SHORT COMMUNICATION

Misidentification of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals in Tripoli, Libya

Mohamed O. Ahmed[1,2*], Abdulbaset R. Abuzweda[2], Mohamed H. Alghazali[2], Asma K. Elramalli[3], Samira G. Amri[4], Ezzeddin Sh. Aghila[4] and Yousef M. Abouzeed[1]

[1]Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, Al Fateh University, Tripoli, Libya; [2]Department of Microbiology, Biotechnology Research Centre, Tripoli, Libya; [3]Tripoli Medical Centre, Tripoli, Libya; [4]Burns and Plastic Surgery Centre, Tripoli, Libya

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a nosocomial (hospital-acquired) pathogen of exceptional concern. It is responsible for life-threatening infections in both the hospital and the community.

Aims: To determine the frequency of MRSA misidentification in hospitals in Tripoli, Libya using current testing methods.

Methods: One hundred and seventy *S. aureus* isolates previously identified as MRSA were obtained from three hospitals in Tripoli. All isolates were reidentified by culturing on mannitol salt agar, API 20 Staph System and retested for resistance to methicillin using the cefoxitin disk diffusion susceptibility test and PBP2a. D-tests and vancomycin E-tests (Van-E-tests) were also performed for vancomycin-resistant isolates.

Results: Of the 170 isolates examined, 86 (51%) were confirmed as MRSA (i.e. 49% were misidentified as MRSA). Fifteen (17%) of the confirmed MRSA strains exhibited inducible clindamycin resistance. Of the 86 confirmed MRSA isolates, 13 (15%) were resistant to mupirocin, 53 (62%) were resistant to ciprofloxacin, 41 (48%) were resistant to trimethoprim-sulfamethoxazole, and none were resistant to linezolid. Although disc-diffusion testing indicated that 23 (27%) of the isolates were resistant to vancomycin, none of the isolates were vancomycin-resistant by Van-E-test.

Conclusions: Misidentification of nosocomial *S. aureus* as MRSA is a serious problem in Libyan hospitals. There is an urgent need for the proper training of microbiology laboratory technicians in standard antimicrobial susceptibility procedures and the implementation of quality control programs in microbiology laboratories of Libyan hospitals.

Keywords: *MRSA misidentification*; *clindamycin resistance*; *E-test*; *vancomycin resistance*

Received: 20 April 2010; Revised: 25 September 2010; Accepted: 12 October 2010; Published: 3 November 2010

The ability of *Staphylococcus aureus* to acquire resistance to antibiotics has resulted in the emergence of methicillin-resistant *S. aureus* (MRSA) (1). MRSA is of great concern, as it causes life-threatening nosocomial and community-acquired infections (2–4). The resistance of MRSA to commonly used therapeutic drugs is widely reported and associated with failed therapy (5). Recent reports by Borg et al. provide evidence of MRSA hyperendemicity in the southeast Mediterranean (6, 7) with important consequences for neighboring countries. However, these reports did not include data from Libya.

Objectives: To evaluate current MRSA detection methods and determine the rate at which *S. aureus* isolates previously characterized as MRSA were misidentified in three hospitals in Tripoli, Libya.

Materials and methods

Source of collection

There were 170 MRSA isolates from clinical and environmental samples collected in the period 2008–2009 that were subjected to antimicrobial susceptibility testing at the Biotechnology Research Center in Tripoli,

Libya. The samples were collected at three hospitals, referred to herein as hospitals A ($n=95$), B ($n=13$), and C ($n=62$), in Tripoli, Libya (Table 1). The collected isolates had been identified previously in each hospital as MRSA, initially based on cultural and microscopic characteristics on blood agar API system and gram staining for species determination as *S. aureus*. A non-referenced disk diffusion susceptibility test against certain and different antibiotics (i.e. oxacillin and cefoxitin) was also used to identify *S. aureus* as MRSA. Notably, none of the three hospitals used a quality control MRSA strain such as a referenced in-house strain or international recognized strain such as EMRSA-15.

Methicillin-resistant S. aureus (MRSA) determination and susceptibility testing

The 170 isolates analyzed at the Biotechnology Research Center were initially identified as *S. aureus* strains based on selective culturing on mannitol salt agar (MSA) and the API Staph test (bioMerieux). The determination of MRSA was based on latex agglutination testing for PBP2a and the cefoxitin disc diffusion susceptibility test in accordance with the British Association of Antimicrobials and Chemotherapy guidelines (BSAC) (8).

Isolates identified as MRSA were cultured overnight on sheep-blood agar, plated on Mueller-Hinton agar, and analyzed in disk diffusion susceptibility tests using the following antibiotics: ciprofloxacin (1 μg), erythromycin (5 μg), clindamycin (2 μg), trimethoprim-sulfamethoxazole (1.25 μg), mupirocin (5 μg), quinupristin/dalfopristin (15 μg), vancomycin (5 μg), or linezolid (10 μg). After a 24-h incubation at 37°C, the zone diameter was measured and compared to MRSA-BSAC guidelines. The D-tests were also performed on isolates that exhibited resistance to erythromycin to test for inducible resistance to clindamycin ($MLSB_i$) (9). An E-test (AB bioMerieux) was performed on MRSA isolates that were vancomycin resistant in the disc diffusion susceptibility test, according to BSAC guidelines. In-house confirmed, positive control MRSA and $MLSB_i$–MRSA isolates were generously provided by Dr. Ahmed MO, Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, Al Fateh University

Table 1. Summary of results showing the percentage of confirmed MRSA isolates from the three different hospitals

Hospital	Number of previously identified MRSA cases	Percentage of confirmed MRSA cases
A	95	53 (56%)
B	13	10 (76%)
C	62	23 (37%)
Total	170	86 (51%)

Results

All isolates were further confirmed as *S. aureus*. Of the 170 isolates previously identified at hospitals as MRSA, only 86 (51%) were confirmed as MRSA in the current study (Tables 1 and 2). Of the 86 confirmed MRSA isolates, 23 (27%) were resistant to erythromycin. The D-testing for inducible clindamycin resistance of erythromycin resistant MRSA isolates revealed that only 15 (17%) exhibited the $MLSB_i$ phenotype. Resistance to ciprofloxacin was identified in 62% (53/86) of the confirmed MRSA isolates, and trimethoprim-sulfamethoxazole resistance was identified in 48% (41/86) of the confirmed MRSA isolates. In contrast to the relatively high levels of resistance to fluoroquinolone and sulphonamide antibiotics, only 13 (15%) of the confirmed MRSA isolates were resistant to mupirocin and none of the confirmed MRSA isolates were resistant to linezolid. Out of the 86 confirmed MRSA isolates, 23 (27%) were vancomycin resistant as determined by the disc diffusion susceptibility test; however, the E-test failed to confirm resistance to vancomycin (Table 2).

Discussion

Until recently, most MRSA infections were acquired in hospital settings. Today, MRSA infections can occur in both rural and urban community settings (10, 11). In the current study, we determined the frequency of MRSA misidentification in hospitals in Tripoli, Libya using current testing methods. We found that only 51% of isolates previously identified as MRSA were confirmed as MRSA using current testing methods and standards (Table 1). A large number of the confirmed MRSA isolates in the current study exhibited resistance to fluoroquinolones. The MRSA resistance to ciprofloxacin is always associated with hospital-acquired MRSA, providing evidence that these isolates were hospital acquired (12). The levels of ciprofloxacin resistance identified in this Libyan study are relatively high relative to the average level of 25% for the Eastern Mediterranean, which ranges from 5% in Algeria to 40% in Turkey (6).

In contrast to the relatively high levels of resistance to fluoroquinolone and sulphonamide antibiotics, only 13 (15%) of the confirmed MRSA isolates were mupirocin resistant and none of the confirmed MRSA isolates were resistant to linezolid. Similar levels of susceptibility to mupirocin (77%) and linezolid (95%) have been reported for MRSA isolates from Saudi Arabian hospitals (13). Our data, showing 100% susceptibility to linezolid, indicates that in Libya, as in Saudi Arabia, linezolid remains a valuable tool for combating MRSA infections, although it should be used cautiously. Resistance to mupirocin, as determined by the E-test, occurred in only a small proportion (i.e. 15%) of the confirmed MRSA isolates, suggesting that mupirocin ointment, the

Citation: Libyan J Med 2010, **5**: 5230 - DOI: 10.3402/ljm.v5i0.5230

Table 2. Resistance phenotypes of confirmed MRSA isolates to antimicrobial agents as determined by the disc diffusion susceptibility test

	AMB resistance of confirmed MRSA isolates (%)									
Confirmed MRSA (%)	CLI	ERY	MU	STX	CIP	QU	VAN	LIN	$MLSB_i$	*E*-test (VAN)
86 (51%)	19 (22%)	23 (27%)	13 (15%)	41 (48%)	53 (62%)	22 (26%)	23 (27%)	0 (0%)	15 (17%)	0 (0%)

Note. AMB, antimicrobial; CLI, clindamycin; ERY, erythromycin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; MU, mupirocin; QU, quinupristin/dalfopristin; VAN, vancomycin; LIN, linezolid; MLSBi, macrolide-lincosamide-streptogramin B inducible resistance.

drug of choice for treating nasal colonization with MRSA, may still be effective in human therapy (14).

Clindamycin is an important drug used for the treatment of staphylococcal infections. The MLSB resistance phenotypes ($MLSB_C$ and $MLSB_i$) confer resistance to multiple antimicrobial drug classes (i.e. macrolides, lincosamides, and streptogramines B). Clindamycin resistance was observed in 19/86 (22%) of the confirmed MRSA isolates in the current study, with inducible resistance seen in an additional 15 isolates. Notably, inducible resistance to clindamycin in MRSA ($MLSB_i$ phenotype) has been shown to compromise therapy (15, 16) indicating the need for routine monitoring and susceptibility testing for both constitutive and inducible clindamycin resistance (17, 18) prior to therapy against MRSA infections in Libyan hospitals.

The importance of including E-testing in determining vancomycin resistance is illustrated by the difference we observed in the number of vancomycin resistant isolates between the disc diffusion susceptibility test (23 resistant isolates) and the E-test (0 resistant isolates, MIC <2 mg/L). The interpretive criteria used here (8) set a break point of <4 mg/l for susceptible strains. Although the use of lower break points can indicate emerging vancomycin resistance (19, 20), our values were well within the susceptible range.

The disc diffusion susceptibility test is a valuable method for the accurate, reliable detection of MRSA and for monitoring resistance trends (21–23). Herein, we found that the results of disc diffusion susceptibility tests indicated that 24% of our isolates were resistant to vancomycin; however, the E-test did not confirm vancomycin resistance for any of the same strains, indicating that disk diffusion susceptibility testing alone is not sufficient (8). Although the disc diffusion susceptibility test should not be relied on to screen for resistance to certain antimicrobials (e.g. vancomycin) it is recommended as a preliminary screening test for resistance to many antimicrobials (23, 24). The results of the current study suggest that microbiologists in Libyan hospitals should not rely on disk diffusion susceptibility tests as a measure of vancomycin resistance. Furthermore, factors such as how disks and media are stored and maintained can certainly affect disk diffusion susceptibility test results. Medium components concentration (i.e. NACl of the MSA for instance) can affect the susceptibility testing results (25). Moreover, many other factors can also influence the results of disk diffusion susceptibility tests such as incubation temperature (25), which could lead to misidentification and misinterpretation. The potential impact of these confounding factors should certainly be considered in Libyan hospitals when testing for MRSA.

It is extremely important for hospitals and microbiologists to follow standardized, reliable methods (e.g. BSAC, CLSI [formerly NCCLS] guidelines) for determining susceptibility to antimicrobials (25). The use and misuse of antimicrobials can lead to serious consequences, which may lead to an increase in the development and dissemination of MRSA (26). Meta-analysis linking antibiotic resistance to antibiotic use, both at the individual and institutional levels, has shown that antibiotic use is associated with a 1.8-fold increased risk of patient-acquired MRSA, and that glycopeptide or quinolone use is associated with a 3-fold increased risk of patient-acquired MRSA (9, 14). These results underscore the need for a conservative approach to antimicrobial use. The results of this study also indicate that tests currently available and used in Tripoli hospitals are generating high false positive rates of MRSA, which could lead to the overuse of various classes of antibiotics and contribute to increased resistance. As the hospital management systems are identical in other parts of Libya, our findings indicate the urgent need for a wider study including a variety of scientific approaches to combat MRSA in Libya. Furthermore, implementing quality control programs in hospital microbiology laboratories and training of laboratory personnel should be mandatory.

Acknowledgements

All of the authors were personally dedicated to this work. We gratefully acknowledge all of the hospital personnel who helped at the three different hospitals. We also offer our special thanks for the help and support we received from members of the Biotechnology Research Center in Tripoli, Libya. The authors acknowledge the

financial contribution of National authority of Scientific Research, Tripoli, Libya.

Conflict of interest and funding

The authors declare no relationship (commercial or otherwise) that may constitute a dual or conflicting interest.

References

1. Jorgensen JH. Laboratory and epidemiologic experience with methicillin-resistant *Staphylococcus aureus* in the USA. Eur J Clin Microbiol. 1986; 5: 693–6.
2. Palavecino E. Clinical, epidemiological, and laboratory aspects of methicillin-resistant *Staphylococcus aureus* (mrsa) infections. Methods Mol Biol. 2007; 391: 1–19.
3. Martins A, Cunha Mde L. Methicillin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci: epidemiological and molecular aspects. Microbiol Immunol. 2007; 51: 787–95.
4. Schmitz FJ, Petridou J, Fluit AC, Hadding U, Peters G, von Eiff C. Distribution of macrolide-resistance genes in *Staphylococcus aureus* blood-culture isolates from fifteen German university hospitals. M.A.R.S. Study group. Multicentre study on antibiotic resistance in staphylococci. Eur J Clin Microbiol Infect Dis. 2000; 19: 385–7.
5. Lewis JS, Jorgensen JH. Inducible clindamycin resistance in staphylococci: should clinicians and microbiologists be concerned? Clin Infect Dis. 2005; 40: 280–5.
6. Borg MA, de Kraker M, Scicluna E, van de Sande-Bruinsma N, Tiemersma E, Monen J, et al. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in invasive isolates from southern and eastern Mediterranean countries. J Antimicrob Chemother. 2007; 60: 1310–5.
7. Borg MA, Cookson BD, Zarb P, Scicluna EA. Antibiotic resistance surveillance and control in the Mediterranean region: report of the ARMed consensus conference. J Infect Dev Ctries. 2009; 3: 654–9.
8. Andrews JM. BSAC standardized disc susceptibility testing method (version 7). J Antimicrob Chemother. 2008; 62: 256–78.
9. Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. J Clin Microbiol. 2003; 41: 4740–4.
10. Fey PD, Said-Salim B, Rupp ME, Hinrichs SH, Boxrud DJ, Davis CC, et al. Comparative molecular analysis of community- or hospital-acquired methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother. 2003; 47: 196–203.
11. Groom AV, Wolsey DH, Naimi TS, Smith K, Johnson S, Boxrud D, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in a rural American Indian community. JAMA. 2001; 286: 1201–5.
12. Klein E, Smith DL, Laxminarayan R. Community-associated methicillin-resistant *Staphylococcus aureus* in outpatients, United States, 1999–2006. Emerg Infect Dis. 2009; 15: 1925–30.
13. Baddour MM, Abuelkheir MM, Fatani AJ. Trends in antibiotic susceptibility patterns and epidemiology of MRSA isolates from several hospitals in Riyadh, Saudi Arabia. Ann Clin Microbiol Antimicrob. 2006; 5: 30–2.
14. Tacconelli E. Antimicrobial use: risk driver of multidrug resistant microorganisms in healthcare settings. Curr Opin Infect Dis. 2009; 22: 352–8.
15. Angel MR, Balaji V, Prakash J, Brahmadathan KN, Mathews MS. Prevalence of inducible clindamycin resistance in gram positive organisms in a tertiary care centre. Indian J Med Microbiol. 2008; 26: 262–4.
16. Oguz VA, Yapar N, Sezak N, Cavus SA, Kurutepe S, Peksel H, et al. The rate of inducible clindamycin resistance and susceptibilities to other antimicrobial agents in staphylococci. Mikrobiyol Bul. 2009; 43: 37–44.
17. Shrestha B, Pokhrel BM, Mohapatra TM. Phenotypic characterization of nosocomial isolates of *Staphylococcus aureus* with reference to MRSA. J Infect Dev Ctries. 2009; 3: 554–60.
18. Ahmed MO, Alghazali MH, Amri SG, Abuzweda AR. Detection of inducible clindamycin resistance (MLSBi) among methicillin-resistant *Staphylococcus aureus* (MRSA) from Libya. Libyan J Med. 2010; 5: 4636.
19. Udo EE, Al-Sweih N, Dhar R, Dimitrov TS, Mokaddas EM, Johny M, et al. A surveillance of antibacterial resistance in *Staphylococcus aureus* isolated in Kuwaiti hospitals. Med Princ Pract. 2008; 17: 71–5.
20. Campanile F, Bongiorno D, Borbone S, Stefani S. Hospital-associated methicillin-resistant *Staphylococcus aureus* (ha-MRSA) in Italy. Ann Clin Microbiol Antimicrob. 2009; 8: 22–4.
21. Kampf G, Weist K, Swidsinski S, Kegel M, Ruden H. Comparison of screening methods to identify methicillin-resistant *Staphylococcus aureus*. Eur J Clin Microbiol Infect Dis. 1997; 16: 301–7.
22. Potz NA, Mushtaq S, Johnson AP, Henwood CJ, Walker RA, Varey E, et al. Reliability of routine disc susceptibility testing by the British Society for Antimicrobial Chemotherapy (BSAC) method. J Antimicrob Chemother. 2004; 53: 729–38.
23. Adaleti R, Nakipoglu Y, Karahan ZC, Tasdemir C, Kaya F. Comparison of polymerase chain reaction and conventional methods in detecting methicillin-resistant *Staphylococcus aureus*. J Infect Dev Ctries. 2008; 2: 46–50.
24. Ahmed MO, Clegg PD, Williams NJ, Baptiste KE, Bennett M. Antimicrobial resistance in equine faecal *Escherichia coli* isolates from North West England. Ann Clin Microbiol Antimicrob. 2010; 7: 12–7.
25. Brown DF, Edwards DI, Hawkey PM, Morrison D, Ridgway GL, Towner KJ, et al. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). J Antimicrob Chemother. 2005; 56: 1000–18.
26. Anupurba S, Sen MR, Nath G, Sharma BM, Gulati AK, Mohapatra TM. Prevalence of methicillin resistant *Staphylococcus aureus* in a tertiary referral hospital in eastern Uttar Pradesh. Indian J Med Microbiol. 2003; 21: 49–51.

***Mohamed O. Ahmed**
Department of Microbiology and Parasitology
Faculty of Veterinary Medicine
Al Fateh University
P.O. Box 30313
Tripoli, Libya
Tel: +21 8925400914
Fax: +21 8214628421
Email: mhmd73@lttnet.net

Citation: Libyan J Med 2010, **5**: 5230 - DOI: 10.3402/ljm.v5i0.5230