

Multicentre surveillance of antibiotic resistance in nosocomial *Staphylococcus aureus* in Cyprus

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التَّصَدُّعُ المتعدد المراكز لمقاومة الجراثيم للمضادات الحيوية في العدوى المستشفوية بالعنقودية الذهبية في قبرص

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الخلاصة: قامت أربع مستشفيات عامة في قبرص بتقييم معدل وجود ذراري العنقودية الذهبية المقاومة للميثيسيلين في مزارع إيجابية للعنقوديات بين مرضى الأقسام الداخلية في الفترة من أيلول/سبتمبر 1999 إلى آذار/مارس 2000. وتم استفراد 193 من الذراري من مزارع للإفرازات التنفسية، وآفات الجلد والأغشية المخاطية، والدم، والبول. وتبين أن 80 ذرية (41.45%) من ذراري العنقودية الذهبية التي شملتها الدراسة كانت مقاومة أيضا للميثيسيلين، و113 ذرية (58.55%) كانت حساسة له. وكانت أكثر من 78.75% من الذراري المقاومة للميثيسيلين مقاومة أيضا للإريثروميسين، و18.75% منها مقاومة للجنتاميسين. أما الذراري الحساسة للميثيسيلين فقد كان 17.69% منها مقاوماً للإريثروميسين و7.08% مقاوماً للجنتاميسين، بينما تبين عدم انخفاض حساسية أي من هاتين الفئتين من الذراري للنانكروميسين.

ABSTRACT Four general hospitals in Cyprus evaluated the incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains in positive cultures of staphylococci among inpatients from September 1999 to March 2000. One hundred and ninety three (193) strains were isolated from cultures of respiratory secretions, skin and mucous membrane lesions, blood and urine. Of the *S. aureus* strains studied, 80 (41.45%) were MRSA and 113 (58.55%) were methicillin sensitive *S. aureus* (MSSA). More than 78.75% of the MRSA were resistant to erythromycin and 18.75% to gentamicin. Of the MSSA, 17.69% were resistant to erythromycin and 7.08% to gentamicin. None of the MRSA and MSSA strains showed reduced sensitivity to vancomycin.

Surveillance multicentrique de la résistance aux antibiotiques pour les souches nosocomiales de *Staphylococcus aureus* à Chypre

RESUME Quatre hôpitaux généraux à Chypre ont évalué l'incidence des souches de *Staphylococcus aureus* résistantes à la méthicilline dans les cultures positives de staphylocoques chez des patients hospitalisés de septembre 1999 à mars 2000. Cent quatre-vingt-treize (193) souches ont été isolées dans des cultures de sécrétions respiratoires, de lésions cutanées et de la membrane muqueuse, de sang et d'urine. Sur les souches de *S. aureus* étudiées, 80 (41,45 %) étaient des *Staphylococcus aureus* résistants à la méthicilline et 113 (58,55 %) des *Staphylococcus aureus* sensibles à la méthicilline. Plus de 78,75 % des *S. aureus* résistants à la méthicilline étaient résistants à l'érythromycine et 18,75 % à la gentamicine. Sur les *S. aureus* sensibles à la méthicilline, 17,69 % étaient résistants à l'érythromycine et 7,08 % à la gentamicine. Aucune des souches de *S. aureus* résistantes et sensibles à la méthicilline n'a montré de sensibilité réduite à la vancomycine.

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Introduction

Bacterial resistance to antibiotics is a serious problem which emerged soon after the discovery of antibiotics [1-5], resulting in increased morbidity and mortality and inflated health care costs. Penicillin-resistant *Staphylococcus aureus* has been identified since 1944 and methicillin-resistant *S. aureus* (MRSA) since 1961 [6]. In the United States of America, MRSA has caused great concern since 1970. This has now become a worldwide problem, as extensive multiresistance to antibiotics has been reported from a large number of countries [8-10]. The appearance of vancomycin-resistant enterococcus (VRE) and of *S. aureus* with reduced susceptibility to vancomycin has helped to understand the mechanisms of transfer of resistance from one microorganism to another. A direct result of this is the appearance of nosocomial isolates against which there are no active antibiotics available [6,10,11].

Given these facts, it is essential to have a clear view of the situation within a given country, through careful recording and assessment of microbial resistance to antibiotics in all therapeutic units. The purpose of this study was to evaluate the incidence of MRSA strains in the total of positive staphylococcus cultures (respiratory secretions, lesions of mucous membrane and skin, blood and urine) from inpatients, and the pattern of staphylococcus resistance to certain groups of antibiotics. The results presented here are the first recorded to date, and will form the basis for the establishment of specific measures for the reduction of MRSA in hospitals in Cyprus.

Methods

The Ministry of Health in Cyprus, together with the recently established National In-

fection Control Committee, has started systematic screening of MRSA strains in the four major district hospitals in the Government-controlled area of Cyprus. The hospitals have a total of 932 beds (the population of Cyprus totals 700 000) and are located in the cities of Nicosia, Limassol, Larnaca and Paphos. The study dealt with the positive results for *S. aureus* from September 1999 to March 2000 and we studied 193 strains from these four hospitals.

The Nosocomial Infection Control Committee of each participating hospital collected and evaluated the positive results for *S. aureus* on a daily basis. It is worth noting that these committees started functioning only in January 1999. The results were collected from the microbiology laboratory, and at the end of each month they were sent to the National Infection Control Committee in the Ministry of Health.

The strains were isolated from cultures of respiratory secretions, lesions of mucous membrane and skin, blood and urine. The isolation was done in accordance with NCCLS (National Committee for Clinical Laboratory Standards) using the production of the enzyme catalase and free or conjugated coagulase, and the Apl 20 Staph Test System (bioMérieux, France). The sensitivity to various antibiotics was evaluated by antibiogram using the Kirby-Bauer disc diffusion method. The methicillin-resistance test was carried out with Müller-Hinton agar in 2% sodium chloride. A 1 µg oxacillin disc was used and the discs were incubated at 35 °C for 24 hours [12,13].

The isolation, identification and sensitivity tests were performed using the same method in all four hospitals. The antibiotics used on the antibiogram were defined by the microbiology laboratories. The number of antibiotics used on the antibiogram was small because of technical difficulties. Differences were analysed for statistical sig-

nificance using the chi-squared test. A P -value < 0.05 was the accepted statistical level of significance.

Results

During the period September 1999 to March 2000, 193 strains of *S. aureus* were isolated from the four general hospitals in Cyprus. Of the 193 isolated strains, 80 (41.45%) were resistant to methicillin (MRSA) and 113 (58.55%) strains were methicillin-sensitive *S. aureus* (MSSA). The pattern of resistance to methicillin in each hospital was as follows. Of the 102 strains of *S. aureus* isolated in Nicosia General Hospital, 60 were MRSA; of the 35 strains isolated in Limassol General Hospital, 11 were MRSA; of the 46 strains isolated in Larnaca General Hospital, 9 were MRSA; and of the 10 strains isolated in Paphos none was methicillin-resistant. The differences were statistically significant $P < 0.01$ (Table 1).

Tables 2 and 3 show the incidence of resistance of MRSA and MSSA to erythro-

Table 1 Incidence of MSSA and MRSA among positive cultures in the four general hospitals in Cyprus, September 1999–March 2000

Hospital	MSSA		MRSA	
	No. strains	%	No. strains	%
Nicosia	42/102	41.17	60/102	58.82
Limassol	24/35	68.57	11/35	31.42
Larnaca	37/46	80.43	9/46	19.56
Paphos	10/10	100.0	0/10	0.00
Total	113/193	58.55	80/193	41.45

$\chi^2 = 30.29$, $P < 0.01$.

MSSA = methicillin-sensitive *Staphylococcus aureus*.

MRSA = methicillin-resistant *S. aureus*.

mycin (15 µg disc), gentamicin (10 µg disc) and vancomycin (30 µg disc) in the four hospitals under study. The differences shown in these tables are not statistically significant.

Table 2 Comparison of the resistance of methicillin-sensitive *Staphylococcus aureus* to different antibiotics in the four general hospitals in Cyprus, September 1999–March 2000

Hospital	Methicillin-sensitive <i>S. aureus</i>					
	Erythromycin-resistant ^a		Gentamicin-resistant ^b		Vancomycin-resistant	
	No. strains	%	No. strains	%	No. strains	%
Nicosia	6/42	14.28	5/42	11.90	0/42	0.0
Limassol	6/24	25.00	0/24	0.00	0/24	0.0
Larnaca	6/37	16.21	3/37	8.11	0/37	0.0
Paphos	2/10	20.00	0/10	0.00	0/10	0.0
Total	20/113	17.69	8/113	7.08	0/113	0.0

^a $\chi^2 = 1.31$, $P > 0.05$.

^b $\chi^2 = 4.14$, $P > 0.05$.

Table 3 Comparison of the resistance of methicillin-resistant *Staphylococcus aureus* to different antibiotics in the four general hospitals in Cyprus, September 1999–March 2000

Hospital	Methicillin-resistant <i>S. aureus</i>					
	Erythromycin-resistant ^a		Gentamicin-resistant ^b		Vancomycin-resistant	
	No. strains	%	No. strains	%	No. strains	%
Nicosia	48/60	80.0	11/60	18.33	0/60	0.0
Limassol	8/11	72.72	1/11	9.07	0/11	0.0
Larnaca	7/9	77.77	3/9	33.33	0/9	0.0
Paphos	–	–	–	–	–	–
Total	63/80	78.75	15/80	18.75	0/80	0.0

^a $\chi^2 = 1.31$, $P > 0.05$.

^b $\chi^2 = 4.14$, $P > 0.05$.

Discussion

The results shown in Table 1 indicate a fairly high incidence of MRSA isolates for the period September 1999 to March 2000. With regard to the samples taken from the four hospitals in Cyprus, the rate of resistance of *S. aureus* to methicillin can be considered indicative of nosocomial infection in the whole country. The highest incidence of isolates was found in the two hospitals with the largest number of beds and which also have intensive care units (ICU). The appearance of a high incidence of MRSA in ICU is associated with the use of assisted ventilation (respirator), tracheostomy and other invasive procedures that involve the upper respiratory system and are often performed on critically ill patients [4,14,15]. This is not unique to Cyprus, since it is evident from the international literature that most countries of the world show an increased incidence of MRSA strains in the ICU [2,4,14,16–18].

Worldwide, the increase in incidence of MRSA correlates with an increase in resistance to other groups of antibiotics. Several studies have reported increased resistance

to gentamicin and erythromycin as well [5]. In our study we recorded very high rates of resistance to erythromycin (78.75%) in the three hospitals where MRSA were isolated. For gentamicin, the incidence of resistance was low (18.75%). One of the hospitals recorded a significantly higher rate of resistance (33.33%). The resistance mechanisms of staphylococcus to aminoglycosides that have been studied to date suggest that if a strain of staphylococcus is resistant to gentamicin then it will also be resistant to tobramycin and kanamycin. If, on the other hand, a strain is sensitive to gentamicin, then it may be either sensitive or resistant to tobramycin and kanamycin. For this reason, and because the epidemiological data for the resistance mechanisms of staphylococcus change frequently and new resistance phenotypes can appear, it is recommended that the microbiology laboratories of the hospitals taking part in the study include the rest of the aminoglycosides in the antibiogram as well.

The incidence of resistance of MSSA to gentamicin is very low (7.08%), while a relatively high resistance to erythromycin

(17.69%) was recorded. No strains of *S. aureus* with resistance to vancomycin were recorded in our study in Cyprus. It was recently reported in Japan that a vancomycin-resistant strain was isolated from an infected surgical wound in a 4-month-old baby [19]. A similar strain was isolated in the United States of America from a 79-year-old man with end-stage renal disease [20].

Since our study confirms a high incidence of MRSA, strict measures should be taken to avoid spread of the MRSA strains. It is known that the resistance of enterococcus to vancomycin (VRE) can be transmitted to *S. aureus* strains [20].

As a result of the high percentage of MRSA cases detected at the start of the study, the National Infection Control Committee of the Ministry of Health recommended to the local Infection Control Committees of the hospitals various mea-

sures [21–23], such as early identification and reporting of patients infected and colonized with MRSA (surveillance), using disposable materials for each of these patients, training personnel responsible for the sterilization of the equipment used for treating the patients, training nursing and medical staff to wash hands with antiseptic soap before and after contact with the patient, using disposable gloves and masks, early discharge of the patients from the hospital in order to avoid contamination of other patients with MRSA, and finally an increase in the number of antibiotics used in the antibiogram.

Continuous surveillance is in use for the early identification and reduction of outbreaks and isolation of unusual antimicrobial resistance in Cyprus. The genotype of the MRSA strains isolated in the three hospitals in Cyprus will be studied in the near future.

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