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Antimicrobial resistance in invasive strains of *Escherichia coli* from southern and eastern Mediterranean laboratories

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ABSTRACT

From January 2003 to December 2005, 5091 susceptibility test results from invasive isolates of *Escherichia coli*, collected from blood cultures and cerebrospinal fluid routinely processed within 58 participating laboratories, were investigated. These laboratories in turn serviced 64 hospitals in Algeria, Cyprus, Egypt, Jordan, Lebanon, Malta, Morocco, Tunisia and Turkey. The median proportion of resistance to third-generation cephalosporins for the duration of the project was 18.9% (interquartile range (IQR): 12.5–30.8%), and for fluoroquinolones 21.0% (IQR: 7.7–32.6%). A substantial proportion of strains reported by laboratories in countries east of the Mediterranean exhibited evidence of multiresistance, the highest proportion being from Egypt (31%). There is clearly a need for further investigation of potential causes of the significant resistance identified, as well as for strengthening of national and international surveillance initiatives within this region.'

Keywords ARMed, cephalosporins, *E. coli*, fluoroquinolones, Mediterranean, resistance

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INTRODUCTION

Serious infections, including septicaemia, caused by resistant Gram-negative bacilli are associated with increased mortality and longer hospital stay, as compared with susceptible strains [1]. One of the most common pathogens isolated from blood cultures in this context is *Escherichia coli* [2]. This organism also exhibits a potential for developing antimicrobial resistance to various antibiotics [3,4]. Significant levels of resistance against first-line antibiotics such as ampicillin, trimethoprim and co-trimoxazole have been noted for the past decade [5]. Moreover, in recent years, reports of increasing resistance to key therapeutic agents such as fluoroquinolones (FQs) and third-generation cephalosporins (3GCs) have started to emerge [6,7].

The epidemiology of resistance varies substantially on a global basis [5,8]. Even within individual regions, major differences may be present. In Europe, resistance proportions tend to be highest among the southern countries, especially those in the Mediterranean region [9]. However, information about the situation in the non-European countries of this region has been sparse. In addition to being few in number, studies have often been unrelated, using different methodologies, and, as a result, are difficult to compare [10].

This lacuna has been addressed by the Antibiotic Resistance Surveillance & Control in the Mediter-

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ranean Region (ARMed) project (http://www.slh.gov.mt/armed) [11]. Over a 3-year period, this European Union-funded study has documented the prevalence of antibiotic resistance in several key pathogens in nine southern and eastern Mediterranean countries. This publication focuses on the work undertaken to evaluate the status of resistance in *E. coli* within participating laboratories and hospitals in these countries.

MATERIALS AND METHODS

ARMed used a methodology identical to that utilized by the European Antimicrobial Resistance Surveillance System (EARSS) (http://www.rivm.nl/earss) [9]. This involved collecting antimicrobial susceptibility test results for *E. coli* strains routinely isolated from blood cultures and cerebrospinal fluid within the 58 participating laboratories of the project. These laboratories in turn serviced 64 hospitals in Algeria, Cyprus, Egypt, Jordan, Lebanon, Malta, Morocco, Tunisia and Turkey. The database included only the first isolate from any individual patient per year. The protocol for *E. coli* susceptibility testing required, as a minimum, a sensitive, intermediate or resistant interpretation of a β-lactam (amoxycillin or ampicillin), an aminoglycoside (AG) (gentamicin and/or tobramycin), an FQ (ciprofloxacin and/or ofloxacin) and a 3GC (cefotaxime or ceftriaxone and/or ceftazidime).

ARMed also accepted antimicrobial susceptibility test data for additional antibiotics if tests were routinely performed by the participating laboratory. These optional antibiotics included imipenem–meropenem, piperacillin, piperacillin with tazobactam, amikacin, co-trimoxazole, tetracycline and nalidixic acid. For the purposes of the study, an isolate was defined as being multiresistant if it was concurrently non-susceptible to AGs, FQs and 3GCs.

All antimicrobial susceptibility testing was performed by the individual laboratories, who interpreted the results according to their own guidelines, which in 70% of laboratories were based on CLSI guidelines and breakpoints. The other laboratories followed the French guidelines of the Comité de l'Antibiogramme de la Société Française de Microbiologie (CA-SFM).

To gain an insight into the comparability of test results, two external quality assessment (EQA) exercises were performed in 2003 and 2004. These exercises were undertaken in collaboration with the EARSS, and utilized strains selected by the EARSS EQA committee and distributed by United Kingdom National External Quality Assessment Scheme (UK NEQAS) [12]. In the 2003 EQA, one *E. coli* strain was distributed. There was 100% concordance for species identification and high concordance (≥88%) for the antibiotics tested. Two extended-spectrum β-lactamase (ESBL)-producing *E. coli* strains were included in the 2004 EQA exercise, including a CTX-M15 isolate. Resistance to 3GCs was tested using cefotaxime in 85% of laboratories and using ceftriaxone in 50%; all laboratories used at least one of these methods. A correct interpretation was given by 95% of those centres using cefotaxime and by all of the laboratories using ceftriaxone. The second strain of *E. coli* in the 2004 EQA was a TEM-26 β-lactamase-producing strain, which all laboratories correctly classified as ceftazidime-susceptible. Under the EARSS methodology, ESBL confirmatory testing was not obligatory, because laboratories followed their own routine procedures. Therefore, resistance to 3GCs was utilized by ARMed as an indicator of ESBL production.

A questionnaire was also sent to the hospitals serviced by the testing laboratories in order to collect information on blood-culturing practices. The blood-culturing rate was calculated from the questionnaire feedback as: total number of blood cultures/total number of patient-days. Laboratories were included in this part of the analysis only if all hospitals within their catchment reported the respective bed information data. Median and interquartile ranges were chosen to describe resistance proportions, so as to avoid potential outlier effects from individual laboratories or countries. Intracountry variations among the results obtained from the different participating laboratories were also considered. Country-specific trend analysis was performed using the Cochrane–Armitage test. Countries were obliged to report at least 20 isolates per year, for all 3 years, to be included in the trend analysis. Most analyses were performed in MEDCALC, version 9.2.1.0 (Medcalc Software, Mariakerke, Belgium).

RESULTS

In total, 5091 *E. coli* isolates were reported to ARMed for the 36-month study period (2003–2005). Table 1 shows the proportions of resistance reported by the laboratories in the participating countries, both overall and for each individual study year.

Aminopenicillin resistance

The highest proportions of resistance were found for the aminopenicillins (median 68.2%; interquartile range (IQR) 57.1–74.1%). In 2005, the proportion of aminopenicillin-resistant *E. coli* isolates varied between 49% (Malta) and 83% (Lebanon). A significant trend of increased resistance was observed for Turkey, from 68% to 75% between 2003 and 2005.

Aminoglycoside resistance

The median AG resistance proportion for all participating laboratories was 24.0% (IQR:17.8–33.1%). By far the highest proportion of AG resistance in 2005 was observed in Egypt (57%), whereas the lowest proportion was reported from Malta (7%). Between 2003 and 2005, a significant increase was observed in Morocco, from 12% to 33%, and a significant decrease in Malta, from 18% to 7%.

Table 1. Number (*n*) of invasive *E. coli* isolates and proportions of resistance to antimicrobial groups, including 95% confidence intervals (95% CI) and questionnaire feedback reported by laboratories and hospitals participating in ARMed (2003–2005)

Country	Hospitals	Population covered (%)	Blood cultures per 1000 patient days	Year	Aminopenicillin		Fluoroquinolones		Third generation cephalosporins		Aminoglycosides		Multiresistance	
					n	%R (95% CI)	*n*	%R (95% CI)	*n*	%R (95% CI)	*n*	%R (95% CI)	*n*	%R (95% CI)
Algeria (DZ)	23	n/a	n/a	2003	104	75 (65–83)	40	3 (0–15)	105	16 (10–25)	106	18 (11–27)	39	0 (0–11)
				2004	310	75 (70–80)	205	7 (4–12)	311	16 (13–21)	307	20 (15–25)	203	1 (0–5)
				2005	234	82 (77–87)	131	2 (1–7)	236	17 (12–22)	238	17 (13–23)	130	0 (0–4)
				Overall	648	78 (74–81)	376	5 (3–8)	652	16 (14–20)	651	18 (16–22)	372	1 (0–2)
Cyprus (CY)	5	100	23.95	2003	19	63 (39–83)	19	32 (14–57)	19	11 (2–35)	19	11 (2–35)	19	5 (0–28)
				2004	46	61 (45–75)	46	22 (11–37)	46	9 (3–22)	46	11 (4–24)	46	1 (0–10)
				2005	74	73 (61–82)	72	29 (19–41)	74	16 (9–27)	74	14 (7–24)	72	4 (1–13)
				Overall	139	68 (59–75)	137	27 (20–35)	139	13 (8–20)	139	12 (7–19)	137	3 (1–8)
Egypt (EG)	9	17	2.48	2003	98	87 (78–92)	44	11 (4–25)*	114	77 (68–84)	124	68 (59–76)	32	6 (1–22)*
				2004	186	73 (65–79)	173	31 (25–39)*	182	67 (60–74)	188	58 (51–65)	170	27 (21–34)*
				2005	223	76 (70–82)	206	48 (41–55)*	222	70 (64–76)	223	57 (50–63)	206	39 (32–46)*
				Overall	507	77 (73–80)	423	37 (33–42)	518	71 (66—75)	535	60 (55–64)	408	31 (27–36)
Jordan (JO)	5	30	18.71	2003	85	65 (54–75)	74	26 (17–37)	132	28 (21–37)	134	27 (20–35)	72	4 (1–13)*
				2004	59	59 (46–72)	72	28 (18–40)	121	30 (22–39)	127	29 (22–38)	66	5 (1–14)*
				2005	24	67 (45–84)	52	31 (19–45)	84	31 (22–42)	105	16 (10–25)	40	18 (8–33)*
				Overall	168	63 (55–70)	198	28 (22–25)	337	29 (25–35)	336	25 (20–29)	178	8 (4–12)
Lebanon (LB)	1	n/a	n/a	2003	29	83 (64–93)	29	45 (27–64)	29	28 (13–47)	29	38 (21–58)	29	14 (5–33)
				2004	34	68 (49–82)	34	32 (18–51)	34	18 (7–35)	34	15 (6–32)	34	6 (1–21)
				2005	36	83 (67–93)	36	53 (36–69)	36	25 (13–43)	36	22 (11–40)	36	11 (4–27)
				Overall	99	78 (68–85)	99	43 (34–54)	99	23 (16–33)	99	24 (16–34)	99	10 (5–18)
Malta (MT)	3	95	15.14	2003	88	39 (29–50)	90	24 (16–35)	91	2 (0–8)	91	18 (11–27)*	90	0 (0–5)
				2004	91	47 (37–58)	91	35 (26–46)	92	4 (1–11)	92	20 (12–29)*	91	3 (1–10)
				2005	87	49 (39–60)	87	30 (21–41)	87	1 (0–7)	87	7 (3–15)*	87	0 (0–5)
				Overall	266	45 (39–51)	268	30 (25–36)	270	3 (1–5)	270	15 (11–20)	268	1 (0–4)
Morocco (MA)	3	4	10.84	2003	59	63 (49–75)	66	14 (7–25)	58	7 (2–18)*	60	12 (5–23)*	53	2 (0–11)*
				2004	67	76 (64–85)	67	24 (15–36)	67	28 (18–41)*	67	30 (20–42)*	67	10 (5–21)*
				2005	65	68 (55–78)	62	24 (15–37)	52	33 (21–47)*	67	33 (22–46)*	51	18 (9–31)*
				Overall	191	69 (62–75)	195	21 (15–27)	177	23 (17–30)	194	25 (19–32)	171	10 (6–16)
Tunisia (TN)	4	12	20.21	2003	152	66 (58–73)	132	20 (13–28)	152	20 (14–27)*	152	19 (13–26)	132	14 (8–21)*
				2004	183	66 (58–72)	156	15 (10–22)	184	16 (11–22)*	184	21 (15–27)	156	10 (6–16)*
				2005	194	68 (61–74)	164	15 (10–21)	194	11 (7–17)*	194	15 (11–22)	164	7 (4–12)*
				Overall	529	67 (62–71)	452	16 (13–20)	530	15 (12–19)	530	18 (15–22)	452	10 (8–13)
Turkey (TR)	11	6	21.17	2003	662	68 (65–72)*	708	38 (35–42)*	716	26 (22–29)*	691	28 (25–32)	681	15 (13–18)
				2004	761	68 (64–71)*	761	43 (40–47)*	764	28 (25–31)*	764	27 (24–31)	759	16 (14–19)
				2005	779	75 (72–78)*	776	44 (40–48)*	779	31 (28–34)*	781	27 (24–31)	773	16 (14–19)
				Overall	2202	71 (69–72)	2245	42 (40–44)	2259	28 (26–30)	2236	28 (26–30)	2213	16 (14–17)

n/a; not available.
*Statistically significant trends ($p < 0.05$).

Third-generation cephalosporin resistance

The largest variation in resistance levels, among both countries and laboratories in the individual countries, was seen for 3GCs. The median proportion of 3GC resistance for the duration of the project was 18.9% (IQR: 12.5–30.8%). A wide range was evident at country level, between 1% in Malta and 70% in Egypt in 2005. From 2003 to 2005, laboratories in Morocco and Turkey showed a significant increase in 3GC resistance, from 7% to 33% and from 26% to 31%, respectively; on the other hand, a significant decrease was seen in Tunisia, from 20% to 11%. With the exception of Malta, at least one ARMed laboratory in each participating country reported 3GC resistance levels in excess of 20%, with this situation being present in the majority of hospitals in Turkey, Algeria, Jordan and, especially, Egypt, where all laboratories, except one, reported 3GC resistance proportions in excess of 30% (Fig. 1).

FQ resistance

Evidence of substantial FQ resistance was also received from many of the participating hospitals. The overall median proportion of FQ resistance was 21.0% (IQR: 7.7–32.6%). In 2005, Algeria reported only 2% FQ resistance in *E. coli*, and this was followed by 15% in Tunisia. Resistance proportions in excess of 40% were observed in Lebanon (53%), Egypt (48%) and Turkey (44%). During the study period, a significant trend of increased resistance was observed in Egypt and Turkey.

Multidrug resistance

The distribution of multiresistance in *E. coli* was also examined, according to our definition. In 2005, multiresistance rates below 5% were found in Algeria, Cyprus and Malta. Multiresistance was highest in Egypt, where more than 30% of isolates were simultaneously resistant to AGs, FQs and 3GCs. The participating laboratories from this country reported only 15.6% of *E. coli* strains as being fully sensitive to the three antimicrobial agents. Over the 3-year period, a significant increase in multiresistance was observed in Egypt, Jordan and Morocco, with a decrease being evident in Tunisian laboratories.

Carbapenem (CP) resistance

Although CP testing was optional, this was performed for 4645 isolates. Resistance to CPs

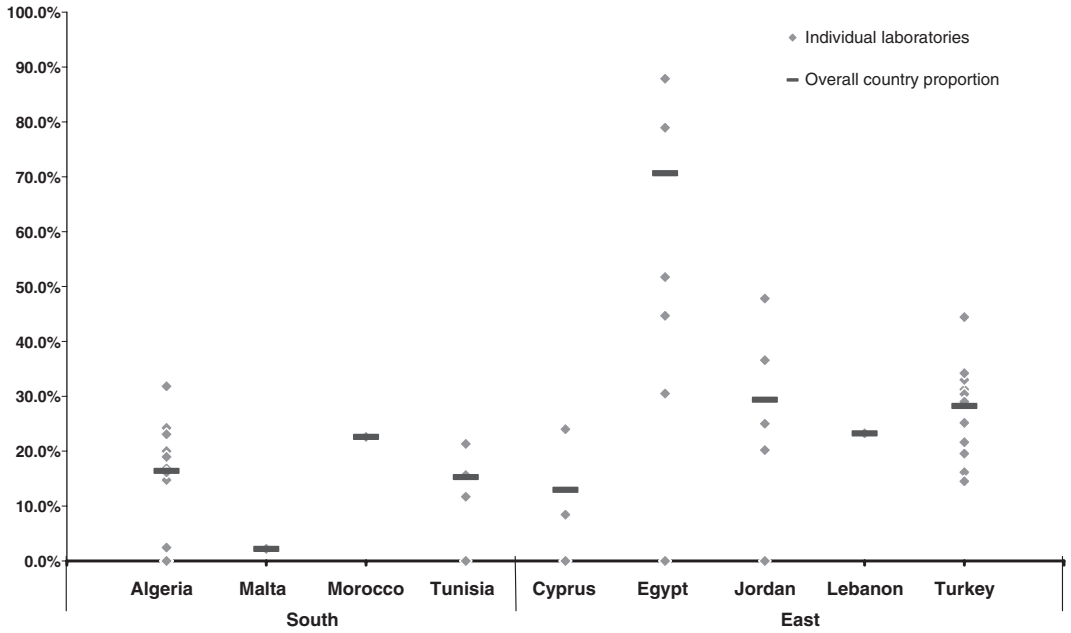

Fig. 1. Proportions of *Escherichia coli* resistance to third-generation cephalosporins at individual laboratory and country levels for the entire surveillance period (2003–2005), including only participating centres reporting at least ten *E. coli* isolates for the entire surveillance period.

was generally low, with all countries showing resistance proportions lower than 5%, except for Egypt (9%).

There was also an interesting geographical difference in the phenotypic resistance profiles between the southern and the eastern Mediterranean laboratories. More than 75% of *E. coli* isolates from Algerian, Tunisian and Maltese laboratories were fully susceptible to AGs, FQs, 3GCs and CPs. On the other hand, more than 30% of isolates from Egypt, Lebanon and Turkey were resistant to at least two of these second-line agents (Fig. 2).

DISCUSSION

The ARMed study is the first pan-regional study of this nature in the south and east of the Mediterranean region, and the information gathered during this study constitutes the largest dataset on the epidemiology of *E. coli* collected in this region. Nevertheless, it is apparent from sporadic earlier publications that reference has already been made to significant levels of resistance in some countries in this region, particularly in Egypt. El Kholy and colleagues reported resistance levels of 48% to 3GCs and 18% to ciprofloxacin in blood culture isolates of *E. coli* from five hospitals in Cairo in the years 1999–2000 [13]. High rates of resistance to both 3GCs and FQs were also described by Saied in Cairo hospitals [14]. The ARMed finding of an IQR of 13.3–37.9% for resistance to ciprofloxacin correlates well with the EARSS results for the southern European countries of Spain, Italy and Portugal, which identified proportions in excess of 25% [9]. This suggests that the whole of the Mediterranean region may be a high-prevalence region for FQ resistance in this important Gram-negative pathogen.

On the other hand, non-susceptibility to 3GCs in *E. coli* remains substantially low, on both a global and a European basis. In fact, networks studying global antimicrobial resistance epidemiology (e.g. MYSTIC, SENTRY and SMART) have reported rates of less than 5% in the majority of participants and rates rarely higher than 10% [15,16]. Similar results have also been reported by the EARSS network, even from their southern European participants [9]. Consequently, the results obtained from ARMed hospitals clearly indicate a substantially higher prevalence of resistance in these participating centres. Resistance to

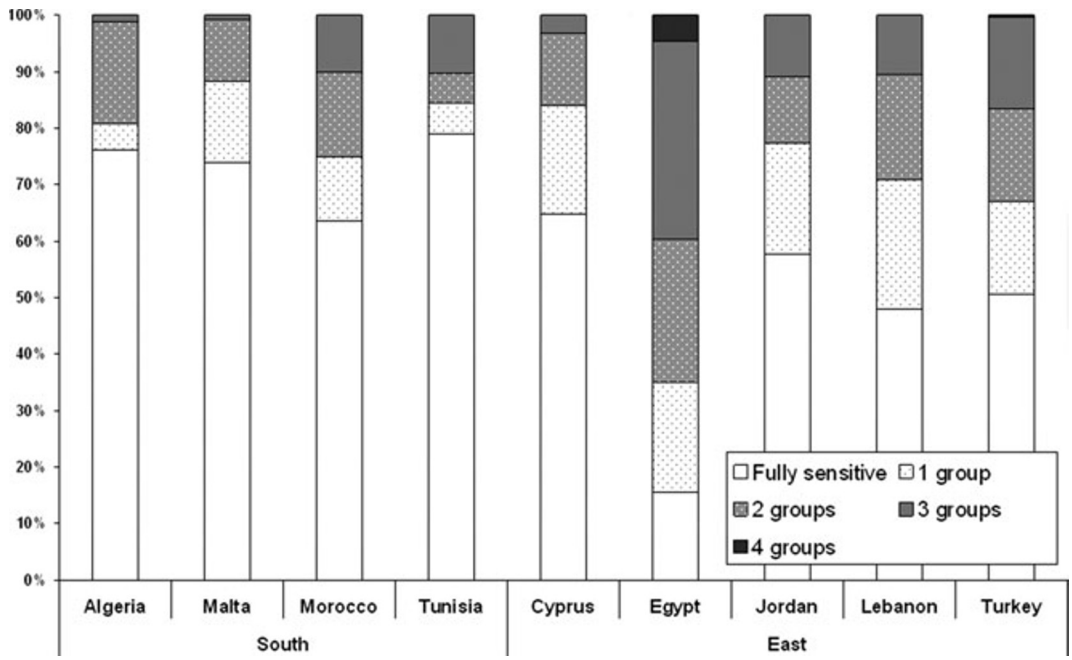

Fig. 2. Percentage frequency of resistance profiles per country for *Escherichia coli* isolates for the entire surveillance period (2003–2005); to be included, isolates had to be tested for all of the following four antibiotic groups: fluoroquinolones, aminoglycosides, third-generation cephalosporins and carbapenems.

3GCs within Enterobacteriaceae is often synonymous with the production of ESBLs [7]. These transferable, plasmid-encoded enzymes hydrolyze and inactivate all β-lactam antibiotics, with the exception of cephamycins and CPs [17]. Furthermore bacteria producing these enzymes are often also resistant to other antimicrobial agents, including AGs and FQs [18]. Several ESBL-producing clonal groups have been identified, but it would appear that CTX-M-15 may predominate within many countries in the southern and eastern Mediterranean region. In fact, strains producing this enzyme have been identified as the predominant 3GC-resistant *E. coli* strains in studies within Egypt [13], Tunisia [19,20], Turkey [21] and Lebanon [22]. Isolates of *E. coli* bearing the $bla_{\text{CTX-M-15}}$ gene, from both inpatient and community sources, have been characterized by a high degree of multiresistance [23]. They have also been identified as being of epidemic potential [24].

The predominance of these *E. coli* clones could explain the extremely high levels of multiresistance found in the isolates from many of the laboratories in this study, particularly those from eastern Mediterranean countries. Considering that only 1% of *E. coli* isolates from the European SENTRY study showed resistance to more than four major antibiotics [25], it is quite significant to find that eastern Mediterranean centres reported 10% or more of their *E. coli* isolates to be concurrently resistant to at least three important antimicrobial classes, those normally reserved for the treatment of serious life-threatening infections in the hospital setting. Furthermore, the level of multiresistance in the ARMed laboratories appears to be significantly higher than that identified by EARSS participants in the European region of the Mediterranean, who (with the exception of those from Israel) reported levels lower than 5% in 2005 [9]. These multiresistant strains are undoubtedly a reason for concern, because of the difficulty in treating serious infections that would be caused by them. This applies not only to the countries themselves, but also to others in the region and further afield, as importation of multiresistant Gram-negative pathogens via returning travellers or migrants to Western countries is becoming an ever more common occurrence [26].

In order to obtain a basic dataset, the ARMed project adopted the well-established 'sentinel' method of identifying individual hospitals and laboratories in the country and/or region [27]. Sentinel surveillance is especially useful in the case of developing countries, where surveillance infrastructure and the funds required are often lacking. Concern has been raised that the quality of routine data may be compromised because susceptibility testing methods and interpretative criteria may not be standardized; thus, many Enterobacteriaceae would be only partially identified, and the panels of antimicrobial agents tested could differ among laboratories [28]. In the case of ARMed, it was ensured that all participating laboratories used a common methodology and an identical basic set of antimicrobial panels. Furthermore, the satisfactory results obtained from the EQA exercise (albeit on a restricted set of strains) can be viewed as an additional indicator of satisfactory diagnostic quality in the participating laboratories. Finally, the comprehensive size of the database lends additional strength to the conclusions reached. Nevertheless, several shortcomings should be kept in mind. The blood-culturing rate varied substantially from country to country, being lowest in Egypt, where the highest resistance levels were identified. Although it is difficult to establish with certainty the extent to which this could have influenced the high resistance proportions, it stands to reason that if blood cultures are taken mainly after therapeutic failure (in accordance with the culture in the country), this will create a bias for isolates with reduced susceptibility. Furthermore, differences in hospital sample mix are inevitable in studies that rely on routine data, especially in some developing countries, where surveillance infrastructure would be absent in smaller district laboratories. Also, the proportion of the population covered differed considerably among countries. A high catchment population was possible in the smaller countries such as Malta and Cyprus, allowing a high level of confidence in the results obtained, but such a high percentage was clearly not possible in the larger nations. However, this does not impede the drawing of relevant conclusions. For example, although the population covered by the Turkish hospitals was low, the spread of participating laboratories across the whole of this country and the inclusion of all hospital types provide sufficient confidence that the results are reasonably indicative of the situation in the whole of the country. On the

other hand, extrapolation of results from a single laboratory (as in Lebanon and Morocco) to a whole country should be undertaken with extreme caution.

A major advantage in the ARMed study was the methodological requirement that only the first patient isolate per year was included. Shannon and French showed significant differences of up to 10% when a 5-day limit for same-patient duplicates was used, as compared with the 365-day limit of ARMed [29]. In addition, the choice of blood cultures and cerebrospinal fluids ensured that only invasive, and therefore clinically relevant, isolates were included. The same authors showed an additional significant difference of 6–10% in their results if isolates from screening specimens were excluded. As the EARSS protocol was adhered to, no investigation of the molecular biology of the multiresistant strains isolated was undertaken. This would have given additional information on linkages and clonal spread among institutions and even countries, as well as possibly identifying the types of ESBL present in the region. This is undoubtedly a future priority in light of the findings of this study.

In conclusion, ARMed data on the status of antimicrobial resistance in *E. coli* within the south-eastern Mediterranean region clearly suggest that a substantial proportion of hospitals in many of the countries in this region face some of the highest rates of multiresistance recorded in this species. This in turn has significant therapeutic implications in the context of serious infections caused by these pathogens, both within and outside the region. In fact, the importation of multiresistant organisms to European hospitals via patients arriving from countries within the Mediterranean region is well documented [30], even resulting in subsequent intra-institutional spread, with the potential for an outbreak [31]. These findings call for further investigation of potential drivers behind resistance trends, as well as for strengthening of both national and international initiatives aimed at improving surveillance of antimicrobial resistance in the region.

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TRANSPARENCY DECLARATION

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