An electronic network for the surveillance of antimicrobial resistance in bacterial nosocomial isolates in Greece

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The present article reports an evaluation of the national electronic network for the continuous monitoring of antimicrobial resistance in Greece. The network employs a common electronic code and data format and uses WHONET software. Our four years' experience with the network confirms its practicality. A total of 22 hospitals in Greece are currently using the software, of which 19 participate in the network. Analysis of the information obtained has greatly helped in identifying the main factors responsible for the emergence of antimicrobial resistance in the participating hospitals. The data collected have also helped to identify priorities for further investigation of the genetic and molecular mechanisms responsible for the emergence of resistance and facilitated development of hospital-based empirical therapy of infections. In conclusion, the implementation of national networks for the surveillance of antimicrobial resistance should be regarded as a priority.

Voir page 600 le résumé en français. En la página 600 figura un resumen en español.

Introduction

Antimicrobial resistance has become a serious public health concern in many parts of the world. Systematic monitoring of such resistance at local, national and international levels is recognized as an integral part of the control strategy by most national and international organizations including WHO (*1–4*).

Several multicentre surveys conducted in Greece over the last 10 years (*5–8*) have identified this problem and underlined the need for immediate action. For example, the Greek Ministry of Health and Welfare has addressed this issue by imposing restrictions on the use of newer antimicrobials (third-generation cefalosporins, aztreonam, imipenem and quinolones) in the hospital setting through an audit-based prescription process. To satisfy the urgent need for an efficient surveillance system to monitor the possible impact of this policy, and to study the epidemiology of antimicrobial resistance, we launched a project in 1995 to establish a national network for continuous monitoring of such resistance.

This project is based on the assumption that the routine results of the antimicrobial sensitivity tests performed daily in each clinical laboratory should be considered to be a major resource for resistance surveillance. Since the quality and compatibility of these data are uncertain, our approach has been to work in parallel, establishing access to the data and assessing its quality. To do this we set up a quality control procedure and employed a standardized electronic code and data format in all hospitals, based on WHONET software. The WHONET software was originally devised by the WHO Collaborating Centre for Surveillance of Antibiotic Resistance, Boston, MA, USA, and further developed by WHO in Geneva. WHONET is distributed free by WHO and facilitates the management of antimicrobial sensitivity test results from routine clinical isolates. A full description of the software and its potential has been published elsewhere (*9, 10*).

In this article we describe the Greek network and present some of the results obtained using it. The results illustrate the positive impact of a national or international surveillance network in preventing and confronting antimicrobial-resistant bacterial diseases. The data presented cover the period January–December 1996.

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Materials and methods

Participating hospitals

A total of 28 hospital microbiology laboratories in Athens, Pereus, Thessaloniki and various other cities in Greece were asked to participate in the network — the main prerequisite being the availability of a PC installed with WHONET software. All data were entered into the program, if possible on a daily basis. Data entry was done either manually (for hospitals using the disc diffusion method for sensitivity testing) or by automatic download with the aid of BACLINK software (available upon request from Dr J. Stelling, Communicable Disease Surveillance and Response Programme, World Health Organization, Geneva), for hospitals using automatic sensitivity systems. Each laboratory was asked to agree to make available all electronic files for purposes of preparing multicentre reports.

Isolation and identification of pathogenic bacteria

Isolation of pathogenic bacteria from clinical specimens and identification to the species level was

performed by standard methods at the microbiology laboratories of each hospital participating in the network.

Sensitivity testing

The sensitivity testing methods used by the participating hospitals were as follows: Kirby–Bauer disc diffusion in 17 hospitals; Sensititre (Sensititre, Salem, NH, USA) in one hospital; Pasco (Difco, Detroit, MI, USA) in 11 hospitals. The zone diameters or minimum inhibitory concentrations (MICs), and not the interpretations of the tests, are entered into WHONET.

Quality control

Escherichia coli (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) strains are used as internal quality controls. Moreover, all laboratories participate in the External Quality Assurance scheme organized by the WHO Collaborating Centre for International Monitoring of Bacterial Resistance to Antimicrobial Agents, Centers for Disease Control and Prevention, Atlanta, GA, USA.

Data reporting

Reports are distributed within each hospital every 6 months. In addition, multicentre reports of resistance data are published monthly in the *Archives of Hellenic medicine*. Data are also published on the network's homepage on the World Wide Web (http://www.mednet.gr/whonet).

Results and discussion

Feasibility of the network

Our first objective was to assess the feasibility of implementing a national network, in view of the associated workload, shortage of resources, and general low level of medical informatics and availability of computer technology in Greek hospitals.

Of the 28 clinical laboratories that were approached, 22 are now routinely using WHONET software: 15 perform disc diffusion methods and 7 use automated systems. Interestingly, although the 15 carrying out disc diffusion methods started using the WHONET software immediately, six of the seven laboratories that based their sensitivity testing on automated systems started using WHONET only after the BACLINK software became available. The six laboratories that are not using the software claim this is due to a shortage of personnel.

Three of the laboratories using the WHONET software refused to distribute their data or participate in the network, and use their data only within their own hospital.

We feel, nevertheless, that our experience is encouraging. Most hospitals in Greece are in practice willing to implement a continuous system of antimicrobial resistance surveillance, despite the

Fig. 1. **Disc diffusion method: distribution of zone diameters around (a) gentamicin and (b) imipenem disks for *Escherichia coli*** (1400 isolates from 13 hospitals included)
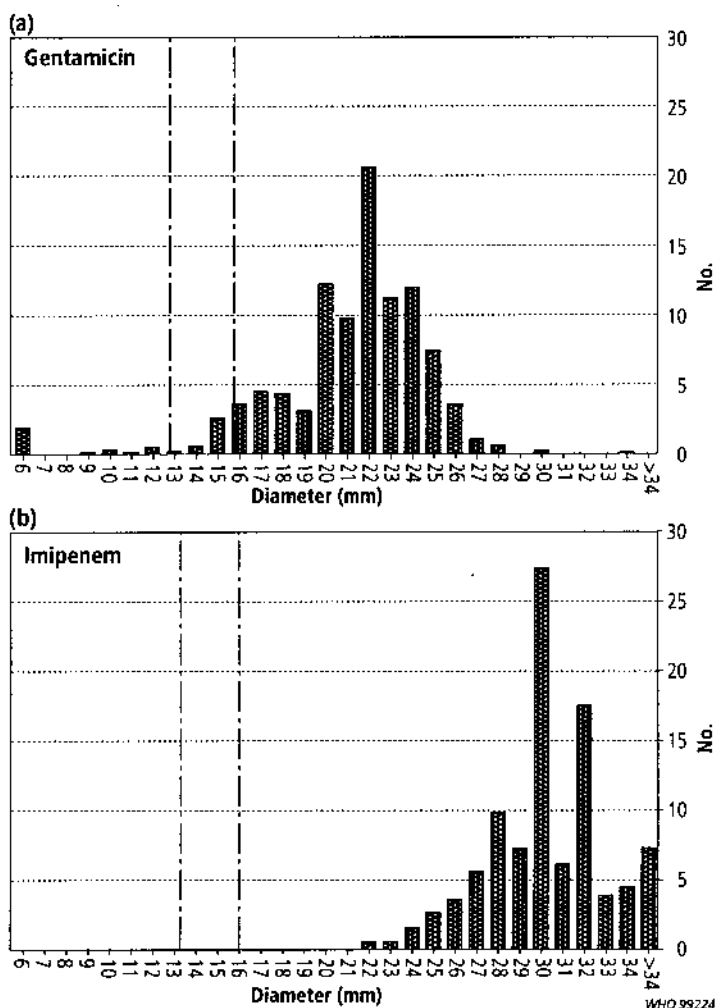
increases in the workload associated with manual data entry. The development of instruments for measuring zone size in the disc diffusion method as well as of software for automatic interfacing with central hospital information systems and for the downloading of data from the automated sensitivity systems to WHONET should solve these difficulties.

Surveillance of subclinical resistance

WHONET software generates a large database of species-specific zone size or MIC distributions for each antibacterial. These distributions are consistent with the existence of two (or more) subpopulations of bacteria: a sensitive one, and one or more populations that carry resistance mechanisms (11, 12). Microbiological breakpoints can be placed to separate the various biologically defined populations, and must be distinguished from the clinical breakpoints.

Monitoring the frequency distribution of zone sizes or MICs provides important information. A reduction in zone size (or an increase in MIC) of the sensitive subpopulation or the emergence of "new" subpopulations could indicate difficulties in quality control or reflect the emergence of a "new" mechanism of resistance. For example, Fig. 1 shows the frequency distribution of zone sizes for E. coli against gentamicin and imipenem from those hospitals using a disc diffusion method for sensitivity testing. With gentamicin, the microbiological breakpoints more or less coincide with the clinical breakpoints. In contrast imipenem's microbiological breakpoints have a disc diameter of 20 mm in most hospitals, whereas the National Committee for Clinical and Laboratory Standards clinical breakpoints for sensitivity are at 16 mm. In this instance, surveillance of the microbiological breakpoints might indicate the emergence of a new resistance mechanism in strains that are still clinically sensitive.

It should be noted that this type of surveillance can only be carried out by laboratories that perform either Kirby–Bauer or full scale MICs, and is not applicable in laboratories that use methods based on breakpoint determination. The need to establish a sentinel network of hospitals performing Kirby–Bauer or full-scale MICs in each country or region is therefore emphasized.

Assessing differences in resistance rates

It is well known that the epidemiology of antimicrobial resistance is regional, probably due to differences in local antibacterial policies and resistance patterns. By their very nature, large-scale surveillance studies might therefore overlook the fact that antimicrobial resistance does not develop uniformly across species, centres and countries. Rather, these changes are the result of a series of interactions between reservoirs of different genomes (species, plasmids and transposons) in different ecological niches with constantly changing selection pressures (13).

Fig. 2. **Rate of isolation of urine samples containing** *Pseudomonas aeruginosa, Enterobacter* spp. and *Enterococci faecalis,* **by individual hospital** (only medical wards are included)
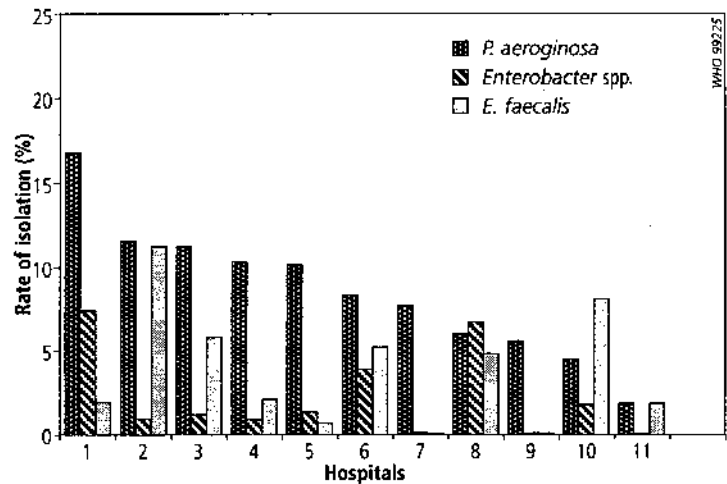

Fig. 3. **Rate of isolation of selective antimicrobial-bacterial combinations, by hospital** (ALL: cumulative mean of all hospitals)
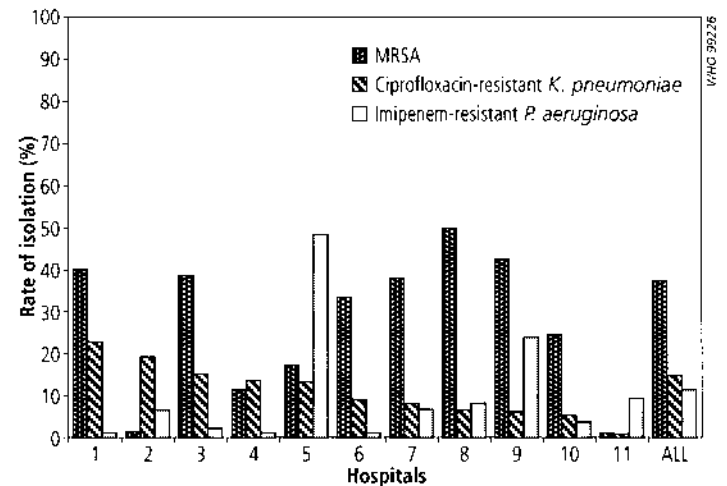
Fig. 4. **Rates of resistance to ciprofloxacin of five major bacteria, by hospital.** Each dot represents a single hospital. The dash represents the cumulative mean of all isolates from all hospitals. Only hospitals with more than 20 isolates per species are included (Reproduced by permission of *Archives of Hellenic medicine*)
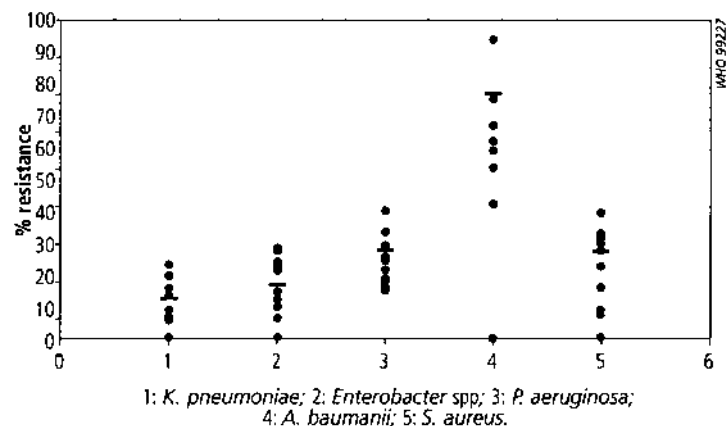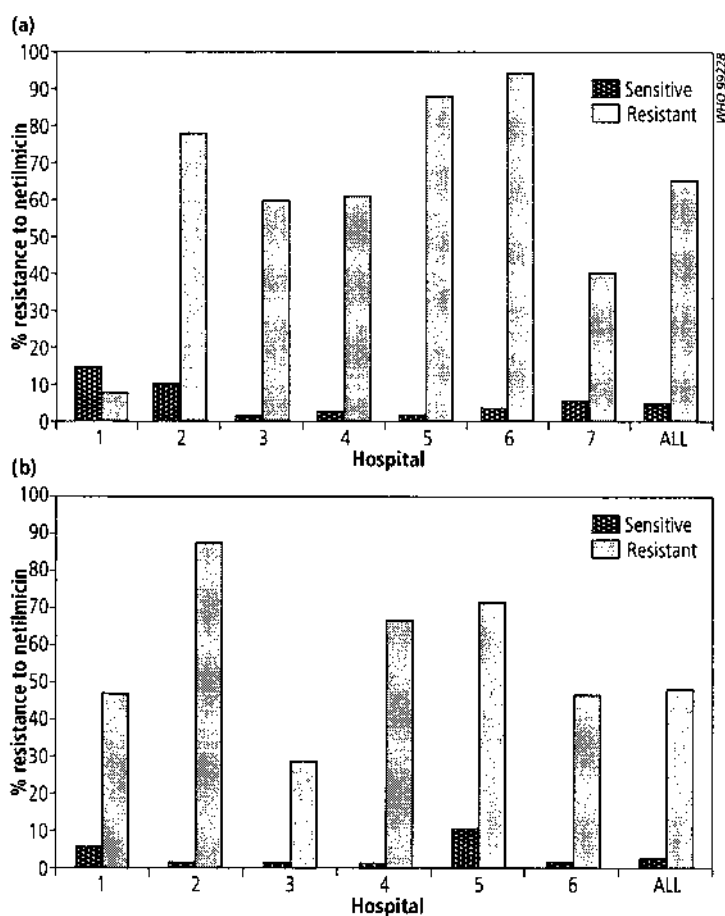

1: *K. pneumoniae*; 2: *Enterobacter* spp; 3: *P. aeruginosa*; 4: *A. baumanii*; 5: *S. aureus*.

Fig. 5. **Percentage resistance to netilmicin of isolates resistant or susceptible to third-generation cefalosporins and aztreonam, by individual hospital; (a)** *Klebsiella pneumoniae* **and (b)** *Enterobacter* **spp.** (ALL: cumulative mean of all hospitals)

Studies of the variation in resistance rates between hospitals of different size, nature, workload and geographical location are therefore valuable for interpreting the relative importance of the various risk factors involved. Nevertheless, national and international data are also useful since they permit the estimation of the overall burden of antimicrobial resistance and its evolution over time, the assessment of risk factors and preventive measures, and the comparison of resistance rates and patterns in different situations.

Although the WHONET software emphasizes local monitoring and management of resistance by each medical centre, its common file format facilitates comparison of resistance rates. Since the species isolated, resistance rates and resistance phenotypes can be compared between hospitals, resistance problems can be recognized, and priorities for further research can be set based on solid epidemiological data.

To illustrate this important function of the network, we show in Fig. 2 the differences in the rate of isolation of the usually multiresistant *P. aeruginosa*, *Enterobacter* spp. and enterococci from urine samples collected in the various participating hospitals. These differences result from variations in patients, nursing practices, duration of stay, and other factors which must be further investigated. The possibility of regional outbreaks or dissemination of resistant strains should also be studied.

In Fig. 3 the proportion of resistant isolates in three sentinel antimicrobial/organism combinations, by hospital, are shown. For each hospital, all wards are included (omitting intensive care units and outpatients). Only the first isolate per patient was included in the analysis. Each hospital appeared to have its own resistance pattern. If confirmed by further molecular and epidemiological studies, this indicates a need not only for nationwide strategies but also of local, hospital-based policies to combat resistance. Fig. 4 shows that the rate of resistance to ciprofloxacin varies considerably between the various hospitals. However, this variation could not be attributed to the different numbers of isolates tested in each hospital (data not shown). This finding indicates that the national mean resistance rate is a very crude indicator and underlines the need for hospital-based studies of factors responsible for the emergence of antimicrobial resistance.

Interpretive reading of the antibiogram

The availability, through WHONET, of a common format for analysis facilitates interpretation of antimicrobial resistance data and deduction, from the prevalent phenotypes, of the main resistance mechanisms and their spread within and between bacterial species, wards, and hospitals (*14*). Similarly, association of these data with molecular studies will facilitate understanding the public health significance of the various biological phenomena. Moreover, through this surveillance system, carefully selected representative strains possessing particular phenotypic resistance patterns can be referred to specialized laboratories for detailed genetic and biochemical studies. Some typical examples of this function of the network are shown in Fig. 5.

In most (but not all) of the study hospitals the *Klebsiella pneumoniae* isolates that were resistant to third-generation cefalosporins and aztreonam (Fig. 5a) seem to be more frequently resistant to aminoglycosides, consistent with the spread of multiresistant R plasmids in this species, as indicated by molecular data (*15, 16*). The same phenomenon is also observed in *Enterobacter* spp. (Fig. 5b), where a different molecular mechanism has been implicated (*17*).

Interestingly, in most hospitals *K. pneumoniae* and *Enterobacter* spp. more frequently exhibit resistance to netilmicin than to gentamicin (Fig. 6 a,b), consistent with the known spread in Greece of the AAC(6)-I aminoglycoside modifying enzyme (*18, 19*). On the other hand, the known predominance of the AAC(6)-II enzyme in *P. aeruginosa* (*19*) is also consistent with the equal rate of resistance to gentamicin and netilmicin observed in most hospitals (Fig. 6c).

Simultaneous resistance to erythromycin and clindamycin in *S. aureus* is in accord with the presence of erythromycin resistance methylase genes (*erm* genes), whereas erythromycin resistance not crossed to clindamycin is consistent with the presence of the *msrA* gene (*20*). The difference in susceptibility of the erythromycin-resistant *S. aureus* to clindamycin observed in the Greek hospitals could be the first indication of a difference in the epidemiology of these two resistance mechanisms, an observation that should be further studied.

Empirical therapy

The development of guidelines for the empirical therapy of infections should be based on an understanding of the epidemiology of antibiotic resistance and the mode of spread of resistant strains and genes within and between hospitals. The data generated by the electronic network are valuable for the development of hospital or even ward-based guidelines, which should eventually replace the countrywide general guidelines. For example, substitution of netilmicin or amikacin by gentamicin in the double therapy regimens for enterobacterial infections seems a reasonable course in hospitals where resistance to netilmicin is more prevalent than resistance to gentamicin (Fig. 6).

Conclusions and future prospects

The establishment of national networks for the continuous monitoring of antimicrobial resistance is recommended by WHO and other national and international bodies (*3*). Although national surveillance systems have been set up in a number of countries (*21*), we believe that Greece is one of the few to have an electronic network based on routine sensitivity results.

In order to increase the efficiency and quality of the network we are continuing to work on the following issues.

- Expansion of the network to include representative hospitals from all parts of Greece.
- Establishment of intensive quality control, especially in hospitals that report unusually high or low resistance rates.
- Adoption of a common minimum set of antimicrobials to be tested by all hospitals, to trace mechanisms of resistance more efficiently.
- Use of the surveillance data for the planning of focused research into the genetic and biochemical mechanisms of resistance that are necessary for the further understanding of the complex epidemiology of the phenomenon.

In conclusion, we believe that our experience demonstrates the potential of the electronic network for studying the patterns and trends in the epidemiology of antimicrobial resistance in the various participating hospitals. This will contribute to identification of the main factors for the emergence of resistance, as well as the priorities for further

Fig. 6. **Resistance rates to gentamicin and netilmicin, by hospital, for (a)** *Klebsiella pneumoniae*, **(b) Enterobacter spp. and (c)** *Pseudomonas aeruginosa* (ALL: cumulative mean of all hospitals)
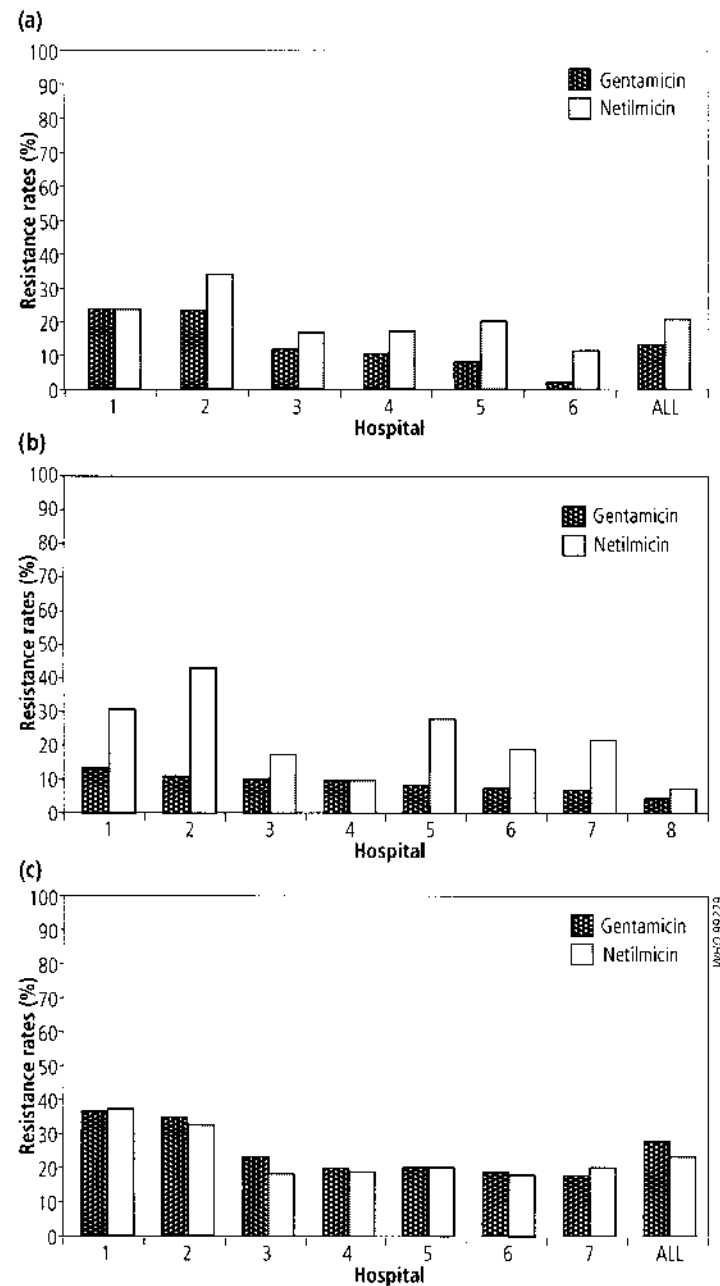
investigating the genetic and molecular mechanisms responsible, and thus for the development of a strategy to confront this major public health problem. ■

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Résumé

Un réseau électronique pour surveiller la résistance aux antimicrobiens des isolats de bactéries dans les hôpitaux en Grèce

La résistance aux antimicrobiens est devenu un problème de santé publique très préoccupant dans de nombreuses régions du monde. La plupart des organisations nationales et internationales, dont l'OMS, reconnaissent la surveillance systématique de cette résistance aux niveaux local, national et international comme un élément important de la stratégie de lutte.

Le présent article décrit le réseau mis en place en Grèce en 1995 pour surveiller en permanence la résistance aux antimicrobiens. Ce réseau, qui s'étend actuellement à 19 hôpitaux, s'appuie sur le principe fondamental que les résultats systématiques des tests de sensibilité aux antimicrobiens effectués quotidiennement dans chaque laboratoire clinique doivent être considérés comme une ressource majeure pour la surveillance de la résistance aux antimicrobiens. Le réseau a défini des méthodes communes de contrôle de qualité et adapté pour tous les hôpitaux un code et des données électroniques inspirés du logiciel WHONET. Ce logiciel, d'abord mis au point par le Centre collaborateur de l'OMS pour la surveillance de la résistance aux antibiotiques, Boston, Massachusetts (Etats-Unis d'Amérique), a été affiné par l'OMS.

Sur les 28 laboratoires cliniques participants qui ont été contactés ces trois dernières années, 22 utilisent maintenant le logiciel WHONET de façon systématique. Quinze de ces hôpitaux utilisent la méthode de la diffusion en gélose et sept des systèmes automatiques. Le logiciel WHONET est installé par les coordonnateurs dans chaque laboratoire d'hôpital et toutes les données sont entrées dans le programme, si possible quotidiennement. Chaque laboratoire est invité à fournir tous les dossiers électroniques ainsi constitués pour les rapports et les études multicentres. Des souches d'*Escherichia coli* (ATCC 25922), de *Staphylococcus aureus* (ATCC 25923) et de *Pseudomonas aeruginosa* (ATCC 27853) sont utilisées pour les contrôles internes de qualité effectués chaque semaine. Tous les laboratoires participent au plan d'évaluation externe de la qualité du programme OMS de surveillance de la résistance aux antimicrobiens organisé par le Centre collaborateur de l'OMS pour la surveillance internationale de la résistance bactérienne aux antimicrobiens, Centers for Disease Control and Prevention, Atlanta, Géorgie (Etats-Unis d'Amérique). Les dossiers WHONET de tous les hôpitaux sont réunis, puis traités électroniquement par les coordonnateurs du réseau, et des rapports multicentres sur les données relatives à la résistance sont établis et publiés chaque mois dans *Archives of Hellenic medicine.* Les données sont aussi accessibles sur le Web à la page d'accueil du réseau (http://www.mednet.gr/whonet).

En conclusion, nos résultats nous semblent démontrer la capacité du réseau électronique à étudier les tendances épidémiologiques de la résistance aux antimicrobiens dans les hôpitaux participants et entre ces hôpitaux. Le système joue donc un rôle clef dans l'identification des principaux facteurs de risque d'émergence d'une résistance et dans la définition des priorités de la recherche sur les mécanismes génétiques et moléculaires en jeu.

Resumen

Grecia: red electrónica de vigilancia de la resistencia a los antimicrobianos en aislados bacterianos nosocomiales

La resistencia a los agentes antimicrobianos ha llegado a ser un gravísimo problema de salud pública en muchas partes del mundo. Casi todas las organizaciones nacionales e internacionales, incluida la OMS, consideran la vigilancia sistemática de esa resistencia en los planos local, nacional e internacional como una parte integrante importante de la estrategia de lucha.

En el presente artículo se describe la red griega de vigilancia continua de la resistencia a los antimicrobianos, que se puso en marcha en 1995. La red comprende actualmente 19 hospitales y su funcionamiento se basa en la idea de que los resultados de las pruebas rutinarias de sensibilidad a los antimicrobianos que se realizan diariamente en cada laboratorio clínico han de considerarse un recurso primordial para la vigilancia de la resistencia a esos agentes. La red funciona con arreglo a un procedimiento común de control de la calidad y a un código electrónico y un formato de datos normalizados en todos los hospitales, merced a la utilización del programa informático WHONET. Este programa fue creado inicialmente por el Centro Colaborador de la OMS para la Vigilancia de la Resistencia a los Antibióticos, Boston (Massachusetts, EE.UU.), y desarrollado luego por la OMS.

Actualmente lo utilizan de manera sistemática un total de 22 de los 28 laboratorios clínicos participantes con los que se tomó contacto en los últimos tres años. De estos hospitales, 15 utilizan métodos de difusión *in vitro* y siete utilizan sistemas automáticos. El programa WHONET lo instalan los coordinadores de cada

laboratorio de hospital, y todos los datos se cargan en el programa a ser posible diariamente. Se pide a cada laboratorio su anuencia para facilitar todos los archivos electrónicos generados con vistas a la preparación de informes y estudios multicéntricos. Semanalmente se utilizan con fines de inspección interna de la calidad cepas de *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) y *Pseudomonas aeruginosa* (ATCC 27853). Todos los laboratorios participan en el plan de garantía externa de la calidad del programa de vigilancia de la resistencia a los antimicrobianos de la OMS, organizado por el Centro Colaborador de la OMS para la vigilancia internacional de la resistencia bacteriana a los agentes antimicrobianos, uno de los Centros de Control y Prevención de Enfermedades, Atlanta (Georgia, Estados Unidos de América). Los archivos WHONET de los distintos hospitales son fusionados y procesados electrónicamente por los coordinadores de la red, tras lo cual se preparan informes multicéntricos sobre los datos de resistencia, que se publican mensualmente en *Archives of Hellenic Medicine*. Los datos también están disponibles en la página de acceso de la red en la Web (http://www.mednet.gr/whonet).

En conclusión, creemos que los resultados que hemos obtenido demuestran el potencial de la red electrónica para estudiar las tendencias de la epidemiología de la resistencia a los antimicrobianos dentro de y entre los diversos hospitales participantes. En consecuencia, el sistema desempeña una función primordial en la determinación de los principales factores de riesgo de aparición de resistencia y de las prioridades de ulteriores investigaciones sobre los mecanismos genéticos y moleculares implicados.

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