Evaluation of different methods for detecting methicillin resistant Staphylococcus aureus in Assiut University Hospital

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ABSRACT

Background: Methicillin resistance in staphylococcus aureus (MRSA) and in Coagulase – negative staphylococci (MRCNS) is wide spread and continues to increase in prevalence particularly in health care settings. MRSA is among the most important causes of nosocomial infections. It possesses a particular ability to spread in hospitals worldwide.

Objectives: In this study we aimed to measure the frequency of clinical MRSA infection in Assiut University Hospital among patients and health care workers, and to compare different methicillin susceptibility methods with mec A gene detection by PCR as a gold standard test. In one year duration study, 660 patients admitted to different ICUs in Assiut University Hospital were included, 20% (132/660) of them developed nosocomial infection. Staphylococci accounted for 58.3% (77/132) of these cases, and 94 staphylococcal isolates were detected. MRSA represented 64.9% (61/94). Also health care workers (HCW) and the patient's environment were screened for MRSA nasal carriage and MRSA contamination respectively. It was found that 20.25% of HCW were colonized with MRSA and 12.3% of patient's environment was contaminated with MRSA. The MRSA screen latex agglutination test (Denk Seiken Co, Niiga Ta, Japan) and oxacillin resistance screening agar base (ORSAB) medium were compared with MIC determination and mec A gene detection by PCR assay, the "gold standard test". In an analysis of 61 mec A positive and 11 mec A negative S. aureus patient isolates, both latex agglutination test and ORSAB medium were reliable in detection of oxacillin resistance with 100% sensitivity and specificity for each. Antimicrobial susceptibility pattern of nosocomial MRSA isolates showed high resistance to ampicillin (95.1%), erythromycin (93.4%) and penicillin (93.4%), while low resistance to amikacin (37.7%), imipenen (29.5%) and vancomycin (18%). We concluded that MRSA-Screen test and ORSAB medium are reliable methods for detection of MRSA. Screening for colonization of MRSA is a key aspect of infection control to limit the nosocomial spread of this organism.

INTRODUCTION

Nosocomial infections are important public health problem in many developing countries, particularly in the ICU settings (1). The incidence of nosocomial infections in ICUs is 4-5 times greater than in general wards (2).*Staphylococcus aureus* (*S. aureus*) is recognized as one of the most important bacterial pathogens seriously contributing to the problem of hospital infections all over the world (3).

The introduction of penicillin offered an opportunity to successfully treat serious staphylococcal infections. However, in the same year an enzyme produced by *S. aureus*, penicillinase (later known as *B-lactamase*) was described. This enzyme was responsible for the clinical failures that appeared soon after the introduction of penicillin (4). During the early 1950s, a series of semi-synthetic penicillins were developed that were stable to destruction by bacterial *B-lactamases*. Methicillin was one of these compounds and was introduced into clinical practice in 1959. One year after its introduction, the first methicillin resistant *S. auerus* (MRSA) was detected (5).The site of action for *B-Lactam* antibiotics is the penicillin binding proteins (PBPs) which catalyse the transpeptiadase reaction that cross links the peptidoglycan of the bacterial cell wall.

The antibacterial effect of the *B-lactam* antibiotics is a result of the inactivation of the high molecular weight PBPs ($PBP_{1,\ 2and3}$), resulting in a stable complex, which is lethal for cell growth. *S. aureus* strains that express high-level resistance to methicillin produce an additional low affinity penicillin binding protein (PBP2a) encoded by the *mecA* gene (6).

At normally inhibitory concentrations of methicillin, PBP2a retains its transpeptidase activity and takes over the role of the normal PBPs in cell wall synthesis (7) The acquisition of *the mec Agene*, which confers resistance to methicillin spawning so-called methicillin-resistant *S. aureus* (MRSA), has resulted in a highly resilient

pathogen that has reached epidemic levels in many parts of the world (8).

Coagulase – negative staphylococci (CNS), particularly *Staphylococcus epidermidis,* are among the most frequently isolated-bacteria in the clinical microbiology laboratory. They have emerged as important nosocomial pathogens during the last few decades (9.) Methicillin resistance in *S.aureus* (MRSA) and the coagulase-negative Staphylococci (MRCNS) are widespread and continue to increase in prevalence, particularly in the health care settings, probably reflecting adaptation of nosocomial clones to the selective pressure caused by widespread use of antibiotics in hospitals.(10).

Risk factors for acquisition of MRSA include chronic deramtoses, underlying medical illnesses, use of antibiotics, surgery and intravenous lines (11). Also there is an association between intensive care unit (ICU), length of stay (LOS), and MRSA acquisition (12).

As MRSA strains are often resistant to other antibiotic classes, surveillance for this important pathogen is a priority (13). Despite guidelines published by National Committee for Clinical Laboratory Standards for testing of susceptibility to Methicillin for staphylococci, the optimal phenotypic method for detection of Methicillin resistance remains controversial (14). The objective of the present study was to determine which of the following susceptibility testing method best detected Methicillin resistance; agar dilution, disk diffusion, and agar screen. The results of these methods were compared to PCR detection of the mec A gene. Also this study aimed to measure the frequency of MRSA and MRCNS in ICUs patients and to provide the clinicians with antibiogram for the proper choice of treatment of these infections.

SUBJECTS AND METHODS

(I) Subjects:-

This was a descriptive study from January 2005 to January 2006.

A) Patients:-This work was performed on 660 patients admitted to different intensive care units in Assiut University Hospital including: Coronary care unit, chest ICU, neurological ICU, internal medicine ICU, general ICU, trauma ICU, post operative ICU, neurosurgery ICU and neonatal ICU. Patients involved in the study were daily monitored for subsequent development of nosocomial infections (any new sign of infection developed more than 48 hrs after admission to the unit) (15).

Nosocomial infections were diagnosed clinically by the attending physicians. They included: Chest infection, urinary tract infection (UTI), wound infection, bed sore and bacteraemia. Different clinical samples were collected from infected patients.

B) Microbiological methods: All samples were examined in infection control unit. They were cultured on mannitol salt agar.

- **1) Identification of staphylococcal isolates**: According to ***Louie et al.,(2002),*** *staphyloccocal* isolates were identified by standard methods including Gram stain, catalase test and tube coagulase test (16).
- **2) Susceptiblity testing**:

I- ***Methods for detection of oxacillin resistance:***

*** Inocula for susceptibility testing**:

High density inocula were made by diluting five colonies grown overnight on blood agar in 5 ml of distilled water to prepare a suspension equivalent in density to 0.5 McFarland barium sulfate standard unit (average turbidity, 10^{8} CFU ml^{-1}**(**17).

i) Disk diffusion test (DD). The disk test was performed as described by NCCLS, (2003) with a 1-ug oxacillin disk on Muller – Hinton agar. The plates were incubated at 35 ^{o}C, and zone diameter was read at 24h – and 48h. Any growth, including light growth, within the 13 mm diameter zone around the disk was indicative of resistance (14).

ii) Oxacillin Resistance Screening Agar Base(ORSAB) (18).

Isolates which showed positive growth on MSA plates were subcultured on ORSAB medium (Oxoid Limited, Bosing stoke, England), that worked on the same principles as MSA. When mannitol fermentation took place the medium changed to an intense blue.

iii) Determination of Oxacillin MICs by the E-test The MICs of oxacillin were determined by the E-test (AB Biodisk, Solna Sweden) according to the manufacture's recommendation.

V) MRSA – Screen latex agglutination test (LA):

This test was used for detection of PBP2a, and based on agglutination of latex particles sensitized with monocloclonal antibodies against PBP2a in accordance with the manufacturer's protocol (Denk Seiken Co, Niiga Ta, Japan) (17)**.**

II- *Antmicrobial Susceptibility Testing:*

The isolated staphylococci were tested for resistance to some antimicrobial agents using commercial discs (Oxoid-Laboratories,UK) by performing the disc diffusion method (modified Kirby-Bauer) on Muller – Hinton agar **(**19). The antibiotics used were:

Ampicillin 10 ug, Pencillin 10 U, Oxacillin 1 ug, Amoxycillin/ clavulanic acid 20/10 ug, Cefotaxime 30 ug, Cephazoline 30 ug, mipenem 10 ug, Amikacin 30 ug, Gentamycin 10 ug, Clindamycin 10 ug, Tetracycline 30 ug, Ciprofloxacin 10 ug, Erythromycin 15ug, Vancomycin 30ug, Chloramphenicol 30ug (15).

III- *Confirmation of Oxacillin Resistance by PCR for detection of the mec A gene.*

i) Extraction of DNA from Bacterial Colonies:-

Cell extracts were made by suspending a single colony in100 ml of sterile distilled H_2O and boiling for 10 minutes (20).

ii) PCR Amplification:

Ten ul of cell lysate were agitated in 40 ul of PCR mixture.This mixture consisted of 0.2 mM concentration of each deoxynucleside triphosphate(AB gene), 0.5 uM concentration of each primer(operon) ,1 U of taq DNA polymerase(Qiagen), 10^x PCR buffer(Qiagen), and 1.5 mM Mg Cl_2(Qiagen). The following primers for detection of mec A gene were used: (5-3) (mecA-1) AAA ATC GAT GGT AAA GGT TGG C and (mecA-2) AGT TCT GCA GTA CCG GAT TTG C ***(Louie et al., 2002).*** DNA amplification was done in a Gene Amp9600 thermal cycler: initial cycle of 95 °c for 5 min , followed by 30 cycles of 95 °c for 30 second, 50 °c for 30 second, and 72 °c for 30 second, with a final extension step of 72 °c for 10 min (20**).**

iii) Detection of PCR Product by Agarose gel Electrophoresis:-

PCR products were visualized in a 0.7% agarose gel stained with ethidium bromide and electrophoresed in TBE buffer (Tris – borate – EDTA) at 100 v for 30 min. for the presence of a 533bp fragment of mec A gene (18**).** Eight microliters of product was adequate to determine the presence and quality of the PCR products (21).

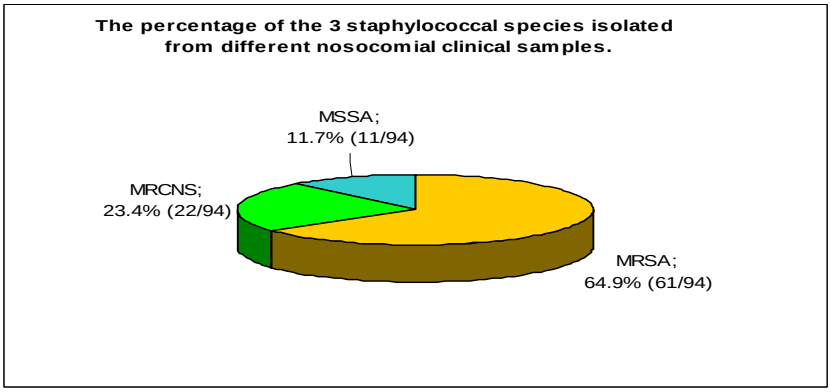
RESULTS

A) Analysis of Nosocomial Infection:

This study included 660 patients admitted to different ICUs in Assiut University Hospital. 20% (132/660) of those patients developed nosocomial infections. Among those patients 77 cases developed NIs by staphylococci with a percentage of 58.3% (77/132). They were 52 males and 25 females with their mean age 50±10 years.

Based on primary isolation of different clinical specimens on mannitol salt agar, 94 staphylococcal isolates were detected and with tube coagulase test 22 CNS isolates and 72 coagulase positive "S.aureus" isolates were identified.

Susceptibility testings to detect oxacillin resistance were performed with oxacillin disc diffusion method, ORSAB medium, oxacillin E-test and the MRSA-screen latex agglutination test. Confirmation of oxacillin resistance was made by PCR for detection the mec A gene. According to the PCR results 61/94 of Staphylococcal isolates were MRSA, 22/94 were MRCNS, and 11/94 were MSSA.



Detection of MRSA :

Based on the results of PCR as the "gold standard" test for detection for MRSA, both latex agglutination and ORSAB medium were reliable phenotypic methods in the detection of oxacillin resistance showing 100% sensitivity and 100% specificity, while the disc diffusion and E-test showed lower sensitivity and specificity (96.7% sensitivity, 81.1% specificity and 98.3% sensitivity, 90.9% specificity respectively) (Table 1)..

Table (1): Sensitivities, specificities, positive and negative predictive values and accuracy for phenotypic methods in comparison with results of PCR for detection of oxacillin susceptibility

Method	PCR for mec A gene				Sensitivity (%)	Specificity (%)	PPV %	NPV %	Accuracy %
	mecA positive (n=61)		mecA negative (n=11)						
	True +ve	False – ve	True -ve	False +ve					
DD	59	2	9	2	96.7	81.8	96.7	81.8	94.4
ORSAB	61	0	11	0	100	100	100	100	100
E.test	60	1	10	1	98.3	90.9	98.3	90.9	97.2
LA	61	0	11	0	100	100	100	100	100

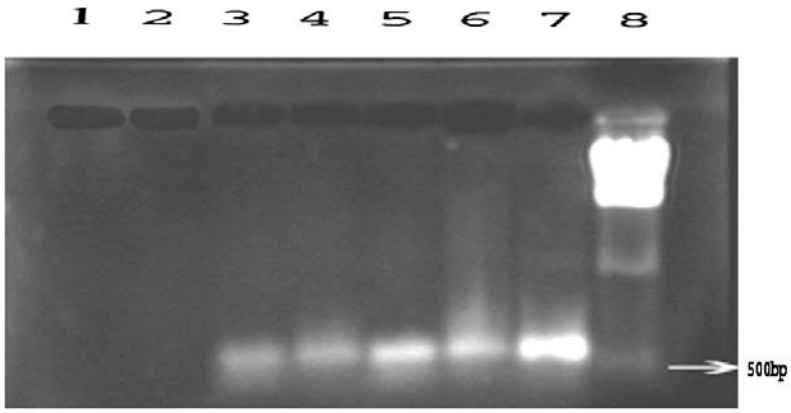


Fig (2):Agarose gel electrophoresis of amplified mec A gene (533 bp). Lanes 3-7 positive MRSA isolates; lane 1 negative control; lane 2 negative isolate and lane 8 100-bp PCR ruler.

C) Analysis of nosocomial staphylococcal isolates :

Table (2) shows the distribution of 94 staphylococcal isolates among different clinical specimens obtained from 77 of nosocomially infected cases. MRSA isolates were most frequently isolated from blood samples representing 52.5% of the total MRSA isolates followed by endotrocheal samples (31.1%) and the least percentage was 1.6% represented by each of rectal swab, urine and bed sore swab samples.

Table (2):- Distribution of 94 staphylococcal isolates among different clinical specimens.

Sample	Organism						Total	
	MRSA		MRCNS		MSSA			
	No.	%	No.	%	No.	%	No.	%
Blood	32	52.5	11	50.0	7	63.6	50	53.2
Endotracheal	19	31.1	4	18.2	3	27.3	26	27.7
Rectal Swab	1	1.6	5	22.7	1	20.0	7	7.4
Urine	1	1.6	1	4.5	0	0.0	2	2.13
Wound Swab	4	6.6	0	0.0	0	0.0	4	4.25
Sputum	3	4.9	0	0.0	0	0.0	3	3.19
Bed Sore Swab	1	1.6	1	4.5	0	0.0	2	2.13
Total	61	100	22	100	11	100	94	100

Distribution of nosocomial staphylococcal isolates among different I.C.Us is shown in table (3). MRSA isolates were most frequently present in trauma ICU of 37.7% (23/61), MRCNS isolates were most frequently present in chest ICU 27.3 %(6/22), whereas the highest percentage of MSSA isolates 36.4% (4/11) were detected in trauma ICU.

Table (3):- Distribution of Nosocomial staphylococcal isolates among different ICUs

Unit	No. Of patients	Organism						Total	
		MRSA		MRCNS		MSSA			
		No.	%	No.	%	No.	%	No.	%
Tropical ICU	60	1	1.6	0	0.0	1	9.1	2	2.1
General ICU	60	6	9.8	3	13.6	1	9.1	10	10.6
Coronary Care Unit	60	10	16.4	5	22.7	3	27.3	18	19.2
Chest ICU	60	10	16.4	6	27.3	1	9.1	17	18.1
Neurology ICU	60	5	8.2	2	9.1	0	0.0	7	7.5
Internal medicine ICU	60	2	3.2	2	9.1	0	0.0	4	4.3
Neonatal ICU	60	0	0.0	0	0.0	1	9.1	1	1.1
Neurosurgery ICU	60	2	3.3	2	9.1	0	0.0	4	4.3
Post Operative ICU	60	2	3.3	0	0.0	0	0.0	2	2.1
Trauma ICU	60	23	37.7	1	4.5	4	36.4	28	29.8
Gynecology and Obstetric department	60	0	0.0	1	4.5	0	0.0	1	1.1
Total	660	61	100	22	100	11	100	94	100

D) Determination of antibiotic susceptibility patterns of staphylococcal isolates:

The overall resistance of nosocomial staphylococcal isolates to antibiotics is shown in table (4).

Antibiotic	MRSA(n=61)		MSSA(n=11)		MRCNS(n=22)	
	NO	%	NO	%	NO	%
Amikacin	23	37.7	6	54.5	12	54.5
Amoxicillin/clavulanic	53	86.9	8	72.7	9	40.9
Ampicillin	58	95.1	10	90.9	17	77.3
Cefotaxime	51	83.6	10	90.9	17	77.3
Cephazoline	42	68.9	4	36.4	14	63.6
Chloramplenicol	42	68.9	7	63.6	15	68.2
Ciprofloxacin	48	78.7	6	54.5	14	63.6
Clindamycin	51	83.6	10	90.9	17	77.3
Erythromycin	57	93.4	10	90.9	16	72.7
Gentamycin	37	60.7	8	72.7	12	54.5
Imipenem	18	29.5	1	9.1	12	54.5
Tetracycline	42	68.9	8	72.7	17	77.3
Oxacillin	59	96.7	2	18.2	22	100.0
Vancomycin	11	18	1	9.1	3	13.6
Penicillin	57	93.4	11	100.0	10	95.5

Apart from oxacillin resistance, it was noticed that the highest resistance of MRSA was to ampicillin (95.1%) and the least resistance was to vancomycin (18%).

E) Assessment of risk factors:

It was found that surgery, previous antibiotic prescription and diabetes were important risk factors for development of MRSA infections.

DISCUSSION

In our study 20% (132/660) of ICUs patients developed nosocomial infections. This result agreed with ***Rosenthal et al., (2003***) and ***Klav et al., (2003)*** who reported that overall nosocomial infection rates were 26.9% and 27% respectively (22,23). However lower rate were reported by ***El-Bialy and El-Sharkawy (2004***) in Zagazig University Hospital with a rate of 8.8% (24) and ***Kandeel, (2000)*** in Mansoura University Hospital with a rate of (13.6%) (25). On the other hand, higher rate was found by ***Yologu et al., (2003***) in the ICUs of a Hospitals in Turkey who reported an over all rate of 33.0% (26). In Alexandria University Hospital ICUs, ***El-Nawawy et al.,(2005*)** reported a high frequency of sepsis (27). Again a higher frequency was reported by ***Singh et al.,(2002***), where the overall incidence of nosocomial infections among 102 ICU patients was 54.9% (2).

In the present study, 94 isolates of staphylococci were detected in 77 cases of staphylococcal nosocomial infections. By tube coagulase test 22 isolates were CNS and 72 were coagulase positive.

In our study analysis of 61 *mec A*-positive *S.aureus* isolates were detected by PCR assay. Both ORSAB medium and Latex agglutination test showed a sensitivity of 100% in detection of MRSA in comparison to PCR which is " the gold standard test " while oxacillin disc diffusion and E-test showed a sensitivity rates 96.7% and 98.3% respectively. Those results more or less agreed with the findings reported by ***Felten et al.,(2002***) who found that the sensitivity rates in detection of 83 clinical isolates of MRSA were 94%, 91.6%, 96.4% and 97.6% for oxacillin agar screen, E-test, oxacillin disc diffusion and MRSA screen, respectively (17).

Also in this work, latex agglutination test showed a sensitivity and specificity of 100% in detection of 11 *mec A* negative *S.aureus* isolates. This coincides with ***Lee et al., (2004***) who reported that in an analysis of 15 *mec A* positive and 11 *mec A* negative *S.aureus* animal isolates, MRSA screen test had a sensitivity and specificity of 100% (28). Also in study by ***Soares et al., (2004),*** MRSA-screen test showed 100% sensitivity and specificity in detection of 61 MRSA strains having the *mec A* gene (29). Moreover our

findings were also supported by those of ***Louie et al., (2000)*** who reported that MSLA showed excellent 98% sensitivity and 99% specificity for the detection of MRSA (30).

Chomvarin et al., (2004), evaluated different phenotypic methods to detect MRSA including oxacillin disk agar diffusion (ODD), oxacillin screening salt agar (OSS) and MRSA screen latex agglutination (MSLA) with the genotypic gold standard PCR *mec A* detection to determine the most appropriate method for routine laboratory use and reported that the sensitivity and specificity of ODD,OSS,MSLA were 96%,100%,97% and 97%,100% and 100% respectively in detecting 106 MRSA isolates (31).

In a study by ***Velasco et al.,(2005)***, both ORSAB medium and PBP2a agglutination for detection of 51 MRSA isolates showed 100% sensitivity when compared to the results of *mec A* gene analysis by PCR (32). ***Dias et al.,(2004)*** reported that among 32 *mec A* gene positive *S.aureus* isolates, ORSAB medium detected 31MRSA isolates (33).

Failure of ODD in detecting 2 *mec A* positive *S.aureus* isolates and the same for E-test that failed to detect one *mec A* positive *S.aureus* isolates may be explained by the statement of ***Marta Knausz et al., (2005)*** who reported that methicillin resistant phenotypes are frequently heterogeneous, causing difficulty in detecting them by the conventional antimicrobial susceptibility methods such as disc diffusion test, measurement of minimal inhibitory concentration by the E-test. Also, they reported that some technical factors, such as the inoculum's size, incubation time and temperature, culture medium, PH and salt concentration, have some effects on the accuracy of these methods, and the detection of *mec A* gene by PCR has been considered as the "gold standard" (34).

In this study we tried to assess the risk factors associated with MRSA infection, they were found to be more among diabetic patients compared to MSSA infections and MRCNS infections which can be explained by the lower immunity and higher vulnerability of diabetic patients to infections. There was a trend towards increased percentage of MRSA infections among patients with previous prescription of antibiotic, previous surgery and with intravenous lines. This is supported by findings of ***Gosbell ,*** (***2004)*** who reported that risk factors for acquisition of MRSA include use of antibiotics, surgery and intravenous lines (11). Also, it was noticed that the longer the duration of hospital stay, the more percentage of MRSA infections. This finding was in agreement with that of ***Marshall et al., (2004)*** who concluded that there is an association between the length of hospital stay in ICU and acquisition on MRSA infection (13). ***Sifuentes and Perez, (2006)*** also stated that, use of IV catheters, long-term hospitalization, surgery and previous use of antimicrobials are considered major risk factors for MRSA infections (35).

Another important finding in our assessment of risk factors for development of MRSA infections was the high percentage of such infection (40%) among patients who were subjected to mechanical ventilation, compared to both MSSA infections and MRCNS infections. This finding coincides with that of ***Pilvinis and Stirbiene, (2003)*** who found that MRSA was among the most etiologic organisms responsible for ventilator associated pneumonia in Europe and North America (36).

In this work, we found that nosocomial MRSA and MSSA were most frequently isolated from blood samples (52.5% and 63.6% respectively). This finding is supported by that of ***Guilarde et al., (2007)*** who reported that among 295 episodes of blood stream infection (BSI), the most common pathogen was *S.aureus* that caused 118 episodes (40%), with 55.9% of MRSA (37).

In another study by ***Lesse and Mylott, (2006),*** 39 episodes of *S.aureus* bacteraemia (SAB) were identified, 15 were due to MSSA and 24 were due to MRSA (38). Also ***Greiner et al., (2007)*** reported that among 49 nosocomially infected patients, 40.8% of them had BSI caused by MRSA (39). In a prospective study by ***Das et al., (2007),*** 147 episodes of SAB were identified. 87 episodes and 60 episodes were due to MRSA and MSSA respectively (40).

In our study, we determined the antibiotic susceptibly patterns of the nosocomially isolated staphylococci by the disk diffusion method. We noticed that MRSA had high overall resistance to penicillin (93.4%), erythromycin (93.4%), clindamycin (83.6%), ampicillin (95.1%), ciprofloxacin (78.7%), tetracycline,(68.9%) cefotaxime (83.1%), gentamycin (60%), and

amoxicillin/clavulanic (86.9%). These results agreed with ***Simor et al., (2001)***, who found that resistance rates of MRSA were: Ciprofloxacin (89%), Clindamycin and erythromycin (94% for each), tetracycline (33%) (18).

In another study done in Korean Hospitals, resistance rates of MRSA isolates were as follows: tetracycline (89.5%), clindamycin (84.3%), erythromycin (97.7%) and gentamycin (95%) with no vancomycin resistance (41).

In Burn Unit study in Mansoura University, resistance rates of MRSA were as follows: gentamycin (58.3%), clindamycin (100%) and erythromycin and ciprofloxacin (33.3% for each), with no evidence of vancomycin resistance (42).

Also ***Leski et al.,(1998)***revealed that MRSA resistance to erythromycin was (48%), tetracycline (40.5%) with no evidence of vancomycin resistance (3). ***Drinka et al., (2004)*** found that all MRSA were usually resistant to quinolones (43).

Differently, we found that MRSA resistance rate to vancomycin was (18%),however this finding was to some extent lower than that of ***Wunderink (2004)*** who reported that vancomycin failed in 40% of MRSA pneumonia (44).

Conclusion

From this study, we concluded that

- Compared to PCR as the gold standard, the MRSA Screen Latex agglutination test was able rapidly and accurately determine the presence of oxacillin resistance mediated by the *mec A* gene.
- Addition of ORSAB plates to the routine protocol, allowed MRSA and CNS identification in 24 hours from sample plating.
- Bacterial resistance to established classes of antibiotics in clinical use is continuing to increase making the need for new agents that can be used for multi-drug resistant organism steadily more urgent.

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تقييم اختبارات مختلفة لإكتشاف مبكروب المكورالعنقودي الذهبي المقاوم للمثيسيللين في مشتشفى أسيوط الجامعى

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إن المكورالعنقودي الذهبي المقاوم للمثيسيللين من أهم مسببات عدوى المستشفيات ويتميز بقدرة خاصة على الإنتشار في المستشفيات على مستوى العالم٠كما يعد المكور العنقودي السلبي لانزيم التجلط سبب رئيسي لعدوى المستشفيات.كما أن النسبة العالية لسوء استخدام المضادات الحيوية يزيد من انتشار مقاومة الميكروب للعقاقير.

تعد أهم ميكانيكية لمقاومة عقار الميثسيللين هى إنتاج بروتين رابط البنسيللين ٢أ والمسئول عنه جين (mecA) الذي يتم الكشف عنه باستخدام تفاعل البلمرة المتسلسل والذي يعد الطريقة الذهبية لتحديد مقاومةهذا الميكروب للمثيسيللين.

ومن الطرق الحديثة لتحديد المكور العنقودي الذهبي المقاوم للميثسيللين، اختبار إى واختبار التلزن والذي يحدد وجود بروتين رابط البنسيللين ٢أ .

و كان **الغرض** من هذه الدراسة تقييم اختبارات ميكروبيولوجية مختلفة لإكتشاف مبكروب المكورالعنقودي الذهبي المقاوم للمثيسيللين في مشتشفى أسيوط الجامعى.

وقد أجريت هذه الدراسة على ٦٦٠ مريض أدخلوا الى وحدات العناية المركزة المختلفة فى مستشفى أسيوط الجامعى. **و خلصت النتائج** الى حدوث ١٣٢ حالة من عدوى المستشفيات ومن بين هذه الحالات وجد أن ٧٧ مريضا نتيجة للإصابة بأحدى أنواع المكور العنقودي، وبإجمالي ٩٤ عزلة تم الحصول عليها من عينات إكلينكية مختلفة.

واستنادا إلى الطرق الظاهرية في التشخيص البكتريولوجي فقد تم عزل ٧٢ من ميكروب المكور العنقودي الذهبي و٢٢ من المكور العنقودي السلبي لانزيم التلزن ٠ ومن بين عزلات المكور العنقودي الذهبي،وجد عدد٦١ ميكروب مقاوم للمثيسيللين وبمقارنة الطرق الظاهرية مع تفاعل البلمرة المتسلسل لتحديد مقاومة المثيسيللين في المكور العنقودي الذهبي وجد توافق كامل لنتائج اختبار التلزن لتحديد بروتين رابط البنسيللين ٢أ ، وبيئة أورساب مع تفاعل البلمرة المتسلسل اضافة إلى ذلك فقد وجد أن جميع عزلات المكور العنقودي السالب لانزيم التلزن مقاومة للمثيسيللين بإستخدام أقراص الاوكاسيللين وبيئة أورساب.

وبإجراء اختبار حساسية المضادات الحيوية لأنواع المكور العنقودي التى تم عزلها ، وجد أنها مقاومة للعديد من أصناف المضادات الحيوية ، بأقل نسبة مقاومة لعقار فانكوميسين.

وخلصت هذه الدراسة إلى أن اختبار التلزن للكشف عن بروتين رابط البنسيللين ٢أ من الطرق السريعة والدقيقة لتحديد مقاومة الميثسيللين بمقارنته بتفاعل البلمرة المتسلسل.أيضا يمكن اضافة بيئة اورساب بطريقة روتينية فى الفحص المعملى كطريقة للتعرف على المكورات العنقودية المقاومة للمثيسيللين فى خلال ٢٤ ساعة.