December 2013, Vol. 7, No. 12, pp. 1255-1259
Journal of Life Sciences, ISSN 1934-7391, USA

High Prevalence of Multidrug-Resistance MRSA and VRSA of Different Infections from Al-Jumhuory Teaching Hospital Patients in Mosul

Ibrahim H. Yaseen[1], Adeeba Y. Shareef[2] and Awwad Sh. Daoud[3]

1. Department of Biology, University of Soran, Soran 44008, Iraq

2. Department of Biology, University of Mosul, Mosul 41001, Iraq

3. Department of Biology, University of Tikrit, Tikrit 34001, Iraq

Received: July 27, 2013 / Accepted: October 10, 2013 / Published: December 30, 2013.

Abstract: *Staphylococcus aureus* is major human pathogen causing large variety of infections worldwide. This study carried out to isolate *S. aureus* from different clinical cases, also detection of MRSA prevalence and VRSA emergence, in addition to shedding light on strains that have to be multidrug resistance against various antibiotics. The clinical samples were collected from Al-Jumhuory Teaching Hospital patients in Mosul, isolates identification were achieved by conventional procedures including biochemical and physiological tests, and the specific latex agglutination test. The sensitivity pattern achieved by using disk diffusion technique, for MRSA and VRSA detection oxacillin-disk (1 μg) and vancomycin-disk (30 μg) were used respectively. Results revealed, among 17 *S. aureus* isolates, 7 (41%) were mostly isolated from patients with wound and burn infections. Isolates had high resistance rate against ampicillin (100%) and cefotaxime (81%), and lower resistance rate against several antibiotics. MRSA was 88% of total isolates, 93.3% of MRSA were multidrug resistance to 3-9 of antibiotics. Six isolates (40%) of MRSA were VRSA. It is concluded that antibiotics other than vancomycin can be used as anti-MRSA agents after a sensitivity test to prevent the prevalence of VRSA, the major cause of this chemotherapy problems maybe irrational and indiscriminate use of broad-spectrum antibiotics.

Key words: Multidrug-resistance, MRSA, VRSA, Mosul.

1. Introduction

Staphylococcus aureus has long been recognized as a major pathogen of hospital acquired infections. Over the last decade, MRSA (methicillin-resistant *Staphylococcus aureus*) strains have become endemic in hospitals worldwide. Moreover, it is now incipient community pathogen in many geographical regions [1].

MRSA accounts for a high proportion of surgical site infections, being responsible for 64% of such infections in 2007/2008 [2]. Fewer than 5% of *S. aureus* isolates are sensitive to penicillin, once the drug of choice for Staphylococcal infections [1].

MRSA was first reported in the UK just two years after the introduction of methicillin in 1959 [3]. Horizontal transfer of the *mec*A gene, which encodes a penicillin-binding protein, results in resistance not only to methicillin, but also to broad spectrum β-lactams such as the third-generation cephalosporins, cefamycins and carbapenems [4].

The proportion of MRSA isolates from blood cultures taken from cases of bacteremia in England has risen dramatically from less than 5% in 1990 to around 40% by the end of the 1990 [2]. As well as mortality rates of almost double those associated with MSSA (methicillin-sensitive *S. aureus*) infections, MRSA has a considerable financial burden on both hospitals and society in general [5].

Corresponding author: Ibrahim H. Yaseen, M.Sc., assist. lecturer, research fields: microbiology and zoology. E-mail: ibrahim86_biology@yahoo.com.

Vancomycin has been the most reliable therapeutic agent against MRSA for the past 3 decades. However, despite its sustained *in vitro* microbiologic inhibitory activity, clinicians continue to debate its utility for MRSA infections [6]. Widespread empirical use of vancomycin to cover Gram-positive organisms, including MRSA, has likely contributed to the growing burden of less susceptible strains or completely resistance as VRSA (vancomycin-resistant *S. aureus*), and many health care facilities have reported an upward trend of vancomycin MICs for MRSA isolates over the past 5 years [7-9].

2. Materials and Methods

2.1 Isolation of Staphylococci from Clinical Specimens

Seventeen *S. aureus* isolates evaluated in this study. These isolates were obtained from different clinical specimens, including samples of urine, swabs from wound and burn infections, ear infections, diabetic foot ulcers, osteomyelitis infections and eye infections. Samples were collected during the period October 2009 till February 2010, from hospitalized and out patients of Al-Jumhuory Teaching Hospital in Mosul, Iraq. Specimens were cultured on Blood and MacConkey agar. Morphological and cultural characteristics identifications were done according to Forbes et al. [10] and Win et al. [11].

2.2 Biochemical Identification of Staphylococcal Isolates

Biochemical and physiological tests that related to *S. aureus* identification, particularly coagulase tube and slide tests; catalase and oxidase tests also the susceptibility to NV (novobiocin) and PB (polymyxin B), were done according to Goldman and Lorrence [12], Forbes et al. [10] and Win et al. [11].

2.3 Growth on Mannitol Salt Agar

All staphylococcal isolates were inoculated on mannitol salt agar (Hi-Media, India) and plates were incubated at 37 °C for 24-48 h. Mannitol fermentation was observed and recorded.

2.4 Latex Agglutination Test

Rapid latex agglutination slide test was performed on all clinical isolates according to the protocols supplied by the manufacturer (MASTASTAPH™, Mast Group, UK). This test based on the test principle described by Essers and Radebold [13], the presence of bound coagulase (clumping factor) and protein A detected using human plasma coated latex particles. Latex reagent contains particles coated with fibrinogen and IgG. Mixed on a slide with *S. aureus* organisms. Cell bound coagulase reacted with fibrinogen and/or protein A reacted with IgG caused rapid agglutination of the latex particles.

2.5 Antimicrobial Susceptibility Testing and MRSA, VRSA Detection

Modified Kirby-Bauer method was used for antibiotic susceptibility test according to Clinical and Laboratory Standards Institute guidelines [14, 15]. Three to five well-isolated colonies of the tested bacteria were selected from an agar plate culture, suspended in 5 mL of N.B. The turbidity of the broth culture was adjusted to 0.5 McFarland standard, which approximately equals ($1\text{-}2 \times 10^8$) CFU/mL. 0.1 mL of the broth culture was added on the surface of the Muller-Hinton agar medium and antibiotic discs that provided by Bioanalyse®-Turkey were applied on the plate after 10 min, including (disk potency, μg/disc) ampicillin (10), clindamycin (2), trimethoprim (5), gentamicin (10), ciprofloxacin (5), levofloxacin (5), erythromycin (15), ceftriaxone (30), cefotaxime (30). Inoculated plates were incubated at 37 °C for 18-24 h. Inhibition zones around antibiotic discs were measured using sliding caliper. In the same way, oxacillin-disk (1 μg) was added to plate for MRSA detection and vancomycin-disk (30 μg) for VRSA detection.

3. Results and Discussion

Staphylococcus aureus isolates were mostly isolated from patients with wound and burn infections (41%), but their prevalence were fewer in patients

with other cases (6%-17%). Table 1 elucidates the number and percentage of isolates for each case.

Results of infection-site cultures in this study reflect the high range of burn and wound infections that were enrolled. The predominance was *S. aureus*, followed by Gram-negative species and coagulase negative *S. aureus*, is similar to the findings in previous bacteriologic studies of such infections [16, 17].

Antibiotic susceptibility results of *S. aureus* showed absolute resistance (100%) against ampicillin, and high resistance rate against cefotaxime (81%), while resistance to ceftriaxone, erythromycin, ciprofloxacin, trimethoprim, gentamicin, levofloxacin and clindamycin were at a rate of 59%, 59%, 41%, 41%, 35%, 23% and 18%, respectively. Whereas MRSA strains as noticeable results exhibited high rate (88%) among *S. aureus*. Table 2 elucidates antibiotic susceptibility among MRSA and MSSA.

The findings of this study are consistent with the worldwide emergence of community associated MRSA [18] and the increasing incidence of community-associated MRSA infections in other regions of Iraq [16], and in across the other regions of the world like United States [19], Europe [20] and India [21].

All isolates demonstrated uniform resistance to oxacillin (a surrogate for methicillin), signifying lack of susceptibility to most of β-lactam antibiotics, including cephalosporins.

However, some were resistant to a number of other non-β-lactam antibiotics, including 46.7% resistant to gentamicn and trimethoprim, 40% resistant to levofloxacin and 60% resistant to ciprofloxacin. The gentamicin susceptibility among our MRSA isolates is similar to the findings of Tiwari et al. [22].

Direct comparison of our findings with other MRSA studies is difficult because we prospectively enrolled an unselected group of patients with different infections, and we report the proportion of MRSA among all infection site cultures. In contrast, previous studies of MRSA have been mostly focused on cultures taken during an outbreak or from a known high-prevalence group. These studies usually report the proportion of MRSA among *S. aureus* isolates only.

MRSA strains revealed a high rate (88%) as indicated in Fig. 1. 93.3% of MRSA were multidrug resistance to 3-9 of antibiotics that used against *S. aureus* strains including β-lactms, aminoglycosides, fluoroquinolones, glycopeptides and trimethoprim. In another study, 72.1% of MRSA were multidrug - resistant MRSA and that by resistant to three or more of selected antibiotics [22].

Whereas VRSA among MRSA showed significant and remarkable rate (40%) included six isolates, this rate considered high resistance to vancomycin (30 μg) and in a high rate according to other studies which no single strain of *S. aureus* was found to be resistant to vancomycin in Sulaimani Burn Hospital in Al-Sulaymaniah, Iraq [16], and in the other regions of the world observed emergence of high-level vancomycin resistant *S. aureus* in Tehran, which 41.85% of *S. aureus* strains were resistant to methicillin, two strains of MRSA were VRSA strains [23].

Recently most notable results of VRSA that recorded by Chakraborty et al. and Tiwari et al. [24, 25] which included eight pathogenic VRSA strains were isolated from post operative pus sample in India and that similar to our results.

These resistance results from inappropriate prescriptions due to lack of standard treatment guidelines, WHO reported that 50% of prescriptions are inappropriate [26]. In developing countries, an

Table 1 Percentages of *S. aureus* isolates from different clinical cases.

Case	UTIs	BWIs	Ear I	DFUIs	Osteo.	Eye I	Total
No. (%)	2 (12%)	7 (41%)	3 (17%)	2 (12%)	2 (12%)	1 (6%)	17 (100%)

UTIs: Urinary tract infections; BWIs: Burn and wound infections; Ear I: Ear infections; DFUIs: Diabetic foot ulcer infections; Osteo.: Osteomyelitis; Eye I: Eye infections.

Table 2 Antibiotic susceptibility (%) among MRSA and MSSA isolates.

Antibiotic	MSSA* ($N = 2$)	MRSA* ($N = 15$)
Oxacillin	0	100
Ampicillin	100	100
Ceftriaxone	50	80
Cefotaxime	100	93.4
Erythromycin	100	80
Gentamicin	50	46.7
Levofloxacin	50	40
Ciprofloxacin	50	60
Trimethoprim	0	46.7

* Percentage of resistance; MSSA: methicillin-sensitive *S. aureus*; MRSA: methicillin-resistant *S. aureus*.

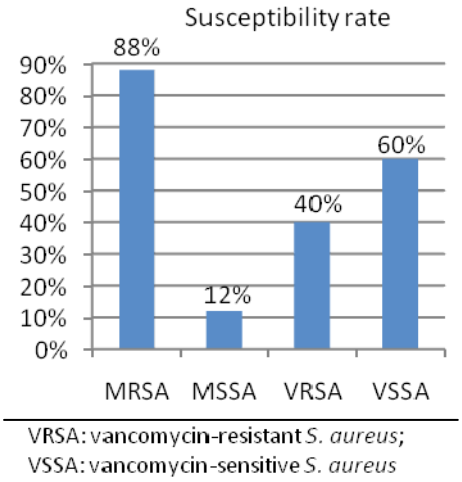

Fig. 1 Percentages of MRSA among *S. aureus* and VRSA among MRSA isolates.

estimated 50% of those who need antimicrobials cannot access due to cost. These drugs are not taken by the patient as recommended or may be self-administered when not required [27]. While data are not available from developed countries, industrialized economies, of the 80%-90% of antibiotics consumption in the community, almost half was due to incorrect indications, and often for viral infections [28].

4. Conclusion

As conclusion, there is high prevalence of MRSA and significant emergence of VRSA in Mosul, and that result from overuse of antibiotics and the selection of broad, rather than narrow spectrum agents, have contributed to the high prevalence of MRSA and VRSA in different clinical cases, and many of these isolates became multidrug resistant. Empiric coverage against MRSA should be considered in institutions with a high MRSA infection, or in patients who are at increased risk for MRSA infection, exposure to previous courses of antibiotics, recent hospitalization or nursing home stay, or by close contact with MRSA infected patients. Therefore, the knowledge of prevalence of MRSA and VRSA and their current antimicrobial profile becomes necessary in the selection of appropriate empirical treatment for different pathogenic cases.

Acknowledgment

Special thanks to staff of Microbiology Lab in Al-Jumhuory Teaching Hospital in Mosul, for valuable assistance and support.

References

[1] F.D. Lowy, *Staphylococcus aureus* infections, New England Journal of Medicine 339 (1998) 520-532.

[2] Health Protection Agency, Surveillance of Health Care Associated Infections Report, http://www.hpa.org.uk/Publications/InfectiousDiseases/AntimicrobialAndHealthcareAssociatedInfections/0807HCAIAnnualReport2008 (accessed July 1, 2008).

[3] D.M. Elston, Community-acquired methicillin-resistant *Staphylococcus aureus*, Journal of the American Academy of Dermatology 56 (1) (2007) 1-16.

[4] T.J. Foster, The *Staphylococcus aureus* "superbug", Journal of Clinical Investigation 114 (12) (2004) 1693-1696.

[5] I.M. Gould, Costs of hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) and its control, International Journal of Antimicrobial Agents 28 (5) (2006) 379-384.

[6] G. Sakoulas, R.C. Moellering Jr., G.M. Eliopoulos, Adaptation of methicillin-resistant *Staphylococcus aureus* in the face of vancomycin therapy, Clinical Infectious Diseases 42 (Suppl. 1) (2006) S40-S50.

[7] H.A. Kirst, D.G. Thompson, T.I. Nicas, Historical yearly usage of vancomycin, Antimicrobial Agents and Chemotherapy 42 (1998) 1303-1304.

[8] G. Steinkraus, R. White, L. Friedrich, Vancomycin MIC creep in non-vancomycin-intermediate *Staphylococcus*

aureus (VISA), vancomycin susceptible clinical methicillin-resistant *S. aureus* (MRSA) blood isolates from 2001-05, Journal of Antimicrobial Chemotherapy 60 (2007) 788-794.

[9] G. Wang, J.F. Hindler, K.W. Ward, D.A. Bruckner, Increased vancomycin MICs for *Staphylococcus aureus* clinical isolates from a university hospital during a 5-year period, Journal of Clinical Microbiology 44 (2006) 3883-3886.

[10] B.A. Forbes, D.F. Saham, A.E. Weissfeld, Bailey & Scott's Diagnostic Microbiology, 12th ed., Mosby Elsevier, USA, 2007.

[11] W.C. Winn, S.D. Allen, W.M. Janda, E.W. Koneman, P.C. Schreckenberger, G.W. Procop, et al., Koneman's Color Atlas and Text Book of Diagnostic Microbiology, 6th ed., Lippincott Williams & Wilkins. Philadelphia, PA, USA, 2006.

[12] E. Goldman, L. Green, Practical Handbook of Microbiology, 2nd ed., Taylor & Francis Group, LLC, USA, 2009.

[13] L. Essers, K. Radebold, Rapid and reliable identification of *Staphylococcus aureus* by a latex agglutination test, Journal of Clinical Microbiology 12 (5) (1980) 641-643.

[14] Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Susceptibility Testing, 17th Information Supplement, CLSI/NCCLS Document M100-S17, Wayne, PA, USA, 2007.

[15] Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Susceptibility Testing, 19th Information Supplement, CLSI/NCCLS Document M100-S19, Wayne, PA, USA, 2009.

[16] A.R. Qader, J.A. Muhamad, Nosocomial infection in Sulaimani Burn Hospital, Iraq, Annals of Burns and Fire Disaster 23 (4) (2010) 177-181.

[17] H. Kaur, J. Bhat, A.R. Anvikar, S. Rao, V. Gadge, Bacterial profile of blood and burn wound infections in burn patients, in: International Symposium on Tribal Health, New Delhi, India, February 27th-March 1st, 2009.

[18] D. Ala'Aldeen, A non-multiresistant community methicillin-resistant *Staphylococcus aureus* exposes its genome, Lancet 359 (2002) 1791-1792.

[19] J.E. Fergie, K. Purcell, Community-acquired methicillin-resistant *Staphylococcus aureus* infections in south Texas children, The Pediatric Infectious Disease Journal 20 (2001) 860-863.

[20] E.W. Tiemersma, S.L. Bronzwaer, O. Lyytikäinen, J.E. Degener, P. Schrijnemakers, N. Bruinsma, et al., Methicillin-resistant *Staphylococcus aureus* in Europe, 1999-2002, Emerging Infectious Diseases 10 (9) (2004) 1627-1634.

[21] J.A. Dar, M.A. Thoker, J.A. Khan, A. Ali, M.A. Khan, M. Rizwan, et al., Molecular epidemiology of clinical and carrier strains of methicillin resistant *Staphylococcus aureus* (MRSA) in the hospital settings of north India, Annals of Clinical Microbiology and Antimicrobials, Published online: Sep. 14, 2006. DOI: 10.1186/1476-0711-5-22.

[22] H.K. Tiwari, D. Sapkota, M.R. Sen, High prevalence of multidrug-resistant MRSA in a tertiary care hospital of northern India, Infection and Drug Resistance 1 (2008) 57-61.

[23] M. Aligholi, M. Emaneini, F. Jabala-Meli, S. Shahsavan, H. Dabiri, H. Sedaght, Emergence of high-level vancomycin-resistant *Staphylococcus aureus* in the Imam Khomeini Hospital in Tehran, Medical Principals and Practice 17 (2008) 432-434.

[24] S.P. Chakraborty, S.K. Mahapatra, M. Bal, S. Roy, Isolation and identification of vancomycin-resistant *Staphylococcus aureus* from post operative pus sample, Al Ameen Journal of Medical Science 4 (2) (2011) 152-168.

[25] H.K. Tiwari, M.R. Sen, Emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA) from a tertiary care hospital from northern part of India, BMC Infectious Diseases, Published online: Oct. 26, 2006. DOI: 10.1186/1471-2334-6-156.

[26] World Health Organization, Antimicrobial Resistance, http://www.who.int/mediacentre/factsheets/fs338/en/index.html (accessed August 21, 2010).

[27] World Health Organization, Medicines: Rational Use of Medicines, http://www.who.Int/mediacentre/factsheets/fs338/en/index.html (accessed June 7, 2010).

[28] G. Cornaglia, A. Lönnroth, M. Struelens, Report from the European conference on the role of research in combating antibiotic resistance, 2003, Clinical Microbiology and Infection 10 (2004) 473-497.