Characterization of methicillin-resistant 

*Staphylococcus aureus* isolated at Tripoli Medical Center, Libya, between 2008 and 2014

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Bacterial pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) represent a well-known public health pathogen with long-standing concern regarding both healthcare-associated, community-associated and livestock-associated infections. Molecular characterization of isolates has been a key element in understanding MRSA persistence and spread, including the recognition of especially epidemic types with national and international public health significance. Notorious examples of such strains include the USA300 community-associated MRSA strain, originally seen in North America but now found internationally (Tenover & Goering, 2009; Nimmo, 2012), and the epidemic EMRSA-15 and EMRSA-16 strains especially common in Europe (Johnson, 2011).

In Libya, detailed information is lacking regarding MRSA persistence and spread in healthcare and community settings (Ahmed et al., 2010, 2012; Buzaid et al., 2011; Falagas et al., 2013; Ghenghesh et al., 2013; Krima et al., 2014; Wareg et al., 2014; Baiu & Al-Abdli, 2015). Previous reports suggest a varying prevalence of MRSA in the Libyan community (i.e. 31 % to ca. 35 %) (Buzaid et al., 2011; Ahmed et al., 2012; Ghenghesh et al., 2013; Wareg et al., 2014) and 18.5 % nasal carriage versus 9.3 % throat carriage.
in healthcare workers (Ahmed et al., 2012; Krima et al., 2014; Baiu & Al-Abdli, 2015). Decreased antimicrobial susceptibility to vancomycin, fusidic acid, ciprofloxacin, streptomyacin, clindamycin, chloramphenicol, erythromycin, trimethoprim/sulfamethoxazole, quinuprisin/dalfopristin and mupirocin has been reported in Libyan MRSA, with some isolates exhibiting constitutive and inducible macro-lide–lincosamide–streptogramin B resistance (Ahmed et al., 2010, 2012; Wareg et al., 2014; Baiu & Al-Abdli, 2015). Genotypic characterization of Libyan MRSA has been limited, with the lukS-PV and lukF-PV genes for Panton–Valentine leukocidin (PVL) detected in one-third of MRSA nasal carriage isolates (n=35) from children and mothers at Tripoli Children’s Hospital and a mixture of five uncharacterized PFGE types found in 12 MRSA (Al-haddad et al., 2014).

To obtain a clearer and more comprehensive picture of MRSA in Libya, the aim of this study was to investigate the epidemiology of MRSA from patients admitted to the Tripoli Medical Center (TMC) over a multi-year period (2008–2014). The data from molecular strain typing were analysed in the context of the associated clinical data, as well as the major environmental changes (i.e. the 2011 Libyan Revolution) occurring during the study period.

**METHODS**

**Bacterial isolates: identification and antibiotic susceptibility.** Two hundred and two MRSA isolates were collected from various clinical specimens (blood, pus, soft tissue, wound swabs, respiratory samples and body fluids) and screening samples (nasal and axillary swabs) from outpatients or individuals admitted to 30 different hospital wards/units at the TMC, Libya during the years 2008, 2009, 2010, 2013 and 2014. The data were generated in connection with routine purposes, the small number of isolates from 2010 and 2013 were grouped with 2009 and 2014, respectively. This resulted in three study periods: 2008, 2009–2010 and 2013–2014. As noted above, the study represented MRSA obtained throughout the TMC facility and included a wide patient demographic and range of hospital units. A summary of the MRSA strain types observed is shown in Fig. 1. Overall, PVL-negative CC5 isolates were most frequently observed (38 %), >90 % of which were similar to the healthcare-associated USA100/800 strain type by PFGE and spa type t002. PVL-positive community-associated CC80 strains were the second most frequent (27 %) followed by CC22 (10 %), 87 % of which were the classic EMRSA-15 PVL type, spa type t022 and PVL positive. The minor groups (<10 % each) were CC15 (75 % closely related, t084 as seen elsewhere in Africa (Egyir et al., 2016), 25 % similar to USA900), CC1 (82 % of which were similar to community-associated USA400 but PVL negative), PVL-negative CC8/ST239, PVL-negative livestock-associated ST291/CC398 (undifferentiated into human or animal clades) and nine other isolates representing a variety of strain types (one or two each) including CC45, CC152, CC30 and CC88 as well as unknown PFGE and spa types.

Clinical samples were cultured onto 5 % sheep blood agar medium (bioMérieux) and incubated at 37 °C for 18–24 h. S. aureus isolates were identified biochemically, and their antibiotic susceptibility was determined by an automated system (Vitek 2 AST-P580 card; bioMérieux) with MIC values based on standard guidelines (NCCLS, 2000). Additional confirmation involved colony morphology, Gram stain, catalase test, coagulase test (Staph plus kit; bioMérieux), growth on MRSA ID chromogenic medium (bioMérieux), and serology using the PBP MRSA detection kit (bioMérieux).

**PFGE analysis.** For each isolate, chromosomal DNA was extracted, embedded in agarose plugs, digested with Smal restriction endonuclease and analysed as previously described by Goering et al. (2011). PFGE profiles with 80 % or higher similarity (using the Dice coefficient and unweighted pair group method with arithmetic mean algorithm) were considered related (Goering et al., 2011).

**Staphylococcal protein A (spa) typing and detection of genes for PVL.** PCR for spa typing was performed using the primers and thermal cycling conditions of the European Network of Laboratories for Sequenced Based Typing of Microbial Pathogens [SeqNet (http://www.seqnet.org)]. The spa typing plug-in tool of BioNumerics v7.6 was used for analysis of spa sequences and assignment of spa types, which were confirmed using the freely available Ridom Spa Server (http://spa.ridom.de/index.shtml). The multilocus sequence type clonal complex (CC) and sequence type (ST) of isolates were inferred from PFGE and spa typing data. PCR for detection of the (lukS-PV and lukF-PV) genes for PVL was performed as previously described by Lina et al. (1999).

**RESULTS**

The 202 isolates available for study were cultured in 2008 (58), 2009 (84), 2010 (2), 2013 (1) and 2014 (57). For summary purposes, the small number of isolates from 2010 and 2013 were grouped with 2009 and 2014, respectively. This resulted in three study periods: 2008, 2009–2010 and 2013–2014. As noted above, the study represented MRSA obtained throughout the TMC facility and included a wide patient demographic and range of hospital units. A summary of the MRSA strain types observed is shown in Fig. 1. Overall, PVL-negative CC5 isolates were most frequently observed (38 %), >90 % of which were similar to the healthcare-associated USA100/800 strain type by PFGE and spa type t002. PVL-positive community-associated CC80 strains were the second most frequent (27 %) followed by CC22 (10 %), 87 % of which were the classic EMRSA-15 PVL type, spa type t022 and PVL positive. The minor groups (<10 % each) were CC15 (75 % PVL positive, t084 as seen elsewhere in Africa (Egyir et al., 2016), 25 % similar to USA900), CC1 (82 % of which were similar to community-associated USA400 but PVL negative), PVL-negative CC8/ST239, PVL-negative livestock-associated ST291/CC398 (undifferentiated into human or animal clades) and nine other isolates representing a variety of strain types (one or two each) including CC45, CC152, CC30 and CC88 as well as unknown PFGE and spa types.

Fig. 2 summarizes the distribution of the different strains over time. CC5 and CC80 MRSA decreased in frequency but remained the two most common strains in all three study periods. Conversely CC22 (especially EMRSA-15), CC15, CC1 and CC8 types increased, resulting in 2013–2014 as the study period with the most diverse population.
of frequently isolated MRSA strains. Interestingly, the frequency of livestock-associated CC398 strains increased from 3% to 7% of total isolates in the years 2008 and 2009–2010, respectively, but was not detected in a similar number of isolates in 2013–2014. Although isolates were cultured from a wide variety of TMC units and patients, no correlation was found between MRSA strain type and patient demographics or TMC location.

As expected, the isolates were uniformly resistant to the β-lactam antibiotics (benzylpenicillin, cefoxitin and oxacillin). Uniform isolate susceptibility was observed for glycopeptide antibiotics (vancomycin and teicoplanin), mupirocin, tigecycline and linezolid. There was no general correlation between antibiotic susceptibility and MRSA strain type or date of isolation. However, CC8/ST239 MRSA were the most multi-resistant (isolated almost exclusively in 2014), and fluoroquinolone resistance tended to increase over time due, in part, to the emergence of CC8/ST239.

DISCUSSION

As noted above, to simplify analysis of the MRSA strain types over time, the isolates were clustered into three groups based on the date of isolation (i.e. 2008, 2009–2010 and 2013–2014). While the most predominant MRSA type (CC5 USA100/800) has been primarily associated with the USA, the strain has been observed worldwide (Monecke et al., 2011). Not surprisingly, the next most common types were the classic European community-associated CC80 and hospital-associated CC22 (EMRSA-15) strains (Monecke et al., 2011). CC5 and CC80 strains predominated from 2008 to 2010, although a trend towards greater strain diversity began to emerge during this period. However, post-2011 samples were markedly more diverse. Significant decreases in CC5 and CC80 strains were accompanied by major increases in CC22 (EMRSA-15), community-associated CC1 (e.g. USA400) isolates especially seen in the USA (McDougal et al., 2003), community-associated CC15-t084 seen in both Europe and the Middle East (Abou Shady et al., 2015) and well-known epidemic and multi-resistant CC8/ST239 strains (Monecke et al., 2011). It is interesting that livestock-associated ST291/CC398 strains, persistent throughout 2008 to 2010, were undetectable in post-2011 sampling.

It is impossible to precisely determine the effect, if any, the Libyan Revolution (2011) may have had on the trends observed here. However, there was a great movement of wounded and other patients from and to Libya post-revolution that could have contributed to the diversity of MRSA strains post-2011 (Elloulou BenDarif, personal communication). Such patients were treated in multiple locations, Europe (Italy, Germany, France, Czech Republic, etc.), Turkey, Tunisia and Jordan among others. Pre-Libyan Revolution (2008, 2009–2010 study grouping), TMC did not receive many patients from outside the Tripoli area; thus, most MRSA isolates were from patients living within Tripoli. Post-Libyan Revolution (2013–2014 study grouping), approximately two million people were displaced from other provinces to Tripoli, which led to an increase in patients exceeding standard capacity at the TMC. This likely contributed to the appearance of strain type (CC8) and the elevated incidences of strain types (CC15 and CC1) in post-Libyan Revolution (2013–2014) statistics. Most Libyan hospitals have no molecular diagnostic capabilities to identify endemic MRSA strain types, which made it difficult to track the sources and distribution of strain types in the TMC. Pre- and post-Libyan Revolution differences in MRSA strains present may be coincidental to the conflict that occurred during that period. Nevertheless, the influence of national and international conflicts on the persistence and spread of infectious diseases (e.g. conditions favouring disease transmission, human introduction of new strains) is well known (Smallman-Raynor & Cliff, 2004).

A review of the data from this study indicates not only the ebb and flow of MRSA over time but also the likelihood of patient-to-patient transmission within the healthcare facility. While infection control issues are common to all healthcare facilities, they are especially troubling in a resource-challenged environment. Thus, strain typing as performed here is especially valuable in focusing attention on infection control practices and techniques among healthcare workers and visitors to healthcare facilities, providing a potential feedback loop to stimulate intervention to reduce behaviour that may contribute to healthcare-associated transmission.

There were several limitations regarding this study. As a result of the Libyan Revolution, it was not possible to consistently collect and ship specimens to the USA for analyses, which resulted in smaller than desired sample sizes within certain years. Additionally, as samples were clinical specimens, the same susceptibility panels were not run on each and every cultured organism. While it was difficult to obtain these strains and the data are not as complete as originally designed, this study paints a valuable picture of MRSA
presence, ebb and flow, in this part of the world which is now coming out of conflict.

ACKNOWLEDGEMENTS

The authors received no external funding for this research. This project was supported only by the authors' own resources. We would like to acknowledge funding from the Council for International Exchange of Scholars, Institute of International Education for the Institutional Fulbright Visiting Scholar Program for Libya, 2013, Public Health, whose funding fostered collaboration between the authors.

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