

STUDY OF METHICILLIN RESISTANCE IN STAPHYLOCOCCUS AUREUS AND SPECIES OF COAGULASE NEGATIVE STAPHYLOCOCCI ISOLATED FROM VARIOUS CLINICAL SPECIMENS

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ABSTRACT

Objective: To identify the Methicillin Resistant Coagulase Negative Staphylococci (MRCONS) and Methicillin Resistant *Staphylococcus aureus* (MRSA) from clinical specimens and their antimicrobial susceptibility patterns.

Methodology: All strains were recovered from clinical specimens according to 0.5 McFarland standard inoculated on Muller Hinton agar. Antibacterial susceptibility was tested by disks diffusion method.

Results: Of 346 Staphylococci, 250 (72.25%) were identified as methicillin resistance. From these strains, ninety three (37.2%) were MRSA and 157 (62.8%) identified as MRCONS. The highest rate of MRCONS identified in *S. haemolyticus* and the lowest rate of MRCONS belonged to *S. intermedius* and *S. schleiferi*. The susceptibility pattern of MRSA and MRCONS against other antimicrobial agents revealed that the lowest resistance was for vancomycin.

Conclusions: The rate of MRCONS was more than MRSA and vancomycin was the most effective antibiotic against those organisms. As such, this antibiotic is the drug of choice for treatment in such cases.

KEY WORDS: Methicillin Resistance *Staphylococcus aureus*, Methicillin Resistant Coagulase Negative Staphylococci, Vancomycin.

Pak J Med Sci October - December 2008 (Part-I) Vol. 24 No. 5 719-724

How to cite this article:

Mehdinejad M, Sheikh AF, Jolodar A. Study of methicillin resistance in staphylococcus aureus and species of coagulase negative staphylococci isolated from various clinical specimens. Pak J Med Sci 2008;24(5):719-24.

INTRODUCTION

Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Resistant Coagulase Negative Staphylococci (MRCONS) are

prevalent worldwide and are an important cause of nosocomial infection, resulting in increased morbidity, mortality, length of hospital stay, and health care costs.¹⁻³ As many as 60-70% of all clinical Coagulase Negative Staphylococci (CONS) and proximally 25% of *S. aureus* isolated from nosocomial infections are resistant to methicillin.⁴ As of early 2005, the number of deaths in the United Kingdom attributed to MRSA has been reported from various sources.⁵ The MRSA prevalence in Saudi Arabia has been reported as 26% among healthy adults.⁶ Its first epidemic was detected during outbreak in the Bellvitge hospital in

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- * Received for Publication: July 17, 2007
- * Revision Received: March 17, 2008
- * 2nd Revision Received: July 8, 2008
- * Final Revision Accepted: July 12, 2008

Barcelona, Spain.⁷ CDC (Centers for Disease Control and prevention) has reported that health-care providers should be aware that MRSA can cause skin infections among healthy newborns.⁸ In the US hospitals, the prevalence of MRSA has increased from 2.4% in 1975 to 29% in 1991.⁹ Hospital connection was the single most important risk factor for the acquisition of MRSA.¹⁰

Some investigators have reported that 20-62% of patients colonized with the MRSA organism were detected within 48-72 hours after admission indicating a possible carrier state.¹¹ It has also been reported that MRSA strains and MRCONS, share the same habitats and permanently or transiently colonize the anterior nares and further regions of skin and mucous membranes which may act as sources of subsequent bacteremia and other infections.¹²

Mechanism of methicillin resistance in *S. aureus* and CONS is due to production of an additional nonnative penicillin binding protein (PBP2a) which is encoded by the *mecA* gene.¹³ MRSA and MRCONS are cross resistance to other β lactam antibiotics such as cephalosporins. These organisms are often resistant to other antibacterial agents such as: macrolides, lincosamides, aminoglycosides, fluroquinolones except vancomycin.^{14,15} In this study, MRSA and MRCONS species from clinical specimens were identified and their susceptibility patterns against some antimicrobial agents tested.

METHODOLOGY

From August 2004 to December 2006 a total of 346 Staphylococci were recovered from various clinical sources (blood, pus, urine, wounds and thraco tube) of patients who were hospitalized and admitted at several wards to the Golestan Hospital in Ahwaz, Iran. All samples were cultured on blood agar and MacConkey's agar plate (Himedia, India & Merck, Germany). These plates were incubated at 37°C for 24 hours; the isolates *S. aureus* were identified via colonial morphology on blood agar plates, gram stain characteristics, Catalase test, Coagulase Positive test, Mannitol fermentation, and the

Coagulase Negative Staphylococci were identified for with using other necessary biochemical tests (produce acid from various sugar, susceptible to novobiocin, resistant to polymyxin B, and bacitracin test and etc.) according to standard microbiological procedures as various Staphylococci.^{16,17}

Antibiotic susceptibility test: Antimicrobial susceptibility testing was done on Mueller-Hinton agar (Merck, Germany) using Disk diffusion (Kirby Bauer's) technique, according to NCCLS guidelines.^{18,19} Suspension of each Staphylococcus was made to turbidity of 0.5 McFarland standards, and cultivated on Muller Hinton agar. The potency of methicillin was 5 μ g/disk and the zone of growth inhibition at least lower than 9 mm in diameter.¹⁹

Finally, the following drugs and concentrations (in brackets) were used to determine the antibiogram of the strains: Vancomycin (30 μ g), Cephalexin (30 μ g), Clindamycin (2 μ g), Erythromycin (15 μ g), Tetracycline (30 μ g), Streptomycin (10 μ g) and Penicillin (10 μ g), (Padtan Teb, Iran and Haimedia, India Company). All tests were performed on Muller-Hinton agar (Oxoid Co., Hampshire, UK) and were interpreted after incubation for 24 hours at 37°C. No supplemental NaCl was added to the agar medium. The zone diameters measured around each disk were interpreted on the basis of guidelines published by the NCCLS.¹⁸

RESULTS

Based on identification method, a total of 346 Staphylococci including 123 (35.54%) *S. aureus* and 223 (64.46%) as sixteen CONS species were identified. Table-I, represents the various strains MRCONS according to clinical specimens identified. The present study showed *S. haemolyticus* as the most prevalent MRCONS strains isolated from clinical specimens, while we could not find any *S. intermedius* or *S. schleiferi* strain to be resistant to methicillin.

The majority of MRSA (35.48%) belonged to specimens isolated from tracheo tube, whereas the majority of MRCONS (57.96%) was isolated from urine samples Table-II. The highest

Table-I: Frequency of 16 species of MRCONS isolated from clinical specimens.

MRCONS	Thrac tube (%)	Pus (%)	Blood (%)	Urine (%)	Wound (%)	Total (%)
<i>S.haemolyticus</i>	4 (16.0)	6 (24.0)	4 (16.0)	10 (40.0)	1 (4.0)	25 (15.9)
<i>S.saprophyticus</i>	0 (0.0)	2 (9.5)	2 (9.5)	17 (81.0)	0 (0.0)	21 (13.4)
<i>S.epidermidis</i>	1 (5.0)	2 (10.0)	0 (0.0)	15 (75.0)	2 (10.0)	20 (12.7)
<i>S.cohnii .spp</i>	3 (15.8)	1 (5.3)	1 (5.3)	14 (73.6)	0 (0.0)	19 (12.1)
<i>S.capitis.spp</i>	0 (0.0)	2 (15.4)	3 (23.1)	8 (61.5)	0 (0.0)	13 (8.3)
<i>S.xylosus</i>	2 (15.4)	0 (0.0)	2 (15.4)	9 (69.2)	0 (0.0)	13 (8.3)
<i>S.simulans</i>	5 (41.6)	2 (16.7)	0 (0.0)	2 (16.7)	3 (25.0)	12 (7.6)
<i>S.lugdunensis</i>	0 (0.0)	0 (0.0)	3 (27.3)	8 (73.7)	0 (0.0)	11 (7.0)
<i>S.hominis</i>	1 (9.0)	4 (36.4)	4 (36.4)	2 (18.2)	0 (0.0)	11 (7.0)
<i>S.arlettae</i>	0 (0.0)	0 (0.0)	0 (0.0)	4 (100)	0 (0.0)	4 (2.5)
<i>S.warneri</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)	2 (1.3)
<i>S.sciuri</i>	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	2 (1.3)
<i>S.caprae</i>	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	2 (1.3)
<i>S.auricularis</i>	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	2 (1.3)
<i>S.schleiferi.spp</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>S.intermedius</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	16 (10.09)	20 (12.73)	22 (14.01)	91 (57.96)	8 (5.11)	157 (100)

MRCONS: Methicillin Resistant Coagulase Negative Staphylococci

frequency of MRSA (39%) and MRCONS (29%) were seen among patients with age of 21-40 years. The prevalence of MRSA and MRCONS among male patients was 55.91% and 56.68%, respectively. The majority of MRSA were seen in neurosurgery (30.10%) and outpatient (23.65%) wards. However, the highest rates of MRCONS were isolated from outpatient (24.84%), pediatric (15.92%) and neurosurgery (14.01%) wards. Moreover, we determined susceptibility of MRCONS and MRSA to other antibacterial agents. The highest resistance among MRCONS and MRSA were identified against penicillin the lowest resistance of MRSA (7.5%) and MRCONS (21.6%) were determined against vancomycin Table-III.

DISCUSSION

Based on antibiotic disk susceptibility pattern against methicillin on 346 Staphylococci including *S. aureus* and CONS (Distributed among 16 species), 250 (72.25%) were identified as methicillin resistance staphylococci (MRS). This included 37.2% *S. aureus* (MRSA) and 62.8% CONS (MRCONS). The percentage of MRSA and MRCONS reported in different countries in France (MRSA 31.5; MRCONS

71.0), Germany (MRSA 17.2; MRCONS 67.4), Greece (MRSA 36.6; MRCONS 83.3), Italy (MRSA 38.8; MRCONS 82.4) and Turkey (MRSA 30.9; MRCONS 74.4).²⁰ This study revealed that the CONS are more resistance to methicillin than *S. aureus*. Our data related to MRSA percentages are in accordance with data from Greece and Italy. However, data which belongs to MRCONS are lower than from other countries. Isolation of MRCONS (70 to 80%) was reported by another investigator in central Europe which is entirely compatible with our data.²¹ The frequency rate of methicillin resistance among staphylococci in Sweden was 42.5%.²⁰ Nevertheless, this rate was low in comparison with our study. Our data is in agreement with the Japoni *et al.*, statement that acknowledged: "If the strategy for antibiotic usage is not changed, we should expect more than 60% of staphylococci isolated from patients be methicillin-resistant in the near future".²²

In our study, the highest rate of MRSA and MRCONS were isolated from the neurosurgery, outpatients and pediatric wards. We suggested that the administrators must consider this issue and necessary measures relating to these wards should be taken.

Table-II: Frequency of MRSA and MRCONS based on various clinical specimens.

Clinical Specimens	MRSA No (%)	MRCONS No (%)
Tracheo tube	33 (35.48)	16 (10.19)
Pus	18 (19.35)	20 (12.73)
Blood	17 (18.29)	22 (14.01)
Urine	14 (15.05)	91 (57.96)
Wounds	11 (11.83)	8 (5.11)
Total	93 (100)	157 (100)

MRSA: Methicillin Resistant *Staphylococcus aureus*
 MRCONS: Methicillin Resistant Coagulase Negative Staphylococci.

The majority of MRCONS were *S. haemolyticus*, *S. saprophyticus*, *S. epidermidis*, and followed by *S. cohnii.spp*, *S. capitis.spp*, *S. lugdunensis* and *S. hominis* and the minority of them were *S. intermedius* and *S. schleiferi.spp*. However, Zilevica *et al.*, reported that the majority of the MRCONS belonged to *S. epidermidis*, *S. saprophyticus*, *S. haemolyticus*, followed by *S. hominis*, *S. warneri*, *S. capitis*.²³ In our data, except *S. capitis*, all MRCONS species had lower percentage than data reported by Caierao *et al* in 2004.²⁴ The majority of MRSA were isolated from tracheo tube, whereas the majority of MRCONS belonged to specimens isolated from urine. Therefore, administration of methicillin antibiotic in patients who had urinary tract infections (UTI) with CONS should be reconsidered.

Since 1996, vancomycin-resistant MRSA strains have also been found.²⁵ However, 0% resistance of MRSA and MRCONS was reported in the studies conducted by Alborzi *et al.*,²⁶ Al-Haodad *et al.*²⁷ and Zilevica.²⁸ Methicillin Resistance of *S. aureus* and CONS strains may have an effect on the resistance of these strains against other antibiotics. As we have shown, the highest resistance rates of MRSA and MRCONS to other antibacterial agents were against penicillin. On the other side, the lowest resistance of MRSA and MRCONS was against vancomycin. In this study, the resistance of MRCONS against vancomycin (21.6%) was more than MRSA (7.5%). Perwaiz *et al* estimated the resistance rate of

Table-III: Antimicrobial resistance pattern against other antibiotics agents of MRSA and MRCONS isolated from clinical specimens.

Antibiotics	Resistance of MRSA No (%)	Resistance of MRCONS No (%)
Vancomycin (Va30)	7 (7.5)	34 (21.6)
Clindamycin (Cc2)	31 (33.3)	96 (61.1)
Cephalexin (Cf30)	48 (51.6)	108 (68.8)
Erythromycin(E15)	58 (62.4)	121 (77.1)
Tetracyclin (Te30)	63 (67.7)	112 (71.3)
Streptomycin (S10)	66 (70.9)	106 (67.5)
Penicillin (P10)	91 (97.8)	144 (91.7)

MRSA: Methicillin Resistant *Staphylococcus aureus*
 MRCONS: Methicillin Resistant Coagulase Negative Staphylococci

MRSA against penicillin, clindamycin, cephalexin, erythromycin over 90% that this percentage except for penicillin, is more than our data. However, our data regarding vancomycin was confirmed by this study.²⁹ Several reports have stated that aminoglycoside resistance is closely related to methicillin-resistance.^{30,31} In our study the resistance rate of MRSA against tetracycline, streptomycin and erythromycin was lower than those reported by Al-Haodad.²⁷ In another study, the resistance rate for these organisms against streptomycin (0%) and penicillin (87.2%) were reported.³²

The early identification of methicillin resistance in Staphylococci, especially from blood isolates, could curtail unnecessary use of vancomycin and allow earlier optimal therapy of infections in few cases related to critically ill patients under the strict clinician supervision. In this study, the rate of MRCONS was lower than other countries and vancomycin was the most effective antibiotic against MRCONS and MRSA.

ACKNOWLEDGMENT

This study was supported by a grant (321) from Jondi Shapour University of Medical Sciences, Ahwaz, Iran. The authors wish to thank Dr. Azar D. Khosravi, Dept. of Microbiology, School of Medicine, Ahwaz Jondi Shapour

University of Medical Sciences, Ahwaz, Iran, for her help in the review.

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