Prevalence of MRSA among *Staphylococcus Aureus* Isolates Recovered From Patients with Otitis Media

Maisra M. El-Bouseary, Tarek E. El-Banna, Fatma I. Sonbol

Department of Microbiology, Faculty of Pharmacy, Tanta University, Tanta, Egypt
maisra_mohammed@outlook.com

Abstract: Emergence of Methicillin Resistant *Staphylococcus aureus* (MRSA) represent a serious clinical and public health problem in Egyptian hospitals. However, little data are available on the prevalence of MRSA in Egypt. During the period between April 2014 to April 2015, the epidemiology of MRSA was studied among Patient admitted to ENT clinic, Tanta University teaching hospital, Egypt. The incidence of MRSA was 26% where 42 MRSA isolates were recovered among 160 *S. aureus* isolated from patients diagnosed with otitis media. Isolation and detection of MRSA was performed using two diverse types of media including Chromogenic MRSA agar plates and Oxacillin Resistance Screening Agar Base (ORSAB) plates. It was observed that ORSAB medium was relatively more accurate in detection of MRSA, where the sensitivity of ORSAB and Chromogenic MRSA agar recorded100% and 97% respectively. One of the characteristics of MRSA is its ability to thrive in the presence of β-lactam antibiotics, which normally prevent bacterial growth by inhibiting synthesis of cell wall. This is due to a resistance gene, *mecA*, which stops β-lactam antibiotics from inactivating transpeptidases enzymes. In the present study, the presence of *mec A*gene, a biomarker gene responsible for resistance to methicillin and other β-lactam antibiotics, was confirmed by PCR. It was found that 38 out of 42 (90.5%) MRSA isolates harbored *mecA* gene. The findings from this study emphasize the bad need for continuous surveillance of MRSA and to set strategies for eradication of such "superbug' to lower mortality, hospitalization and treatment costs of this infection.
[Maisra M. El-Bouseary, Tarek E. El-Banna, Fatma I. Sonbol. **Prevalence of MRSA among *Staphylococcus Aureus* Isolates Recovered From Patients with Otitis Media.** *Nat Sci* 2018;16(6):48-55]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 8. doi:10.7537/marsnsj160618.08.

Keywords: MRSA, ENT, Epidemiology, *mecA*, Egypt.

1. Introduction

Methicillin Resistant *Staphylococcus aureus* (MRSA) strains are “subspecies” of *S. aureus* which showed a distinctive characteristic of being Methicillin resistant. Numerous phenotypic and genotypic characteristics distinguish methicillin-susceptible *S. aureus* (MSSA) from MRSA. MRSA usuallyare multi-drug resistant, that show resistance not only to β-lactam antibiotics but also to a wide range of antibiotic classes, such as fluoroquinolones, tetracyclines, macrolides, lincosamides and aminoglycosides (Livermore et al. 2008; Pantosti and Venditti 2009). Methicillin-resistant *Staphylococcus aureus* (MRSA) cause several illnesses such as pneumonia, infective endocarditis, osteomyelitis, septic arthritis and skin and soft tissue infections, including nasal and ear infections. Numerous risk factors contribute to the emergence of MRSA causing ear and sino-nasal infections. The major factors are widespread use of broadspectrum antibiotics and previous nasal surgeries **(Sachithanandam 2014)**.

Since two decades, vancomycin has been prescribed asa magic therapy for MRSA. Nevertheless, decreased susceptibility limits vancomycin usage to eradicate serious infections caused by MRSA especially for MRSA pneumonia, due to suboptimal penetration of vancomycin in the alveolar lining fluid(Tenover and Moellering Jr. 2007; Stevens 2006). Resistance to the newest antibiotics licensed to treat MRSA infections, linezolid and daptomycin has already emerged (Marty et al. 2006; Murthy et al. 2008; Pantosti and Venditti 2009). The Delayed diagnosis and treatment of MRSA infections lead to poorer clinical outcomes. By enabling optimized management strategies, rapid diagnostic tests may lower mortality, hospitalization, and costs (Hassoun, Linden, and Friedman 2017).

The acquisition of *mecA* gene coding for the penicillin-binding protein 2a (PBP 2a), involved in bacterial cell wall synthesis is the major evidence for the detection of resistance to methicillin and to all β-lactam antibiotics in *S. aureus* (Shopsin and Kreiswirth 2001). The *mecA* gene is located on a mobile staphylococcal cassette chromosome (SCC) element known as *SCCmec*, twelve different types of *SCCmec* (I to XII) have been defined to date, five of which (I to V) are globally distributed(Shore and Coleman 2013). The detection of methicillin resistance, however, is complicated by the fact that itsphenotypic expression in many strains is heterogeneous **(Melter et al. 1999)**. This fact required development of more specific and more sensitive laboratory techniques such as slidelatex agglutination test, various disc diffusion methods and *mecA* gene detection tests based on polymerase chain reaction (PCR) which enhance the expression of this

resistance in vitro (Odonkor, Newman, and Addo 2012).

Because of increasing incidence of MRSA in ENT diseases and a reported year to year variations in the incidence of MRSA and the emergence of resistance to several antimicrobial drugs, there is a continuous need for monitoring of this alarming pathogen. Therefore, our study was conducted to update relevant epidemiologic data to determine the incidence, reveal the risk factors and rapid detection of infection for instituting effective management of MRSA and improve patient well-being in Egypt.

2. Materials and Methods

Sample collection

A total of 202 fresh specimens were collected randomly from patients admitted to ENT department of Tanta University Teaching hospitals during the period between April 2014 to April 2015. Collection of the clinical samples included specimens from inpatients and outpatients. Clinical samples were collected by physicians and nurses and they were sputum, nose swabs or ear swabs. Handling of these samples were performed according to guidelines for the collection of clinical specimens **(Hopkins 2016)**.

For each patient, a full patient questionnaire was filled in and all recorded data were entered onto a Microsoft EXCEL spread sheet. The designed patient questionnaire is comprehensive and covers demographics such as age, gender, education and place of residence as well as clinical details such as collected sample site, diagnosis and antibiotic therapy.

Isolation and Identification of MRSA isolates

Each clinical specimen was inoculated onto Mannitol Salt Agar (MSA) plates and after incubation at 37^{o}C for 24 h, Golden yellow colonies indicating *S. aureus* growth were further streaked onto both Chromogenic MRSA agar plates (CHROMagar^{TM} MRSA) and Oxacillin Resistance Screening Agar Base (ORSAB) agar (Oxoid™ ORSAB) for confirmation of MRSA.

MRSA growth was detected by intense mauve color of produced colonies on Chromogenic MRSA agar. Furthermore, only isolates showed intense blue colonies on ORSAB were recorded as MRSA.

Screening of the tested MRSA isolates for *mecA* gene

Polymerase Chain Reaction (PCR) technique wasperformed to detect the presence of *mecA* gene. PCRs were run on G-Storm (GS1) Thermal Cycler.

DNA extraction procedures:

Genomic DNA of the tested MRSA isolates was extracted by denaturation of a few colonies suspended in sterile water at 98°C for 15 min and then centrifuged at 13,000 rpm for 30 seconds. The supernatant was used as template for amplification in PCR**(Berglund et al. 2005)**.

The PCR conditions used were as follows:

Initial Denaturation (at 94ºC for 5 Min)**,**35 Cycles of Denaturation (at 94ºC for 1 Min)**,** Annealing (at 52ºC for 1 Min)**,** Extension (at 72ºC for 1 Min)**,** Final extension (at 72ºC for 5 Min)**,** Store at 4°C**(Al-talib et al. 2009)**.

The primers used were:

Forward primer sequence (*mecA*-F):"ACGAGTAGATGCTCAATATAA".

Reverse primer sequence (*mecA*-R): "CTTAGTTCTTTAGCGATTGC".

Gel Electrophoresis: .

The PCR products were run on 1.5% agarose gel to visualize the amplified bands. The gels were visualized, and photographs recorded under UV using Syngene G-BOX documentation system.

3. Results

Isolation and Identification of MRSA isolates

Out of 202 clinical specimens 160*S. aureus* isolates were recovered after culture on MSA. MRSA growth was confirmed by using two different selective media; Chromogenic MRSA agar and Oxacillin Resistance Screening Agar Base (ORSAB) media. Out of 160 *S. aureus*isolates, 42, 41MRSA isolates (26.3%, 25.6%) were recovered using ORSAB medium and Chromogenic MRSA agar respectively (Figure 1).

Prevalence of MRSA isolates according to sample type, age groups or gender

The clinical information and physical examination of the specimens of 202 cases were subjected for interpretation. As shown in Table (1), a total number of 42 isolates out of160*S. aureus* isolates tested positive for MRSA. The highest number of MRSA were isolated from ear swabs (57%) followed by sputum specimen (24%) then nasal swab (19%).

The distribution of 42 MRSA isolates recovered in terms of out-patients and in-patients cases was represented in Table (2). Outpatients had the highest carriage of MRSA representing (67%) whiles in-patients had a total of 14 isolates representing33% of total MRSA isolates. No MRSA (0%) was detected in nasal swabs collected from out-patients.

Regarding different age groups, the highest incidence of MRSA (24%) was recorded from the age group 0-10 years old followed by those of 21-30 years old. (21%). On the other hand, the lowest incidence (5%) was recorded in age group 61-70 years old as shown in Table (3).

Concerning distribution of MRSA in both sexes, the overall incidence of MRSA was found higher in females (62%) than in males (38%).

The effect of fourrisk factors for MRSA including immunosuppression, diabetes mellitus (DM),

Chronic obstructive pulmonary disease (COPD), prior surgery was statistically determined. Analysis of risk factors revealed that MRSA cases associated with DM (23.8%), prior surgery (21.4%), immunosuppression (19%) and COPD (11.9%).

Screening of recovered MRSA isolates for *mecA* gene

In the present study, detection of *mecA* among the recovered MRSA isolates was performed using PCR technique. The PCR electrophoregram showed distinct DNA band of *mecA* gene with amplicon size of 293 bpin 38out of 42 (90.5%) tested MRSA isolates (Figure 2).

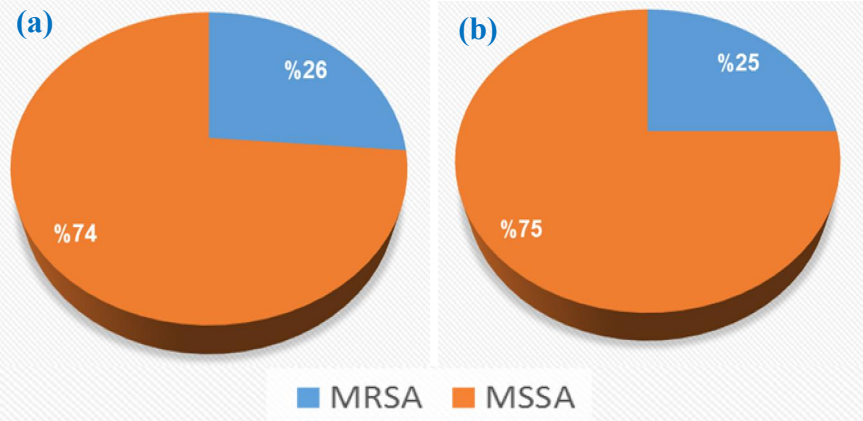

Figure 1: "Percentage of MRSA and MSSA among recovered *S. aureus* isolates recovered on (a) ORSAB (b) Chromogenic MRSA agar".

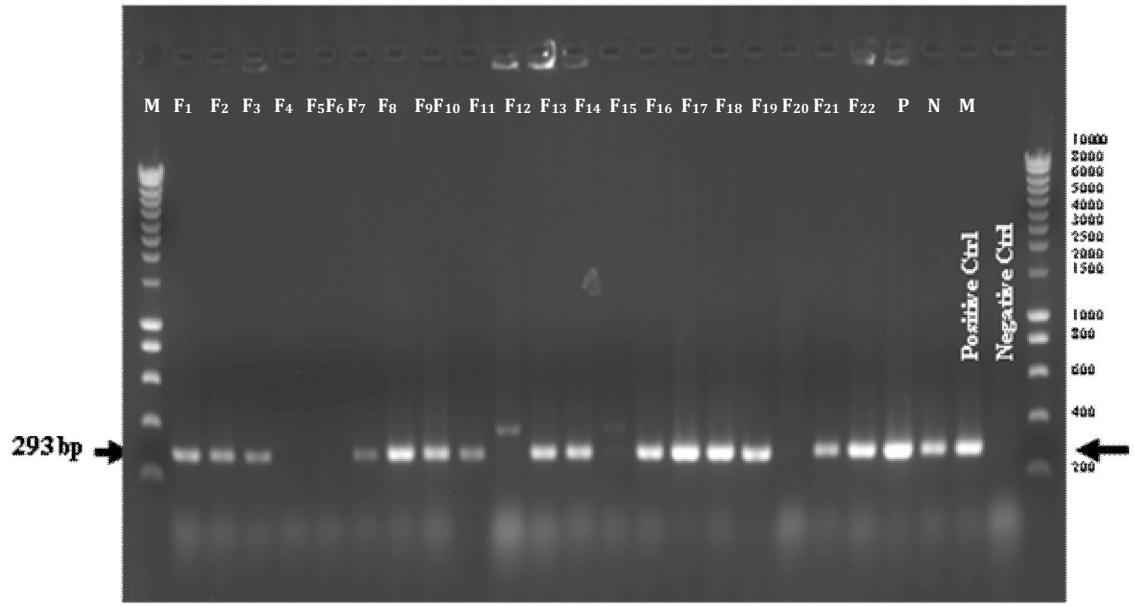

Figure 2: "A representative electrophoregram of *mecA* gene amplicons. Lane M 100 bp DNA ladder. Lane P is positive control. Lane N is negative control. Lanes F1-F22 are amplified products of tested MRSA isolates".

Table 1: Isolates of methicillin-resistant *S. aureus* (MRSA) from different clinical specimen.

Clinical specimen	No. of *S.aureus*	No. of MRSA	% MRSA
Sputum (n=50)	31	10	24%
Ear swab (n=104)	86	24	57%
Nasal swab (n=48)	43	8	19%
Total (n=202)	160	42	100%

Table 2: Distribution of methicillin-resistant *S. aureus*isolates among outpatient and inpatient cases.

Clinical specimen	No. of MRSA	Outpatients No. (%)	Inpatients No. (%)
Sputum	10	8 (80%)	2 (20%)
Ear swab	24	20 (83%)	4 (17%)
Nasal swab	8	0 (0%)	8 (100%)
Total	42	28 (67%)	14 (33%)

Table 3: Distribution of methicillin-resistant *S. aureus* isolates according to different age groups.

Age Range	No. of MRSA (%)*
0-10	10 (24%)
11-20	4 (10%)
21-30	9 (21%)
31-40	8 (19%)
41-50	6 (14%)
51-60	3 (7%)
61-70	2 (5%)

* Percent calculated relative to total number (42) of MRSA isolates.

4. Discussion

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a nosocomial pathogen of major worldwide importance. Epidemiological studies of MRSA undoubtedly has continued to evolve since its discovery more than three decades ago (Odonkor, Newman, and Addo 2012). Several reports were stablished regarding MRSA incidence and spreading rates amongst nosocomial *S. aureus* isolates. Recent studies reported an increases of prevalence of nosocomial as well as community acquired MRSA isolates**(Goodrich et al. 2009; Espadinha et al. 2013; Alaklobi et al. 2015)**.

MRSA growth was confirmed by using two different selective media; Chromogenic MRSA agar media (CHROMagar MRSA) and Oxacillin Resistance Screening Agar Base (ORSAB) media. It was observed that ORSAB medium was more accurate in detection of MRSA. This finding was congruent with previous studies who evaluated the specificity and accuracy of ORSAB versus CHRO Magar MRSA, and they reported that % Sensitivity of ORSAB and CHROMagar MRSA was 78% and 72% respectively and % Specificity was 93.1% in case of ORSAB and 92.1% in case of CHRO Magar MRSA **(Perry et al. 2004; Cherkaoui et al. 2017)**. Also, out of 360 *S. aureus* isolates 117, 109MRSA isolates (33%, 30%) were recovered from various clinical specimens using ORSAB medium and Chromogenic MRSA agar respectively (ongoing publication).

In the present study, we investigate the incidence of MRSA among *S. aureus* isolates recovered from patients admitted to ENT clinic of Tanta university teaching hospital. It was found that 26% of *S. aureus* isolates were found to be resistant to methicillin. Similar rates were reported in different studies conducted in Egypt including Cairo (25%)**(See et al. 2013)** and Menoufia (23%) **(Melake et al. 2014)**. Comparing our findings with that obtained by other workers in Egypt, a controversary was obvious where a higher incidence of MRSAin Cairo (50%) **(Borg et al. 2007)** and 60.5% **(Morsy et al. 2017)** was reported. Similarly Abdel-maksoud et al. reported an overall incidence rate of 76.6% in 12 hospitals in Egypt from 2005 to 2013**(Abdel-maksoud et al. 2016)**. However, a markedly lower incidence of MRSA was reported in Assiut (18.9%)**(Ahmed et al. 2009)** and in Sohag (4.6%) Egyptian governorate**(Abu-Gharbia et al. 2015; Abouelfetouh 2017)**.

The isolation rate of MRSA was comparable to that reported (21-30%) by Kesah and other researchers in some parts of Africa (including Nigeria; Cameroon, Kenya and Algeria) and Malta between 1996-1997 **(Kesah et al. 2003)**. Meanwhile, reports emanating from Middle East countries also revealed higher rates in the incidence of MRSA: in Saudi Arabia (77.5%) (Baddour, Abuelkheir, and Fatani 2006)and in Libya (54–68%)**(Ghenghesh et al. 2013)**. However, lower rates of below 10% in North Africa (e.g. Algeria) and Malta were reported **(Azeez-Akande 2010; Ghebremedhin et al. 2009)**. In Tunisia, the prevalence of MRSA increased from 16% to 41% between 2002–2007 **(Mastouri et al. 2006)**, while in Libya it was 31% in 2007**(Buzaid et al. 2011)**. In South Africa, the incidence rate decreased from 36% in 2006 to 24% during 2007–2011 (Jansen van Rensburg, Whitelaw, and Elisha 2012). In Botswana, the prevalence varied from 23–44% between 2000–2007**(Wood et al. 2009; Falagas et al. 2013)**.

The former findings indicate that the incidence of MRSA keeps changing every year. These variations in results might be explained by differences in geographical location, hygienic measures and cross infection by the hand of the medical personnel, air and other materials.

Staphylococcal cassette chromosome mec (SCCmec) is a genomic island of unknown origin containing the antibiotic resistance gene *mecA*(Katayama, Ito, and Hiramatsu 2000; Shore and Coleman 2013). SCCmec contains additional genes beyond *mecA*, including the cytolysin gene psm-mec, which may suppress virulence in MRSA strains **(Kaito et al. 2011)**. Other new *mecA* gene homologs including *mecB* and *mecC* were detected in other species, *mec C* has also been found on the chromosome of *S. xylosus* while *mec B* has not been reported yet in staphylococcal species **(Hiramatsu et al. 2013)**. Therefore, recognition of *mecA* gene is the

major evidence for the detection of MRSA isolate. This was approved by many investigators all over the world: including Egypt (Khairalla, Wasfi, and Ashour 2017), Europe (Deplano et al. 2014; Paterson, Harrison, and Holmes 2014)and USA**(Murakami et al. 1991)**. Interestingly, *mecA*-positive MRSA strains were recovered from hand and nasal specimens of the outpatients as well as inpatients under investigations. This might be explained by the widespread use of non-prescription antimicrobial agents in Egypt. In the present study, it was found that 38MRSA isolates (90.5%) were *mecA*-positive. Only four MRSA isolates were negative *mecA*. Similarly, Atoum et al. reported strains of negative *mecA* being methicillin resistant and positive *mecA* being methicillin sensitive. These findings might be explained due to non-functional *mecA* gene or inactive PBP2a protein(Atoum, Akel, and Battikhi 2003). Although, PCR based detection of MRSA is highly recommended. The absence of *mecA* gene in a considerable percentage of MRSA isolates requires investigating the alternative genetic options related to the resistance phenomena **(Elhassan et al. 2015)**.

Conclusion

This study reports the epidemiology incidence rate, and occurrence of *mecA* gene among MRSA isolates associated with ENT infections in Tanta, Egypt. Our data indicate that:

1. MRSA might be considered as one of the important nosocomial pathogens among patients admitted to ENT department, Tanta University teaching hospital.
2. ORSAB mediumwas more accurate and sensitive for rapid detection of MRSA isolates compared to chromogenic MRSA agar.
3. Absence of *mecA* gene in some MRSA isolates recommended the investigating of alternative genetic options relate to methicillin resistance phenomena.
4. The need for continuous surveillance of MRSA in endemic regions to obtain a more comprehensive and detailed knowledge of epidemiology of MRSA is highly recommended.

References

1. Abdel-maksoud, Mohamed, Mona El-shokry, Ghada Ismail, Soad Hafez, Amani El-kholy, Ehab Attia, and Maha Talaat. 2016. "Methicillin-Resistant *Staphylococcus aureus* Recovered from Healthcare- and Community-Associated Infections in Egypt" *International Journal of Bacteriology*2016. Hindawi Publishing Corporation. doi:10.1155/2016/5751785.
2. Abouelfetouh, Alaa. 2017. "The Status of Methicillin Resistance Among Egyptian *Staphylococcus aureus* Isolates: An Overview." *Infectious Disorders Drug Targets*. United Arab Emirates. doi:10.2174/1871526516666160802111200.
3. Abu-Gharbia, Magdy; Michael N. Agban; Rasha Z., Abdelmasieh; 2015. "The Enviromental Contamination in Some Intensive Care Units of Assiut and Sohag University Hospitals Department of Immunology and Microbiology, Faculty of Medicine,." *Egyptian Journal of Medical Microbiology* 24 (1): 31–35.
4. Ahmed, Shaaban H, Enas A Daef, Mohammed S Badary, Mohammed A Mahmoud, and Alaa A Abd-Elsayed. 2009. "Nosocomial Blood Stream Infection in Intensive Care Units at Assiut University Hospitals (Upper Egypt) with Special Reference to Extended Spectrum Beta-Lactamase Producing Organisms." *BMC Research Notes* 2: 76. doi:10.1186/1756-0500-2-76.
5. Al-talib, Hassanain, Chan Yean Yean, Alyaa Al-khateeb, Habsah Hassan, Kirnpal-kaur Banga Singh, Karim Al-jashamy, and Manickam Ravichandran. 2009. "A Pentaplex PCR Assay for the Rapid Detection of Methicillin-Resistant *Staphylococcus aureus* and Panton-Valentine." *BMC Microbiology* 8: 1–8. doi:10.1186/1471-2180-9-113.
6. Alaklobi, F., F. Aljobair, a. Alrashod, R. Alhababi, M. Alshamrani, W. Alamin, Lyubov Lytvyn, F. Alrogi, and D. Mertz. 2015. "The Prevalence of Community-Associated Methicillin-Resistant *Staphylococcus aureus* among Outpatient Children in a Tertiary Hospital: A Prospective Observational Study in Riyadh, Saudi Arabia." *International Journal of Pediatrics and Adolescent Medicine* 2 (3–4). Elsevier Ltd: 136–40. doi:10.1016/j.ijpam.2015.09.001.
7. Atoum, Manar F, Hazem Akel, and Mohamed N Battikhi. 2003. "Comparison of PCR and Disc Diffusion Methods in Detecting Methicillin Resistance among *Staphylococcus* Species from Nosocomial Infections." *Saudi Medical Journal* 24 (12). Saudi Arabia: 1410–12.
8. Azeez-Akande, O. 2010. "Global Trend of Methicillin-Resistant *Staphlococcus aureus* and Emerging Challenges for Control." *African Journal of Clinical and Experimental* 11 (3): 150–58. http://www.ajol.info/index.php/ajcem/article/view/57771.
9. Baddour, Manal M, Manal M Abuelkheir, and Amal J Fatani. 2006. "Trends in Antibiotic Susceptibility Patterns and Epidemiology of MRSA Isolates from Several Hospitals in Riyadh, Saudi Arabia." *Annals of Clinical*

Microbiology and Antimicrobials 11: 1–11. doi:10.1186/1476-0711-5-Received.

10. Berglund, Carolina, Paula Mölling, Lennart Sjöberg, and Bo Söderquist. 2005. "Multilocus Sequence Typing of Methicillin-Resistant *Staphylococcus aureus* from an Area of Low Endemicity by Real-Time PCR." *Journal of Clinical Microbiology* 43 (9). American Society for Microbiology: 4448–54. doi:10.1128/JCM.43.9.4448-4454.2005.
11. Borg, Michael A., Marlieke De Kraker, Elizabeth Scicluna, Nienke van de Sande-Bruinsma, Edine Tiemersma, Jos Monen, and Hajo Grundmann. 2007. "Prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Invasive Isolates from Southern and Eastern Mediterranean Countries." *Journal of Antimicrobial Chemotherapy* 60 (6): 1310–15. doi:10.1093/jac/dkm365.
12. Buzaid, Najat, Abdel-Naser Elzouki, Ibrahim Taher, and Khalifa Sifaw Ghenghesh. 2011. "Methicillin-Resistant *Staphylococcus aureus* (MRSA) in a Tertiary Surgical and Trauma Hospital in Benghazi, Libya." *Journal of Infection in Developing Countries* 5 (10). Italy: 723–26.
13. Cherkaoui, Abdessalam, Gesuele Renzi, Patrice Franc, and Jacques Schrenzel. 2017. "Comparison of Four Chromogenic Media for Culture- Based Screening of Meticillin-Resistant *Staphylococcus aureus*." *Journal of Medical Microbiology*, no. 2007: 500–503. doi:10.1099/jmm.0.46981-0.
14. Deplano, Ariane, Stien Vandendriessche, Claire Nonhoff, and Olivier Denis. 2014. "Genetic Diversity among Methicillin-Resistant *Staphylococcus aureus* Isolates Carrying the mecC Gene in Belgium." *The Journal of Antimicrobial Chemotherapy* 69 (6). England: 1457–60. doi:10.1093/jac/dku020.
15. Elhassan, Mogahid M, Hani A Ozbak, Hassan A Hemeg, Miskelyemen A Elmekki, and Leila M Ahmed. 2015. "Absence of the Mec A Gene in Methicillin Resistant *Staphylococcus aureus* Isolated from Different Clinical Specimens in Shendi City, Sudan." *BioMed Research International* 2015.
16. Espadinha, Diana, Nuno A. Faria, Maria Miragaia, Luís Marques Lito, José Melo-Cristino, Hermínia de Lencastre, and Médicos Sentinela Network. 2013. "Extensive Dissemination of Methicillin-Resistant *Staphylococcus aureus (*MRSA) between the Hospital and the Community in a Country with a High Prevalence of Nosocomial MRSA." *PLoS ONE* 8 (4): 1–8. doi:10.1371/journal.pone.0059960.
17. Falagas, Matthew E, Drosos E Karageorgopoulos, John Leptidis, and Ioanna P Korbila. 2013. "MRSA in Africa : Filling the Global Map of Antimicrobial Resistance." *Plos One* 8 (7). doi:10.1371/journal.pone.0068024.
18. Ghebremedhin, B., M. O. Olugbosi, A. M. Raji, F. Layer, R. A. Bakare, B. König, and W. König. 2009. "Emergence of a Community-Associated Methicillin-Resistant *Staphylococcus aureus* Strain with a Unique Resistance Profile in Southwest Nigeria." *Journal of Clinical Microbiology* 47 (9): 2975–80. doi:10.1128/JCM.00648-09.
19. Ghenghesh, Khalifa Sifaw, Amal Rahouma, Khaled Tawil, Abdulaziz Zorgani, and Ezzedin Franka. 2013. "Antimicrobial Resistance in Libya: 1970-2011." *Libyan Journal of Medicine* 8 (1): 1–8. doi:10.3402/ljm.v8i0.20567.
20. Goodrich, Jennifer S., Tameaka N. Sutton-Shields, Alan Kerr, Joel P. Wedd, Melissa B. Miller, and Peter H. Gilligan. 2009. "Prevalence of Community-Associated Methicillin-Resistant *Staphylococcus aureus* in Patients with Cystic Fibrosis." *Journal of Clinical Microbiology* 47 (4): 1231–33. doi:10.1128/JCM.00255-09.
21. Hassoun, Ali, Peter K. Linden, and Bruce Friedman. 2017. "Incidence, Prevalence, and Management of MRSA Bacteremia across Patient Populations—a Review of Recent Developments in MRSA Management and Treatment." *Critical Care* 21 (1). Critical Care: 211. doi:10.1186/s13054-017-1801-3.
22. Hiramatsu, K, T Ito, S Tsubakishita, T Sasaki, F Takeuchi, Y Morimoto, Y Katayama, et al. 2013. "Genomic Basis for Methicillin Resistance in *Staphylococcus aureus*." *Infect.Chemother.* 45 (2093–2340 (Print)): 117–36. doi:10.3947/ic.2013.45.2.117.
23. Hopkins, Johns. 2016. "Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 6 / 2016 Overview Contents : By Specimen Type Johns Hopkins Medical Microbiology Specimen Collection Guidelines – Updated 6 / 2016," 1–27.
24. Jansen van Rensburg, Melissa J, Andrew C Whitelaw, and Brenda G Elisha. 2012. "Genetic Basis of Rifampicin Resistance in Methicillin-Resistant *Staphylococcus aureus* Suggests Clonal Expansion in Hospitals in Cape Town, South Africa." *BMC Microbiology* 12 (March). England: 46. doi:10.1186/1471-2180-12-46.
25. Kaito, Chikara, Yuki Saito, Gentaro Nagano, Mariko Ikuo, Yosuke Omae, Yuichi Hanada, Xiao Han, et al. 2011. "Transcription and Translation Products of the Cytolysin Gene Psm-Mec on the Mobile Genetic Element SCCmec

Regulate *Staphylococcus aureus* Virulence." *PLoS Pathogens* 7 (2). doi:10.1371/journal.ppat.1001267.

26. Katayama, Y, T Ito, and K Hiramatsu. 2000. "A New Class of Genetic Element, Staphylococcus Cassette Chromosome mec, Encodes Methicillin Resistance in *Staphylococcus aureus*." *Antimicrob. Agents Chemother.* 44 (0066–4804): 1549–55.
27. Kesah, C., S. Ben Redjeb, T. O. Odugbemi, C. S B Boye, M. Dosso, J. O. Ndinya Achola, S. Koulla-Shiro, M. Benbachir, K. Rahal, and M. Borg. 2003. "Prevalence of Methicillin-Resistant Staphylococcus Aureus in Eight African Hospitals and Malta." *Clinical Microbiology and Infection* 9 (2). European Society of Clinical Infectious Diseases: 153–56. doi:10.1046/j.1469-0691.2003.00531.x.
28. Khairalla, Ahmed S, Reham Wasfi, and Hossam M Ashour. 2017. "Carriage Frequency, Phenotypic, and Genotypic Characteristics of *S. aureus* Isolated from Dental Health- Care Personnel, Patients, and Environment." *Scientific Reports*, no. April. Springer US: 1–16. doi:10.1038/s41598-017-07713-8.
29. Livermore, David M., Russell Hope, Geraldine Brick, Mark Lillie, and Rosy Reynolds. 2008. "Non-Susceptibility Trends among Enterobacteriaceae from Bacteraemias in the UK and Ireland, 2001-06." *Journal of Antimicrobial Chemotherapy* 62 (SUPPL. 2).
30. Marty, Francisco M, Wendy Yeh, Christine B Wennersten, Lata Venkataraman, Esperanza Albano, Edwin P Alyea, Howard S Gold, Lindsey R Baden, and Satish K Pillai. 2006. "Emergence of a Clinical Daptomycin Resistant *Staphylococcus aureus* Isolate during Treatment of Methicillin Resistant Staphylococcus Aureus Bacteremia and Osteomyelitis." *Journal of Clinical Microbiology* 44 (2): 595–97. doi:10.1128/JCM.44.2.595.
31. Mastouri, M, M Nour, M Ben Nejma, O Bouallegue, M Hammami, and M Khedher. 2006. "Antibiotics resistance of meticilline-resistant Staphylococcus aureus: detection of the first glycopeptides low sensibility strains in Tunisia." *Pathologie-biologie* 54 (1). France: 33–36. doi:10.1016/j.patbio.2004.10.009.
32. Melake, Nahla A, Naira A Eissa, Tarek F Keshk, and Asmaa S Sleem. 2014. "Prevalence of Multidrug-Resistant Bacteria Isolated from Patients with Burn Infection." *Menoufia Medical Journal*, 677–84. doi:10.4103/1110-2098.167888.
33. Melter, O., I. Santos Sanches, J. Schindler, M. Aires De Sousa, R. Mato, V. Kovárova, H. Zemlicková, and H. De Lencastre. 1999. "Methicillin-Resistant *Staphylococcusaureus* Clonal Types in the Czech Republic." *Journal of Clinical Microbiology* 37 (9): 2798–2803.
34. Morsy, Mervat, Abbas Ahmed, Ahmed Mohamed, Ahmed El-bondkly Abeer, Ali Keera, and Amal Mohamed Ali. 2017. "Incidence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Microbial Community of Cancer Patients and Evaluation of Their Resistant Pattern." *Arab J Sci Eng*, 4–5. doi:10.1007/s13369-017-2670-4.
35. Murakami, K, W Minamide, K Wada, E Nakamura, H Teraoka, and S Watanabe. 1991. "Identification of Methicillin-Resistant Strains of Staphylococci by Polymerase Chain Reaction." *Journal of Clinical Microbiology* 29 (10). United States: 2240–44.
36. Murthy, Madhukiran H., Michael E. Olson, Robert W. Wickert, Paul D. Fey, and Ziba Jalali. 2008. "Daptomycin Non-Susceptible Meticillin-Resistant *Staphylococcus aureus* USA 300 Isolate." *Journal of Medical Microbiology* 57 (8): 1036–38.
37. Odonkor, Stephen T., Mercy J. Newman, and Kennedy K. Addo. 2012. "Prevalence and Antibiotic Susceptibility Profile of Methicillin Resistant *Staphylococcus aureus* in Accra, Ghana." *Microbiology Research* 3 (2): 20. doi:10.4081/mr.2012.e20.
38. Pantosti, A., and M. Venditti. 2009. "What Is MRSA?" *European Respiratory Journal* 34 (5): 1190–96. doi:10.1183/09031936.00007709.
39. Paterson, Gavin K, Ewan M Harrison, and Mark A Holmes. 2014. "The Emergence of mecC Methicillin-Resistant *Staphylococcusaureus*." *Trends in Microbiology* 22 (1). England: 42–47. doi:10.1016/j.tim.2013.11.003.
40. Perry, John D, Amie Davies, Lynne A Butterworth, Andrew L J Hopley, Audrey Nicholson, F Kate Gould, and Perry E T Al. 2004. "Development and Evaluation of a Chromogenic Agar Medium for Methicillin-Resistant *Staphylococcus aureus*." *Journal of Clinical Microbiology,* 42 (10): 4519–23. doi:10.1128/JCM.42.10.4519.
41. Sachithanandam, Sangeetha. 2014. "Rising Methicillin-Resistant *Staphylococcus aureus* Infections in Ear, Nose, and Throat Diseases." *Case Reports in Otolaryngology* 2014 (July 2013). Hindawi Publishing Corporation: 1–3. doi:10.1155/2014/253945.
42. See, Isaac; Fernanda C. Lessa; Omar Abo ElAta; Soad Hafez; Karim Samy; Amani El-Kholy; Mervat Gaber El Anani; Ghada Ismail; Amr Kandeel;, and Talaat Katherine Ellingson and

Maha. 2013. "Incidence and Pathogen Distribution of Healthcare-Associated Infections in Pilot Hospitals in Egypt." *Infect Control Hosp Epidemiol. 2013* 34 (12): 1281–88. doi:10.1086/673985.Incidence.

43. Shopsin, Bo, and Barry N. Kreiswirth. 2001. "Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus*." *Emerging Infectious Diseases* 7 (2): 323–26. doi:10.3201/eid0702.700323.
44. Shore, Anna C, and David C Coleman. 2013. "Staphylococcal Cassette Chromosome Mec: Recent Advances and New Insights." *International Journal of Medical Microbiology : IJMM* 303 (6–7). Germany: 350–59. doi:10.1016/j.ijmm.2013.02.002.
45. Stevens, Dennis L. 2006. "The Role of Vancomycin in the Treatment Paradigm." *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America* 42 Suppl 1 (Suppl 1): S51–57. doi:10.1086/491714.
46. Tenover, F C, and R C Moellering Jr. 2007. "The Rationale for Revising the Clinical and Laboratory Standards Institute Vancomycin Minimal Inhibitory Concentration Interpretive Criteria for *Staphylococcusaureus*." *Clin.Infect.Dis.* 44 (1537–6591 (Electronic)): 1208–15. doi:10.1086/513203.
47. Wood, Sarah M, Samir S Shah, Margaret Bafana, Adam J Ratner, Peter A Meaney, Kolaatamo C S Malefho, and Andrew P Steenhoff. 2009. "Epidemiology of Methicillin-Resistant *Staphylococcus aureus* Bacteremia in Gaborone, Botswana." *Infection Control and Hospital Epidemiology : The Official Journal of the Society of Hospital Epidemiologists of America*. doi:10.1086/599003.

5/10/2018