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Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in invasive isolates from southern and eastern Mediterranean countries

Michael A. Borg[1]*, Marlieke de Kraker[2], Elizabeth Scicluna[1], Nienke van de Sande-Bruinsma[2], Edine Tiemersma[2], Jos Monen[2] and Hajo Grundmann[2] on behalf of the ARMed Project members and collaborators[†]

[1]Infection Control Unit, Mater Dei Hospital, Msida MSD05, Malta; [2]National Institute of Public Health and the Environment, Center for Infectious Disease Control, Epidemiology and Surveillance, Bilthoven, The Netherlands

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***Objectives*: Efforts aimed at curtailing the ever increasing spread of methicillin-resistant *Staphylococcus aureus* (MRSA) require effective information of its epidemiology. However, knowledge about the situation in southern and eastern countries of the Mediterranean is incomplete since reports have been sporadic and difficult to compare.**

***Methods*: Over a 36 month period from 2003 to 2005, the ARMed project collected more than 5000 susceptibility test results of invasive isolates of *S. aureus* from blood cultures routinely processed within participating laboratories servicing 62 hospitals situated in Algeria, Cyprus, Egypt, Jordan, Lebanon, Malta, Morocco, Tunisia and Turkey.**

***Results*: Overall, the median MRSA proportion was 39% (interquartile range: 27.1% to 51.1%). The highest proportions of MRSA were reported by Jordan, Egypt and Cyprus, where more than 50% of the invasive isolates were methicillin-resistant. Considerable variation was identified in the proportion of MRSA in hospitals within the same country.**

***Conclusions*: It appears that most of the countries in the Mediterranean region are experiencing a surge in MRSA infections. This requires a greater focus to identify relevant drivers of resistance and implement effective practices in order to address them, especially improved infection control and antibiotic consumption practices.**

Keywords: surveillance, epidemiology, resistance, ARMed

Introduction

Since the early 1980s, methicillin-resistant *Staphylococcus aureus* (MRSA) strains have proliferated in hospitals throughout the world, mainly through clonal spread.[1] The epidemiology of MRSA varies considerably on a global basis and even shows remarkable differences at regional level.[2] In Europe, a north to south gradient has been reported, with the highest proportions of resistant isolates found in the Mediterranean countries.[3] Among the countries participating in the European Antimicrobial Resistance Surveillance System (EARSS) (www.rivm.nl/earss), Greece, Spain, Italy, Israel and Croatia have all reported prevalence of 25% or more for methicillin resistance within *S. aureus* blood culture isolates.[4]

The Mediterranean region, therefore, appears to be a hyperendemic geographic area for MRSA. However, equivalent information about the epidemiology in the non-European countries of the southern and eastern Mediterranean has been sparse. In addition to being few in number, studies have used different methodologies and, as a result, are difficult to compare.[2,5]

This lacuna has been addressed by the Antibiotic Resistance Surveillance and Control in the Mediterranean Region (ARMed)

*Correspondence address. ARMed Project, Infection Control Unit, St Luke's Hospital, G'Mangia MSD08, Malta. Tel: +356-2545-4528; Fax: +356-2545-4529; E-mail: michael.a.borg@gov.mt
[†]ARMed Project members and collaborators are listed in the Acknowledgements section.

project (www.slh.gov.mt/armed) that started in January 2003.[6] Over a 3 year period, this study has documented the prevalence of antibiotic resistance in several key pathogens isolated in the southern and eastern Mediterranean countries as well as attempted to investigate potential influencing factors such as antibiotic consumption and infection control.

Materials and methods

Using the methodology adopted by the EARSS network,[4] ARMed collected antimicrobial susceptibility test results for *S. aureus* isolated from routine blood cultures as sent to the participating laboratories by identified hospitals. Oxacillin susceptibility was determined by using an oxacillin screening plate at 6 mg/L, an oxacillin (1 or 5 μg) disc and/or a cefoxitin (10 μg) disc. Our protocol requested participating laboratories to confirm non-susceptible isolates through *mecA* PCR, PBP2a agglutination or oxacillin MIC. Susceptibility testing of other antibiotics (tetracycline, erythromycin, ciprofloxacin, gentamicin and rifampicin) was performed on an optional basis. All antimicrobial susceptibility testing was performed by the individual laboratories who interpreted the results according to their own guidelines, in which 70% of the cases were based on those of the CLSI (formerly the NCCLS). Only the first isolate from any individual patient was included in the database. Any susceptibility data lacking mandatory information (including date of sample, laboratory code and patient identifier) were rejected together with duplicate isolates from the same patient within the same year.[4]

Two external quality assessment (EQA) exercises were performed, in 2003 and 2004, to obtain an insight into the validity and comparability of test results. The exercise, undertaken in conjunction with the laboratories in the EARSS network, utilized a mixture of different organisms, distributed by UK NEQAS (United Kingdom National External Quality Assessment Service) based at the Health Protection Agency (HPA).[7] The *S. aureus* in the 2003 EQA exercise was fully susceptible, with oxacillin MIC between 0.25 and 0.5 mg/L. The overall concordance for this strain was 100% for both species identification as well as for oxacillin susceptibility. A methicillin-heteroresistant *S. aureus* was also distributed in 2003 for which a 69% concordance for oxacillin (MIC 2–4 mg/L) was reported, whereas concordances for other antibiotics were higher than 92%. In 2004, the overall concordance for detection of methicillin resistance was 74% when tested with either oxacillin (MIC 64 to >256 mg/L) or cefoxitin (MIC 16–32 mg/L) or both. The results obtained were broadly equivalent to those obtained by the European EARSS laboratories.

To be able to determine the MRSA incidence and understand the frequency of utilization of blood culture as a diagnostic tool, a questionnaire was sent to all participating hospitals and laboratories. The questionnaire included queries about the total number of patient days in the hospital and the total number of blood cultures processed in the laboratory. The MRSA incidence was calculated as: total number of MRSA isolates (database)/total number of patient days (questionnaire). Only hospitals with information on both variables were included in this analysis. The blood-culturing rate was calculated from the questionnaire feedback as: total number of blood cultures/total number of patient days. Laboratories were only included in this part of the analysis if all their catchment hospitals reported the respective bed information data.

Country-specific trend analysis was performed using the Cochrane-Armitage test. Countries had to report at least 20 isolates per year, for all 3 years, to be included in the trend analysis. Correlation of the blood-culturing rate, or the size or type of hospital with resistance proportions or incidences, was undertaken using the Spearman correlation. *Z*-score evaluation was used to analyse the range of intra-country variance of MRSA resistance. Most analyses were performed in Medcalc, version 9.2.1.0 (Medcalc Software, Mariakerke, Belgium).

Results

An overall total of 5353 *S. aureus* isolates were reported to ARMed for the 36 month study period (2003–05), by 62 hospitals serviced by 59 laboratories in all 9 participating countries. The overall median MRSA proportion was 39% (interquartile range: 27.1% to 51.1%). The highest overall proportions of MRSA were reported by Jordan, Egypt and Cyprus, where more than 50% of the *S. aureus* blood cultures isolates were methicillin-resistant (Table 1). Over the 3-year duration of data collection, a statistically significant increasing trend ($P < 0.05$) was observed in hospitals within Egypt as well as Malta. On the other hand, significant decreases were seen in Jordan and in Turkey. In order to eliminate possible sampling bias due to changes in the composition of reporting laboratories over the time of the project, trend analysis was repeated, assessing only the labs that reported consistently for all 3 years; the results did not change.

Within most of the participating countries, considerable variation was evident between the MRSA prevalence rates in individual hospitals of the country (Figure 1). In fact, the majority of countries with at least three hospitals showed intra-country *Z*-score MRSA differences in excess of 2; the biggest difference was found in Egypt and Turkey where the divergence was almost 3.5. The smallest variance was found in Morocco and Tunisia, the two countries with the lowest overall MRSA proportion. Resistance proportions were not related to the type or size of the hospital.

We also examined the distribution of single methicillin resistance and multiresistance (defined as resistance to methicillin together with three or more of ciprofloxacin, tetracycline, gentamicin and erythromycin) of *S. aureus* for the total length of the study period for isolates in which all five antibiotics were tested. The highest rate of multiresistance was found in Turkey, reaching levels of 73% of all MRSA isolates reported. Resistance to methicillin and two other antimicrobial groups was predominant in MRSA isolates from Cyprus (63%), Morocco (47%) and Tunisia (42%). On the other hand, the majority of MRSA isolates from Algeria were resistant to methicillin alone.

The blood-culturing rate varied from 2.5 blood cultures per 1000 patient days in Egypt to 24.0 blood cultures per 1000 patient days in Cyprus (Table 1). The varying blood-culturing rates in the different countries, however, were not significantly associated with MRSA resistance proportions, yielding a Spearman correlation (r) of 0.14 ($P = 0.76$). As shown in Figure 2, MRSA proportions and MRSA incidences in the ARMed database were found to correlate (Spearman's r: 0.71; $P < 0.01$). This implies that the ranking of countries and hospitals is similar when using resistance proportions instead of incidences.

Discussion

The information collected by our study constitutes the largest data set on the epidemiology of MRSA collected in the south and

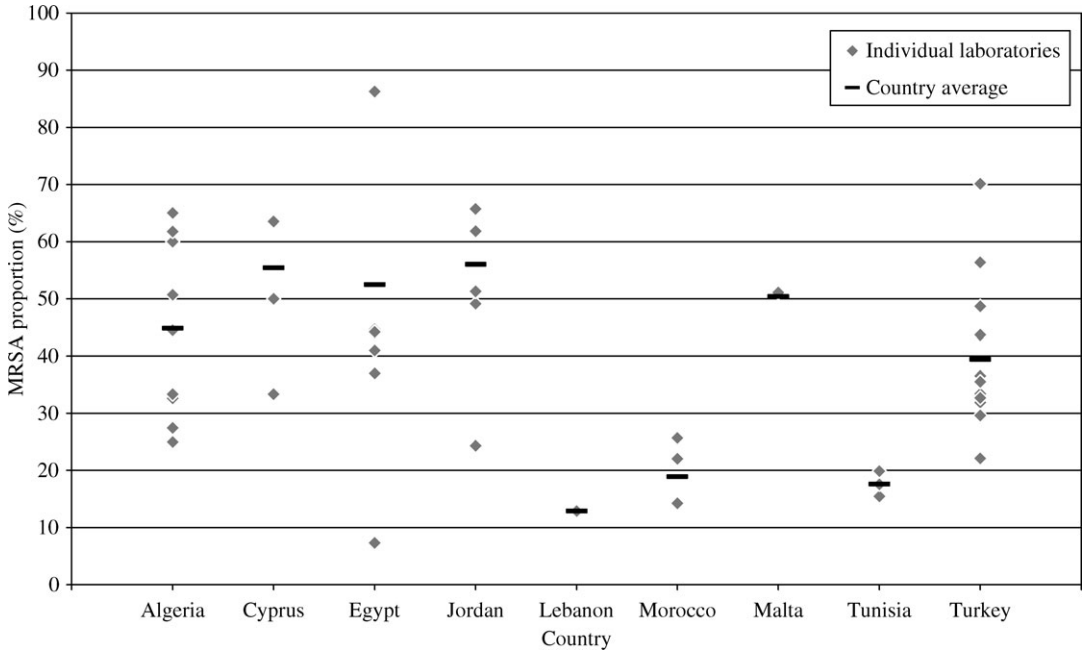

Figure 1. Individual MRSA proportions for hospitals reporting at least 10 *S. aureus* isolates for the whole surveillance period (2003–05) together with the country average.

east of the Mediterranean. The results unequivocally show that methicillin-resistant *S. aureus* is significantly present in this part of the world. This concurs well with prior anecdotal information from the same region. In fact, the MRSA proportions reported by ARMed centres correlate closely with previous sporadic reports already published from Algeria,[8] Cyprus,[9] Jordan,[10] Lebanon,[11] Turkey[12] and Tunisia.[13] Even the very high prevalence identified in Egyptian hospitals is well in line with a previous study which reported oxacillin resistance >60%.[14] This agreement is quite remarkable considering that, unlike ARMed, these studies used a multitude of methodologies and collected isolates from a variety of different clinical specimens. The advantage of blood culture isolates is that, although they represent a small subset of clinical infections, they are all fully relevant from a clinical viewpoint since they represent true infection.

In contrast to resistance proportions, MRSA incidence rates provide patient-based risk estimates. However, this may not be easily achievable for epidemiologists in low resource countries where major difficulties may be encountered in sourcing the relevant clinical and demographic information from individual hospitals to establish accurate incidence rates. In such cases, it is much easier to calculate MRSA proportions from laboratory data. Our results would support the utility of such calculations to assess trends in resistance proportions, which can then be used to track the results of MRSA control programs.

When comparing the ARMed results with results from a similar, albeit less standardized, study in 1996/7,[15] significant increases in MRSA prevalence in Tunisia, Algeria and Malta can be identified over the 6 year interval. Particularly, in the latter two countries, MRSA proportions have increased 10-fold. Although temporal increases in MRSA prevalence over the past decade have been reported in many countries,[3,16] such a level of proliferation over a relatively short period of time is undoubtedly a cause for concern.

Considerable variation was also shown in the proportions of MRSA between individual hospitals in the same country. It is unlikely, from the feedback of the individual country collaborators, that this is the result of diagnostic differences, culturing anomalies or laboratory errors which have all been noted in other studies to increase intra-country variation.[17] Tiemersma *et al.*[4] hypothesize that such a variation of MRSA between hospitals in one country suggests that the country is experiencing a surge of MRSA infections in their hospitals. This conclusion would fit in with the rapid increase in prevalence in the different ARMed countries noted above.

One major factor that could drive regional MRSA dissemination could be the ineffectiveness of infection prevention and control (IC). Previous data published by the ARMed project suggest that significant progress still needs to be achieved at a national level to foster improvement in this respect.[18] Although our results additionally indicated that the development of IC at individual hospital level varied significantly throughout the south-eastern Mediterranean, it was generally evident that most aspects of IC are still at an early developmental stage in most of the hospitals reviewed.[19] A recent study on the worldwide variation of MRSA control identified that the southern Mediterranean participants were reasonably at par with other healthcare facilities in surveillance programmes, but significantly inferior in adoption of standard isolation precautions at ward level.[20]

In order to obtain its basic data set, the project adopted the well-established 'sentinel' method of identifying individual hospitals and laboratories and extrapolating the results obtained to those of the country and/or region.[21] Sentinel surveillance is especially useful in the case of developing countries where surveillance infrastructure and the money to set it up is often lacking. In the ARMed project, the proportion of the population covered differed considerably between countries. In the case of Malta and Cyprus, there is no doubt that a high level of

Table 1. Number of invasive *S. aureus* isolates and the annual proportions of MRSA, including 95% confidence intervals (95% CI), and questionnaire-derived denominator information (2003–05)

Country	No. of hospitals	Percentage of population covered	Blood culture sets per 1000 patient days	Year	No. of *S. aureus* isolates	% MRSA	95% CI
Algeria (DZ)	23	NA	NA	2003	93	41	(31–52)
				2004	298	46	(40–51)
				2005	178	46	(38–53)
				overall	569	45	(41–49)
Cyprus (CY)	5	100	23.95	2003	28	64	(44–81)
				2004	39	49	(33–65)
				2005	54	56	(41–69)
				overall	121	55	(46–64)
Egypt[a] (EG)	9	17	2.48	2003	98	33	(24–43)
				2004	216	50	(43–57)
				2005	243	63	(56–69)
				overall	557	52	(48–57)
Jordan[a] (JO)	5	30	18.71	2003	234	66	(60–72)
				2004	154	61	(53–69)
				2005	130	32	(24–40)
				overall	518	56	(51–60)
Lebanon (LB)	1	23	NA	2003	10	0	(0–34)
				2004	10	20	(4–56)
				2005	11	18	(3–52)
				overall	31	12	(4–31)
Malta[a] (MT)	3	95	15.14	2003	122	43	(34–52)
				2004	94	56	(46–66)
				2005	78	55	(43–66)
				overall	294	50	(45–56)
Morocco (MA)	3	4	10.84	2003	157	18	(13–26)
				2004	195	20	(15–26)
				2005	113	18	(11–26)
				overall	465	19	(16–23)
Tunisia (TN)	4	12	20.21	2003	171	15	(10–22)
				2004	219	18	(14–24)
				2005	196	19	(14–25)
				overall	586	18	(15–21)
Turkey[a] (TR)	11	6	21.17	2003	749	43	(39–47)
				2004	703	40	(36–44)
				2005	760	35	(32–39)

NA, not available.
[a]Statistically significant MRSA trend over the 3 years ($P < 0.05$), only calculated for countries reporting at least 20 isolates per year.

confidence can be assumed since the percentage catchment of the total population is very high. Within the larger countries, it is clear that such a high percentage is not possible. However, even where the catchment population is low, relevant conclusions can be still made if the hospital mix is sufficiently representative of the different types of hospitals, patients and regions. Therefore, although the population covered by the Turkish hospitals is low, the spread of participating laboratories across the geographical span of this expansive nation and the inclusion of all different hospital types provide reasonable confidence that the results are reasonably indicative for the whole country. On the other hand, any extrapolation of results from a single laboratory (as in Lebanon) or a few hospitals in one city (Morocco) to the whole country should be undertaken with extreme caution. It should also be pointed out that healthcare in developing countries is more concentrated in urban areas and the reported catchment populations can therefore be underestimated because it is difficult to estimate the proportion of the population coming from the rural areas to a particular hospital in the city.

In conclusion, ARMed data on the MRSA epidemiology in the south-eastern Mediterranean have clearly established that many hospitals in this region show evidence of hyperendemicity. This has major consequences not only for the countries themselves but also for neighbouring nations, since importation of resistant strains, via travellers, can result in dissemination and outbreaks in the host country at both hospitals,[22] as well as community level.[23] It is therefore critical for countries

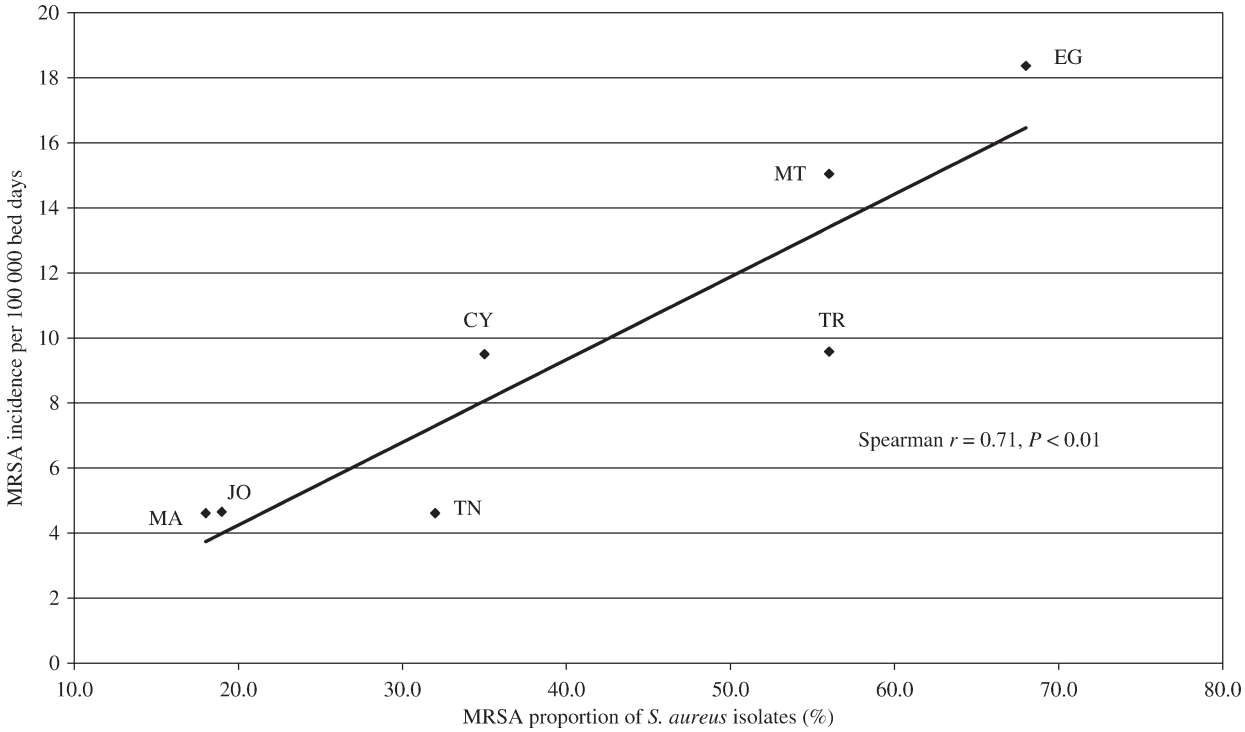

Figure 2. Correlation between the overall country MRSA incidences and proportions for 2005 (only including isolates from hospitals that provided demographic data). MA, Morocco; JO, Jordan; TN, Tunisia; CY, Cyprus; MT, Malta; TR, Turkey; EG, Egypt.

within the region to strengthen both their national as well as international initiatives aimed at improving surveillance of antimicrobial resistance as well as address possible drivers, including ineffective infection control and/or inappropriate antibiotic use.

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Transparency declarations

None to declare.

References

1. Livermore DM. Antibiotic resistance in staphylococci. *Int J Antimicrob Agents* 2000; **16** Suppl 1: S3–10.

2. Grundmann H, Aires-de-Sousa M, Boyce J *et al.* Emergence and resurgence of meticillin-resistant *Staphylococcus aureus* as a public-health threat. *Lancet* 2006; **368**: 874–85.

3. Stefani S, Varaldo PE. Epidemiology of methicillin-resistant staphylococci in Europe. *Clin Microbiol Infect* 2003; **9**: 1179–86.

4. Tiemersma EW, Bronzwaer SL, Lyytikainen O *et al.* Methicillin-resistant *Staphylococcus aureus* in Europe, 1999–2002. *Emerg Infect Dis* 2004; **10**: 1627–34.

5. Gur D, Unal S. Resistance to antimicrobial agents in Mediterranean countries. *Int J Antimicrob Agents* 2001; **17**: 21–6.

6. Borg MA, Scicluna E. Antibiotic resistance in the Mediterranean region: the ARMed project. *Eurosurv Weekly* 2004; http://www.eurosurveillance.org/ew/2004/040212.asp#5 (16 March 2007, date last accessed).

7. Bronzwaer S, Buchholz U, Courvalin P *et al.* Comparability of antimicrobial susceptibility test results from 22 European countries and Israel: an external quality assurance exercise of the European Antimicrobial Resistance Surveillance System (EARSS) in collaboration with the United Kingdom National External Quality Assurance Scheme (UK NEQAS). *J Antimicrob Chemother* 2002; **50**: 953–64.

8. Ramdani-Bouguessa N, Bes M, Meugnier H *et al.* Detection of methicillin-resistant *Staphylococcus aureus* strains resistant to multiple antibiotics and carrying the Panton-Valentine leukocidin genes in an Algiers hospital. *Antimicrob Agents Chemother* 2006; **50**: 1083–5.

9. Gourni M, Kontou M, Hadjipanayiotou C *et al.* Multicentre surveillance of antibiotic resistance in nosocomial *Staphylococcus aureus* in Cyprus. *East Mediterr Health J* 2001; **7**: 744–9.

10. Al-Zu'bi E, Bdour S, Shehabi AA. Antibiotic resistance patterns of *mecA*-positive *Staphylococcus aureus* isolates from clinical specimens and nasal carriage. *Microb Drug Resist* 2004; **10**: 321–4.

11. Kanj SS, Ghaleb PA, Araj GF. Glycopeptide and oxacillin activity against *Staphylococcus aureus* isolates at a tertiary care center in Lebanon. *J Med Liban* 2004; **52**: 8–12.

12. Erdinc FS, Yetkin MA, Ataman Hatipoglu C *et al*. Five-year surveillance of nosocomial infections in Ankara Training and Research Hospital. *J Hosp Infect* 2006; **64**: 391–6.

13. Ben Jemaa Z, Mahjoubi F, Ben Haj H'mida Y *et al*. Antimicrobial susceptibility and frequency of occurrence of clinical blood isolates in Sfax-Tunisia. *Pathol Biol (Paris)* 1993–1998; **52**: 82–8.

14. El Kholy A, Baseem H, Hall GS *et al*. Antimicrobial resistance in Cairo, Egypt 1999–2000: a survey of five hospitals. *J Antimicrob Chemother* 2003; **51**: 625–30.

15. Kesah C, Ben Redjeb S, Odugbemi TO *et al*. Prevalence of methicillin-resistant *Staphylococcus aureus* in eight African hospitals and Malta. *Clin Microbiol Infect* 2003; **9**: 153–6.

16. Panlilio AL, Culver DH, Gaynes RP *et al*. Methicillin-resistant *Staphylococcus aureus* in U.S. hospitals, 1975–1991. *Infect Control Hosp Epidemiol* 1992; **13**: 582–6.

17. Harbarth S, Albrich W, Goldmann DA *et al*. Control of multiply resistant cocci: do international comparisons help? *Lancet Infect Dis* 2001; **1**: 251–61.

18. Borg MA, Cookson BD, Scicluna EA *et al*. National infection control initiatives within countries of the southern and eastern Mediterranean. *J Hosp Infect* 2005; **60**: 182–5.

19. Borg MA, Cookson BD, Scicluna E *et al*. Survey of infection control infrastructure in selected southern and eastern Mediterranean hospitals. *Clin Microbiol Infect* 2007; **13**: 344–6.

20. Richet HM, Benbachir M, Brown DFJ *et al*. Are there regional variations in the diagnosis, surveillance and control of methicillin-resistant *Staphylococcus aureus*? *Infect Control Hosp Epidemiol* 2003; **24**: 334–41.

21. Schrag SJ, Zell ER, Schuchat A *et al*. Sentinel surveillance: a reliable way to track antibiotic resistance in communities? *Emerg Infect Dis* 2002; **8**: 496–502.

22. Bernards AT, Frenay HM, Lim BT *et al*. Methicillin-resistant *Staphylococcus aureus* and *Acinetobacter baumannii*: an unexpected difference in epidemiologic behavior. *Am J Infect Control* 1998; **26**: 544–51.

23. Urth T, Juul G, Skov R *et al*. Spread of a methicillin-resistant *Staphylococcus aureus* ST80-IV clone in a Danish community. *Infect Control Hosp Epidemiol* 2005; **26**: 144–9.