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CONCISE ARTICLE

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Multicenter pilot survey of resistant bacteria in the Mediterranean area

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Abstract With the aim of testing the feasibility of a multiresistant bacteria (MRB) surveillance methodology and evaluating the level of antimicrobial resistance and dissemination of resistant pathogens in the Mediterranean area, a pilot study was carried out in nine university hospitals in Algeria, Tunisia and France. The results indicate that third-generation cephalosporin-resistant *Enterobacteriaceae* comprise the major MRB in Algerian and Tunisian hospitals. In France, the highest incidence rates were found for methicillin-resistant *Staphylococcus aureus*, while in Tunisian hospitals, imipenem-resistant *Acinetobacter baumannii* seems to be a particularly prevalent organism. Although the data were not representative of the participating countries as a whole, the results show the importance and ubiquity of the problem in the area and the feasibility of surveillance.

Keywords Multiresistant bacteria · NosoMed · Mediterranean region

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Introduction

Antibiotic resistance is an increasing problem in hospitals and it has a real impact on morbidity, mortality and costs in healthcare settings. Several national and international surveillance systems have been developed to improve the monitoring of antibiotic resistance [1–3]. In the Mediterranean area, some initiatives have begun but, overall, surveillance efforts are still very limited [4].Within the framework of the NosoMed project, designed to strengthen healthcare epidemiology for the investigation of nosocomial infections in the Mediterranean area, a relatively simple surveillance methodology based on microbiological laboratory data was designed and tested. The main objective of this study was to evaluate (a) the feasibility of performing such surveillance and (b) its ability to provide information on the level of resistance and dissemination of resistant pathogens within hospitals.

Materials and methods

This prospective, multicenter study was conducted between March 2003 and January 2004 with the voluntary participation of nine university hospitals: four from Algeria (3-month period), two from Tunisia (6-month period) and three from France (3-month period). The French participating centers were members of a broader regional surveillance network, "BMR Sud-Est" [5].

The study was performed using microbiological laboratory data from the participating hospitals. The surveillance focused on methicillin-resistant *Staphylococcus aureus* (MRSA), ceftazidime-resistant *Pseudomonas aeruginosa*

(CRPA), imipenem-resistant *Acinetobacter baumannii* (IRAB) and third-generation cephalosporin-resistant *Enterobacteriaceae* (CRE). Isolates with intermediate susceptibility were considered resistant. Species were identified using routine bacteriological procedures. Antimicrobial susceptibility testing was performed using a disk diffusion method on Mueller–Hinton agar. *Enterobacteriaceae* were submitted to the extended-spectrum β-lactamase (ESBL) production test with double-disk synergy. Quality control was performed using standard norms in a routine microbiology laboratory.

The antimicrobial susceptibility testing results were interpreted according to approved breakpoints issued by the Clinical and Laboratory Standards Institute (CLSI) (formerly National Committee for Clinical Laboratory Standards) [6] in Algerian hospitals and the French Society for Microbiology (CA-SFM) [7] in Tunisia and France. Minimum inhibitory concentration (MIC) breakpoints were the same for MRSA (susceptible if MIC ≤2 mg/l) and IRAB (susceptible if MIC ≤4 mg/l) and different for CRPA and CRE (susceptible if MIC ≤4 mg/l in CA-SFM guidelines and MIC ≤8 mg/l in CLSI guidelines).

For each type of MRB, only one isolate from the same specimen source per patient and per stay (subjects hospitalized for a period of at least 24 h) was included. All duplicate (i.e., multiple isolates of the same species from the same patient), screening, and environmental samples were excluded. Only strains recovered from diagnostic specimens were included. They were collected from blood cultures, subcutaneous punctures (i.e. pus, hematoma, liquid, tissue, or prosthetic material obtained from sterile anatomic sites by surgery or biopsy, ascites or peritoneal liquid, cerebrospinal fluid, pleural or articular liquid), protected and non-protected respiratory samples, intravascular devices (peripheral or central catheters, implanted access ports), and urine.

For each MRB isolate included in the analysis, the following data were collected: the date and site of sample acquisition, the specialty of the unit, and the age and gender of the patient. At the end of the surveillance period, the following variables (denominator data) were noted: number of beds per hospital, number of admissions, number of days of hospitalization, total number of each type of bacterial strain collected during the study period. Statistical analyses were performed using Epi Info software version 6.04. The following rates were calculated: (a) percentage of MRB: defined as the number of resistant bacterial isolates divided by the total number of bacterial isolates of the same type (×100); (b) MRB attack rate: defined as the number of resistant bacterial isolates divided by the number of all hospital admissions for the same period (×100); (c) MRB incidence rate: defined as the number of resistant bacterial isolates divided by the number of hospital days for all patients for the same period (×1,000).

Results and discussion

Overall, 970 MRB strains were identified during the study period. Distribution of MRB differed between countries, with the majority of MRB isolates being obtained from medical units in France (46.5%), from intensive care units in Tunisia (38.2%) and from surgical units in Algeria (36.7%). Similarly, the sample sites from which MRB were collected differed among countries (Table 1).

The main indicators for the MRB isolated per country are presented in Table 2. Of a total of 1,522 *S. aureus* strains isolated, 339 (22.3%) were resistant to methicillin. The proportion of MRSA collected from patients in

Table 1 Distribution of multiresistant bacteria in three Mediterranean countries according to hospital unit and sampling site

Sample source	Number (%) of isolates			
	Algeria (*n*=286)	Tunisia (*n*=288)	France (*n*=396)	Total (*n*=970)
Unit				
Medicine	56 (19.6)	52 (18.1)	184 (46.5)	292 (30.1)
Surgery	105 (36.7)	69 (24.0)	96 (24.2)	270 (27.8)
Intensive care	29 (10.1)	110 (38.2)	85 (21.4)	224 (23.1)
Pediatrics	56 (19.6)	33 (11.5)	11 (2.8)	100 (10.3)
Maternity	26 (9.1)	3 (1.0)	1 (0.3)	30 (3.1)
Other	14 (4.9)	21 (7.2)	19 (4.8)	54 (5.6)
Site				
Blood culture	31 (10.8)	73 (25.3)	24 (6.1)	128 (13.2)
Subcutaneous puncture	143 (50.0)	83 (28.8)	10 (2.5)	236 (24.3)
Protected respiratory sample	14 (4.9)	42 (14.6)	7 (1.8)	63 (6.5)
Non-protected respiratory sample	25 (8.7)	4 (1.4)	77 (19.4)	106 (10.9)
Intravascular device	1 (0.3)	17 (5.9)	1 (0.3)	19 (2.0)
Urine	48 (16.8)	55 (19.1)	85 (21.5)	188 (19.4)
Other[a]	24 (8.4)	14 (4.9)	192 (48.5)	230 (23.7)

[a]Superficial suppurations and cultures from pressure sores

Table 2 Characteristics of multiresistant bacteria in participating countries

Strain type and country	Total number	No. resistant[a]	Percent resistant within species	Attack rate[b]	Incidence[c]	Incidence in ICU[d]
S. aureus						
Algeria	203	72	35.5	0.21	0.35	0.61
Tunisia	336	42	12.5	0.16	0.20	0.54
France	983	225	22.9	0.76	1.17	1.63
Total	1522	339	22.3	0.38	0.55	1.10
P. aeruginosa						
Algeria	193	40	20.7	0.12	0.20	2.74
Tunisia	254	34	13.4	0.13	0.16	1.54
France	449	46	10.2	0.16	0.24	1.05
Total	896	120	13.4	0.13	0.20	1.40
A. baumannii						
Algeria	97	5	5.2	0.01	0.02	0.30
Tunisia	191	55	28.8	0.21	0.26	3.0
Total	288	60	20.8	0.10	0.14	2.46
Enterobacteriaceae						
Algeria	658	169	25.7	0.49	0.83	5.18
Tunisia	1192	157	13.2	0.60	0.73	3.38
France	–	125	–	0.42	0.65	2.26
Total	–	451	–	0.50	0.74	2.98

[a]For *S. aureus* strains resistant to methicillin, for *P. aeruginosa* strains resistant to ceftazidime, for *A. baumannii* strains resistant to imipenem, and for *Enterobacteriaceae* strains resistant to third-generation cephalosporins
[b]Attack rate per 100 admissions
[c]Incidence per 1,000 days of hospitalization
[d]*ICU* intensive care unit

maternity units was especially high in Algeria (31.9% of total MRSA), particularly in one of the participating hospitals.

A total of 896 *P. aeruginosa* strains was obtained from all participating hospitals, and 13.4% was shown to be resistant to ceftazidime. Given that the percentages of resistance for IRAB and CRE were not available for France, our results for these microorganisms were based only on data from the participating Algerian and Tunisian hospitals. Overall, the percentage of IRAB was higher in Tunisia (28.8%) than in Algeria (5.2%), while the percentage of CRE was higher in Algeria (25.7%) compared with Tunisia (13.2%). Pediatric services were principally affected by CRE; in Algeria and Tunisia, 28.4% (48 strains) and 17.2% (27 strains) were CRE in these units, respectively. The most common types of CRE in these two countries were *Klebsiella pneumoniae* (32.5 and 35.7%, respectively), *E. coli* (15.4 and 30.6%, respectively), and *Enterobacter cloacae* (14.2 and 14.0%, respectively). In France, the primary CRE organisms were *Enterobacter cloacae* (33.6%), *Enterobacter aerogenes* (20.8%), and *Citrobacter* spp (14.4%). In Algeria and Tunisia 92% and 72.7% of CRE isolates, respectively, were ESBL, while this proportion was only 26% in France.

In France, the highest incidence and attack rates were found for MRSA (incidence, 1.17/1,000 hospitalization days), while in Algeria and Tunisia CRE represented the principal MRB (incidence, 0.83 in Algeria and 0.73 in Tunisia) (Table 2).

The main objective of the present work was to set up and evaluate the feasibility of a standardized protocol for monitoring MRB in the Mediterranean region. This objective was largely accomplished as the protocol was judged to be straightforward and to contain very few missing values (0.2% for specialty of units, 0.4% for age, and 0.6% for gender).

The percentage of *S. aureus* strains demonstrating resistance to methicillin differed among countries and ranged from 12.5% in Tunisia to 35.5% in Algeria. This variability has been demonstrated previously in other multicentric studies carried out in the Mediterranean area, with resistance rates of 41% in Greece [8] and 71% in Egypt [9], 45% in Morocco [10], and 35.4% in Turkey [11]. Compared to our data for CRPA, a study carried out in Egypt found a higher percentage of these organisms [9], while similar data were found in a study conducted in Tunisia [12].

The findings concerning IRAB are particularly alarming in Tunisia, especially in ICUs. The importance of IRAB has already been raised in the Tunisian hospitals participating in the NosoMed Network [13] and in other studies [12], highlighting that this microorganism warrants more surveillance in the Mediterranean area. Currently, *A. baumannii* is not included in antibiotic resistance survey programs in European countries despite its recent implication in an epidemic in French hospitals [14]. In the present study, CRE emerged as having the highest incidence among the microorganisms studied in Algeria and Tunisia, while in France these rates were lower than that observed for MRSA.

Our results might be sensitive to some bias or limitations. For example, the variation among the sources of specimens in the three countries reflects variable densities of sample collection and thus biases the distribution of organisms. Furthermore, the number of organisms identified was higher in France compared to other countries. This difference might be due to a higher proportion of infections,

a larger number of samples collected (in the context of better laboratory facilities) or to both causes. Hence, sampling procedures should be standardized to allow better comparability of data among participating centers. This current lack of standardization also explains why information from France was incomplete for IRAB and percentage of resistance of CRE, since this data was not routinely collected by the "BMR Sud-Est" network. One possible limitation of the study was the absence of internationally standardized MIC breakpoints, which lead us to accept both the French and American standards, even if this does not explain the observed differences. An epidemic at the time of the study (as may have been the case in an Algerian hospital presenting particularly high rates of MRSA) could also have influenced the data.

Our data is intended to encourage further participation of hospitals in defining targets for the control of MRB and to stimulate surveillance and prevention on local and national levels. The performance of continual or periodic studies using the same surveillance methodology will allow participating hospitals to improve their surveillance tools and generate more valid data; moreover, it will help identify the temporal evolution of MRB.

In conclusion, antibiotic resistance is a significant problem in Mediterranean hospitals. In order to have reliable data, it is important to focus on MRSA [15], but it is also necessary to track other bacteria that appear to have significant epidemiologic implications. This study will be augmented by audits on the prescription of antibiotics and infection control measures.

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