UK epidemic strains of meticillin-resistant *Staphylococcus aureus* in clinical samples from Malta

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Since 1999, the European Antimicrobial Resistance Surveillance System (EARSS) has monitored the rise in infection due to a number of organisms, including meticillin-resistant *Staphylococcus aureus* (MRSA). The EARSS reported that MRSA infections within intensive care units account for 25–50 % of infections in many central and southern European countries, these included France, Spain, Great Britain, Malta, Greece and Italy. Each country has defined epidemic MRSA (EMRSA) strains; however, the method of spread of these strains from one country to another is unknown. In this current study, DNA profiles of 473 isolates of MRSA collected from the UK and Malta were determined by PFGE. Analysis of the data showed that two countries separated by a large geographical distance had a similar DNA profile pattern. Additionally it was demonstrated that strains of EMRSA normally found in the UK were also found in the Maltese cohort (EMRSA 15 and 16). A distinct DNA profile was found in the Maltese cohort, which may be a local EMRSA, and accounted for 14.4 % of all Maltese isolates. The appearance of the same MRSA and EMRSA profiles in two separate countries suggests that MRSA can be transferred out of their country of origin and potentially establish in a new locality or country.

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INTRODUCTION

Staphylococcus aureus and particularly meticillin-resistant *S. aureus* (MRSA) have become a major problem in hospital-acquired infections worldwide. Recently the World Health Organization recognized that antibiotic resistance is one of the major threats facing the world in the future (WHO, 2000). Diseases of concern include AIDS, tuberculosis, malaria and hospital-acquired infections, including vancomycin-resistant enterococci and MRSA (WHO, 2000). Between 1991 and 2000 the Health Protection Agency reported that bacteraemia cases caused by MRSA in the UK rose from 2 to 40 % (HPA, 2000).

The typing of MRSA beyond the species level has become an important tool in aiding the identification and control of disease outbreaks, and monitoring the spread of different strains of MRSA, both nationally and internationally (Tenover *et al.* 1995). There are a number of different

genotyping methods utilized for the characterization of MRSA; however, PFGE is currently accepted as the 'gold standard' method due to the high degree of reproducibility and the excellent discriminatory power this method provides (Witte, 2000).

Malta and the UK are included in the top five European countries showing high levels of MRSA infection (EARSS, 2007). The aim of this study was to genetically type and compare MRSA isolate populations within the Maltese and UK clinical setting. This is believed to be the first report of strain profile comparisons between these two geographically separated locations.

METHODS

Bacterial strains. Clinical isolates of MRSA were collected from three London-based hospitals (LBH) in the UK and one general hospital in Malta. These were Kingston Hospital, Surrey, the Royal Brompton Hospital, London, the Royal Marsden Hospital, London and St Luke's Hospital, Malta (the main general hospital in Malta serving a population of approximately 400 000 people). A total of 257 isolates of MRSA were collected from the LBH over an 18 month

Abbreviations: EARSS, European Antimicrobial Resistance Surveillance System; EMRSA, epidemic meticillin-resistant *Staphylococcus aureus*; EU, European Union; LBH, London-based hospitals; MAR, multiple antibiotic resistance; MRSA, meticillin-resistant *Staphylococcus aureus*.

period (07/2002–12/2004). A total of 216 isolates were collected from St Luke's Hospital Malta, over an 8 month period (02/2003–10/2003). Duplicate isolates from patient samples were excluded from the study.

Antibiotic-sensitivity testing. A panel of 14 different antibiotics were used for testing, 13 using standard operating procedures as defined by the British Society for Antimicrobial Chemotherapy (BSAC, 2004). The antibiotics selected as determined by BSAC methodology were: amikacin, chloramphenicol, ciprofloxacin, clarithromycin, clindamycin, erythromycin, gentamicin, linezolid, meticillin, penicillin G, rifampicin, teicoplanin and tetracycline (MAST Diagnostic). In addition, sensitivity to vancomycin (Sigma) was assayed using a well established methodology described by Hubert *et al.* (1999). The following *S. aureus* strains were used as controls: MRSA NCTC 12493 (control 1) and Oxford *S. aureus* NCTC 06571 (control 2).

PFGE. PFGE was performed as previously described by Bannerman *et al.* (1995) with modifications taken from the HARMONY protocol (Murchan *et al.*, 2003). Electrophoresis was carried out using a CHEF-DR II system (Bio-Rad) using a standard 23 h two block run method as detailed in the HARMONY protocol (Murchan *et al.*, 2003).

The gels were stained with ethidium bromide (BDH) as described by Murchan *et al.* (2003). Band patterns were loaded into the Bionumerics [Jaccard (Tol 2.7-2.7)(H70.0 % s >0.0 %) (0.0%-100.05)] analysis program producing a dendrogram. Banding patterns were interpreted using the criteria devised by Tenover *et al.* (1995).

RESULTS AND DISCUSSION

The aim of this study was to type MRSA isolates collected from the two European study groups (LBH and Maltese hospital), by PFGE using *Sma*I. Antibiotic susceptibility testing demonstrated that all isolates retrieved from this study were susceptible to linezolid, teicoplanin and vancomycin (data not shown). All MRSA isolates were resistant to between 2 and 10 antibiotics (out of a panel of

14), with a mean resistance to 6 antimicrobials (data not shown). Multiple antibiotic resistance (MAR) profiles were generated for each isolate, the most predominant MAR profiles from both countries can be seen in Table 1, which also includes the strain type to which these isolates belonged to (the predominant strain types are shown in bold). The MAR profiles accounted for 66 % (170/256) of the isolates from the LBH and 48 % (104/216) of the isolates from the Maltese hospital, with the remaining isolates falling into other MAR profiles containing less than 5 % of isolates within a given profile. The most common MAR profiles show resistance to six antibiotics (ciprofloxacin, clarithromycin, clindamycin, erythromycin, meticillin and penicillin G).

Of the 473 isolates tested, PFGE banding patterns were determined for 90.5 % (428/473) of isolates. Profiles were determined for 91.4 % (235/257) of LBH isolates and 89.4 % (193/216) of Maltese hospital isolates. A dendrogram (Fig. 1) was constructed using these banding patterns and a total of 32 different PFGE profile groups were identified, with a varying number of subtypes within each group. PFGE profile groups were determined using three criteria: the guidelines formulated by Tenover *et al.* (1995), the similarity (in the number and size of bands) to published banding patterns and the demographic similarity (60 % or greater) within a group of related isolates (Murchan *et al.*, 2003).

Of the 32 identified PFGE profile groups, 11 were found to be present in both the LBH and Maltese hospital cohorts (groups A–K) (Table 2), of which approximately 90 % of the isolates (91 % LBH and 89 % Maltese hospital) tested from both cohorts were grouped into one of these profiles. The remaining 10 % of isolates from each cohort were

Table 1. Predominant MAR profiles and strain types, as determined by PFGE, in the MRSA isolates from the LBH and Maltese hospital cohorts

The predominant strain types are shown in bold.

MAR profile	Percentage of isolates showing the indicated resistance pattern		PFGE band profile	
	LBH (n=256)	Maltese hospital (n=216)	LBH (n=256)	Maltese hospital (n=216)
AK, G, PG, M, CD, E, Clar, Cip	5.1		D, L, E, G, U16, unknown	–
AK, PG, M, C, CD, E, Clar, Cip	4.7		D, J, U6, U15, U16	–
AK, PG, M, CD, E, Clar, Cip	4.3		D, unknown	–
G, PG, M, CD, E, Clar, Cip	12.8		E, A, D, G, U1, U2, U16, unknown	–
PG, M, C, CD, E, Clar, Cip	8.2	20.8	E, D, U9, G, unknown	E, B, C, F, U20, U13, U21, unknown
PG, M CD, E, Clar, Cip	31.1	21.8	E, A, B, C, D, F, H, K, U16, U2, U9, unknown	E, F, L, U5, U8, U10, U17, U21, unknown
PG, M, Cip		5.6	–	E, G, H, L, U10, unknown

AK, Amikacin; C, chloramphenicol; Cip, ciprofloxacin; Clar, clarithromycin; CD, clindamycin; E, erythromycin; G, gentamicin; M, meticillin; PG, penicillin G.

Fig. 1. A dendrogram showing the position of both the shared and unique strain profiles determined for isolates of MRSA collected from the UK and Malta. The position of each strain is illustrated by a representative isolate within that strain type.

found to belong to one of 21 unique profiles, which contained between one and five isolates only.

Two PFGE profile groups (D and E) were identified as being similar to published banding patterns for epidemic MRSA (EMRSA) clones. Approximately 60 % of isolates from both cohorts belonged to PFGE profile group E, which was determined to be closely related to the published banding pattern of EMRSA 15 (Murchan *et al.*, 2003). PFGE profile group E contained a main group and 37 subtypes, these subtypes varied from the main group by either insertion or deletion compared to the main banding pattern. The subtypes contained between 2 or 3 and 38 isolates.

Profile group D was determined to be closely related to the published banding pattern of EMRSA 16 (Murchan *et al.*, 2003). The majority of the isolates within profile group D were from LBH MRSA (14.3 %), and only a small percentage was from the Maltese hospital cohort (2.1 %). This profile group contained a main group and 13 subtypes. Conversely, profile group K contained predominantly Maltese hospital isolates (14.4 %); however, one isolate (0.4 %) was from the LBH cohort and was grouped into a subtype of this profile. Profile group K contained a

main banding pattern and 13 subtypes. Additionally, this profile was not comparable to any currently published banding patterns. It is therefore tempting to speculate that this isolate might be a local Maltese strain. However, the observation was not confirmed in this study and more work on this specific point is required.

Of the remaining seven PFGE profiles groups that were identified in both countries, each contained between 0.4 and 4.8 % of the isolate population. These profiles were not found to be similar to known banding profiles for EMRSA isolates and varied in the number of subtypes that they contained.

Twenty-one PFGE profiles unique to a single cohort were identified (U1–U21). The LBH cohort was found to have 13 unique profiles, whereas the Maltese hospital group contained 9 unique profiles. Approximately 10 % of isolates from the LBH and Maltese hospital groups produced unique banding patterns.

Comparison of MAR profiles and PFGE profiles showed some clustering, strain type E, which has a similar banding pattern to EMRSA 15, was the predominant strain type for isolates that showed the most common MAR profile containing resistances to penicillin G, meticillin, clinda-

Table 2. Summary overview of the PFGE profiles obtained for the 473 isolates tested

A total of 32 strains with closely related profile groups were identified, consisting of 10 groups (A–J) found in both cohorts and 22 profiles unique to a single cohort (U1–U22).

Profile		Frequency (%)	
Main type	Subtype	LBH	Maltese hospital
Both cohorts			
A	A1–A3	2.98	0.52
B	B1–B3	1.7	1.04
C	C1–C2	0.85	0.52
D	D1–D13	18.3	6.22
E	E1–E37	52.77	58.55
F	F1–F3	0.85	4.66
G	G1–G6	5.11	0.52
H	H1–H3	0.85	1.04
I	I1–I5	3.83	0.52
J	J1–J5	2.98	0.52
K	K1–K13	0.43	15.54
Single cohort			
U1	–	0.43	–
U2	–	0.85	–
U3	–	0.43	–
U4	–	0.43	–
U5	–	–	0.52
U6	–	1.28	–
U7	–	–	0.52
U8	–	–	0.52
U9	–	0.85	–
U10	–	–	1.55
U11	–	–	0.52
U12	–	0.43	–
U13	–	0.85	–
U14	–	–	0.52
U15	–	1.7	–
U16	–	1.28	–
U17	–	–	1.04
U18	–	–	2.59
U19	–	0.43	–
U20	–	0.43	–
U21	–	–	2.59

mycin, erythromycin, clarithromycin and ciprofloxacin (Table 1). The second most common MAR profile contained the same resistances as listed previously, with the addition of gentamicin, and again the predominant strain type in this group was type E.

In the LBH cohort strain type D (which has a similar banding pattern to EMRSA 16) demonstrated a slightly different MAR profile. The basic profile contained seven antibiotics (amikacin, ciprofloxacin, clarithromycin, clindamycin, erythromycin, meticillin and penicillin G) and two different profiles contained the above antibiotics with the addition of either gentamicin or chloramphenicol.

To the best of our knowledge this is the first report of the identification of strains related to EMRSA 15 and 16 in the Maltese MRSA isolate population. The spread of dominant MRSA strains from one country to another is not unique to this study as demonstrated by the inter-continental spread of the Iberian and Brazilian clone (Aires de Sousa *et al.*, 1998; Heym *et al.*, 2002; da Silva Coimbra *et al.*, 2003). These clones were for some time the dominant type of MRSA in Europe; however, a number of recent studies have highlighted the spread of EMRSA 15 and 16 into European countries, and the rise of these clones as the dominant type. Two recent reports from both Portugal and the Canary Islands (Spain), describe the changing type of the dominant MRSA clone over the past decade. Montesinos *et al.* (2006) reported the rise of EMRSA 16 in the Canary Islands; the group showed the predominant clone in 1997 was the Iberian clone. Both EMRSA 15 and 16 first appeared in the Canary Islands in approximately 1999, after the introduction of these clones the percentage of cases due to the Iberian clone started to fall rapidly. In 1999 approximately 80 % of cases were attributed to the Iberian clone; however, just 2 years later, in 2001, EMRSA 16 had emerged as the predominant clone, with less than 10 % of cases reported being due to the Iberian clone. Amorim *et al.* (2007) reported similar findings in a Portuguese hospital, where, in 1993, the Iberian clone was predominant. However, after its introduction in 1995, the Brazilian clone replaced the Iberian clone and became the predominant clone until about 2000 when it was replaced by EMRSA 15. Increases in the number of cases due to EMRSA 15 have also been reported in Germany and the Czech Republic (Witte *et al.*, 2001; Melter *et al.*, 2006).

In conclusion, this study has demonstrated that two countries separated by a large geographical distance have closely related clones of EMRSA, and the predominant clone in both countries was found to be EMRSA 15. Recent reports have highlighted the rise of both EMRSA 15 and 16 in a number of European countries; however, two questions that need to be addressed are: how were the clones spread originally, and how have they become established and risen to become the predominant clones in such a short time?

In addition to low cost and rapid travel, freedom of movement within the European Union (EU) has meant it is now easier to travel and work in different European countries. This may have resulted in increased movement of people within the EU member states; it can therefore be hypothesized that this movement has helped to disseminate EMRSA 15 and 16 from the UK to other EU countries. Both Montesinos *et al.* (2006) and Melter *et al.* (2006) have highlighted travel as a possible reason for the introduction of EMRSA 15 and 16 into their countries. Montesinos *et al.* (2006) point out that over 5 million tourists a year visit Tenerife, of which approximately 2 million are from Britain, and therefore suggest this may be a route by which the clone has moved between the two countries. Melter *et al.* (2006), however, suggest that EMRSA 15 may have

spread from Germany to the Czech Republic as these two countries share a border. Although the spread of the clones may be explained by increased travel, why these clones should have become established and then become the predominant clone in a few years is hard to explain. Antibiotic resistance evidence from both Montesinos *et al.* (2006) and Amorim *et al.* (2007) demonstrated that the EMRSA clones are more sensitive to antibiotics such as gentamicin and tetracycline than the Iberian or Brazilian clone. However, similar resistance levels to antibiotics such as ciprofloxacin, clindamycin, erythromycin and rifampicin were shown by all clones. It could therefore be speculated that both EMRSA 15 and 16 may be more pathogenic than the other clones, with a possible increased ability to adhere and proliferate within a wound or survive within this particular environment. However, further research would need to be carried out to support this suggestion. What is clear from this current study and other work is that EMRSA 15 and 16 appear to be spreading across a number of European countries, and that once established appear to rise to become the predominant clone.

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REFERENCES

- Aires de Sousa, M., Sanches, I. S., Ferro, M. L., Vaz, M. J., Saraiva, Z., Tendeiro, T., Serra, J. & de Lencastre, H. (1998). Intercontinental spread of a multidrug-resistant methicillin-resistance *Staphylococcus aureus* clone. *J Clin Microbiol* **36**, 2590–2596.
- Amorim, M. L., Faria, N. A., Oliveira, D. C., Vasconcelos, C., Cabeda, J. C., Mendes, A. C., Calado, E., Castro, A. P., Ramos, M. H. & other authors (2007). Changes in the clone nature and antibiotic resistance profiles of methicillin-resistance *Staphylococcus aureus* isolates associated with spread of the EMRSA-15 clone in tertiary care Portuguese hospital. *J Clin Microbiol* **45**, 2881–2888.
- Bannerman, T. L., Hancock, G. A., Tenover, F. C. & Miller, J. M. (1995). Pulse-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. *J Clin Microbiol* **33**, 551–555.
- BSAC (2004). *BSAC Disc Diffusion Method for Antimicrobial Susceptibility Testing*, version 3. Birmingham: British Society for Antimicrobial Chemotherapy. http://www.bsac.org.uk/_db/_documents/jan_2004_website_version_3.pdf.
- da Silva Coimbra, M. V., Silve-Carvalho, M. C., Wisplinghoff, H., Hall, G. O., Tallent, S., Wallace, S., Edmond, M. B., Figueiredo, A. M. & Wenzel, R. P. (2003). Clonal spread of methicillin-resistant *Staphylococcus aureus* in a large geographic area of the United States. *J Hosp Infect* **53**, 103–110.
- EARSS (2007). *EARSS Annual Report 2006*. Bilthoven: European Antimicrobial Resistance Surveillance System. http://www.rivm.nl/earss/Images/EARSS%202006%20Def_tcm61-44176.pdf
- Heym, B., Le Moal, M., Armand-Lefevre, L. & Nicolas-Chanoine, M.-H. (2002). Multilocus sequence typing (MLST) shows that the “Iberian” clone of methicillin-resistant *Staphylococcus aureus* has spread to France and acquired reduced susceptibility to teicoplanin. *J Antimicrob Chemother* **50**, 323–329.
- HPA (2000). *Surveillance of Hospital-Acquired Bacteraemia in English Hospitals 1997–2002*, Nosocomial Infection National Surveillance Service and the Public Health Laboratory Service joint report. London: Health Protection Agency (formerly the Public Health Laboratory Service). http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1194947379958
- Hubert, S. K., Mohammed, J. M., Fridkin, S. K., Gaynes, R. P., McGowan, J. E., Jr & Tenover, F. C. (1999). Glycopeptide-intermediate *Staphylococcus aureus*: evaluation of a novel screening method and results of a survey of selected U.S. Hospitals. *J Clin Microbiol* **37**, 3590–3593.
- Melter, O., Urbášková, P., Jakubů, V., Macková, B. & Žemličková, H. (2006). Emergence of EMRSA-15 clone in hospitals throughout the Czech Republic *Euro Surveill* **11**, E060803.6.
- Montesinos, I., Delgado, T., Riverol, D., Salido, E., Miguel, M. A., Jimenez, A. & Sierra, A. (2006). Changes in the epidemiology of methicillin-resistant *Staphylococcus aureus* associated with the emergence of EMRSA-16 at a university hospital. *J Hosp Infect* **64**, 257–263.
- Murchan, S., Kaufmann, M. E., Deplano, A., de Ryck, R., Struelens, M., Zinn, C. E., Fussing, V., Salmenlinna, S., Vuopio-Varkila, J. & other authors (2003). Harmonisation of pulse-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. *J Clin Microbiol* **41**, 1574–1585.
- Tenover, F. C., Arbeit, R. D., Goering, R. V., Mickelsen, P. A., Murray, B. E., Persing, D. H. & Swaminathan, B. (1995). Interpreting chromosomal DNA restriction patterns produced by pulse-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* **33**, 2233–2239.
- WHO (2000). *Overcoming Antimicrobial Resistance, World Health Organization Report on Infectious Diseases 2000*. Geneva: World Health Organization. http://www.who.int/infectious-disease-report/2000/other_versions/index-rpt2000_text.html
- Witte, W. (2000). Diagnostics, typing and taxonomy. In *Gram-Positive Pathogens*, pp. 309–317. Edited by V. A. Fischetti, R. P. Novick, J. J. Ferretti, D. A. Portnoy & J. I. Rood. Washington, DC: American Society for Microbiology.
- Witte, W., Enright, M., Schmitz, F. J., Cuny, C., Bräulke, C. & Heuck, D. (2001). Characteristics of a new epidemic MRSA in Germany ancestral to United Kingdom EMRSA-15. *Int J Med Microbiol* **290**, 677–682.