, performance of confirmative diagnostics for smaller laboratories, as well as guidance and education regarding STI diagnostics and quality assurance issues; and (vi) patient insurance be introduced, to cover expenses for thorough laboratory-based diagnostics for each STI patient.

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ORIGINAL ARTICLES

Surveillance report

ANTIBIOTIC RESISTANCE IN THE SOUTHEASTERN MEDITERRANEAN – PRELIMINARY RESULTS FROM THE ARMED PROJECT

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Sporadic reports from centres in the south and east of the Mediterranean have suggested that the prevalence of antibiotic resistance in this region appears to be considerable, yet pan-regional studies using comparable methodology have been lacking in the past.

Susceptibility test results from invasive isolates of *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Enterococcus faecium* and *faecalis* routinely recovered from clinical samples of blood and cerebrospinal fluid within participating laboratories situated in Algeria, Cyprus, Egypt, Jordan, Lebanon, Malta, Morocco, Tunisia and Turkey were collected as part of the ARMed project.

Preliminary data from the first two years of the project showed the prevalence of penicillin non-susceptibility in *S. pneumoniae* to range from 0% (Malta) to 36% (Algeria) [median: 29%] whilst methicillin resistance in *Staphylococcus aureus* varied from 10% in Lebanon to 65% in Jordan [median: 43%]. Significant country specific resistance in *E. coli* was also seen, with 72% of isolates from Egyptian hospitals reported to be resistant to third generation cephalosporins and 40% non-susceptible to fluoroquinolones in Turkey. Vancomycin non-susceptibility was only reported in 0.9% of *E. faecalis* isolates from Turkey and in 3.8% of *E. faecium* isolates from Cyprus.

The preliminary results from the ARMed project appear to support previous sporadic reports suggesting high antibiotic resistance in the Mediterranean region. They suggest that this is particularly the case in the eastern Mediterranean region where resistance in *S. aureus* and *E. coli* seems to be higher than that reported in the other countries of the Mediterranean.

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Introduction

Data from the European Antimicrobial Resistance Surveillance System (EARSS) [www.earss.rivm] indicate that the highest levels of antibiotic resistance have been found within the Mediterranean countries participating in the system. On the other hand, information about the prevalence of antimicrobial resistance in the non-European countries of the southern and eastern Mediterranean has, in the past, been sparse. Nevertheless, high levels of resistance have been reported in *Streptococcus pneumoniae* [1,2], *Staphylococcus aureus* [3] as well as within species of the Enterobacteriacae [4,5]. Unfortunately, besides being few in number, these studies have been totally unrelated, using different methodologies and, as a result, are difficult to compare [6].

This deficiency has been addressed by the Antibiotic Resistance Surveillance & Control in the Mediterranean Region (ARMed) project [www.slh.gov.mt/armed] which began in January 2003,7 and is funded by the European Commission under the INCOMED programme of the DG Research Fifth Framework Protocol (ICA3-CT-2002-10015). Over its four year funding period, this study is documenting the prevalence of antibiotic resistance in southern and eastern Mediterranean countries, as well as attempting to investigate potential factors such as antibiotic consumption and infection control. We report on the midway findings of ARMed-EARSS, the resistance epidemiology subcomponent of the project.