

In Vitro Studies on Antibiotic Susceptibility Pattern of Methicillin Resistant and Sensitive *Staphylococcus aureus* isolates

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ABSTRACT

Background: Methicillin Resistant *Staphylococcus aureus* (MRSA) is a variant of *Staphylococcus aureus* which is resistant to all β -lactams. Infections due to (MRSA) strains have been detected worldwide and it makes the treatment of common infections much more difficult. **Objectives:** This study was carried out to determine and compare the Antibiotic susceptibility patterns in Methicillin Resistant and Sensitive *Staphylococcus aureus* isolates (MRSA, MSSA). **Material and methods:** 395 clinical samples were collected from National Cancer Institute (NCI), Cairo, Egypt. Identification of all isolates and detection of its susceptibility was determined with Microscan system, standard biochemical tests and disc diffusion method using Kirby-Bauer disk diffusion method and the interpretation of the results was done using Clinical and Laboratory Standards Institute guidelines (CLSI)⁽¹⁾. MRSA was detected using resistant to Cefoxitin and oxacillin. **Results:** Out of identified 408 isolates collected from all clinical samples, 43 isolates found to be *Staphylococcus aureus* were comprising 16 (37.2%) were MRSA strains and 27 (62.8%) were MSSA strains, 100% of MRSA and 77.8% of MSSA isolates were multidrug resistant (MDR). However, no *Staphylococcus aureus* strains were found to be resistant to vancomycin [100% VA sensitive], [MRSA and MSSA] were [38% and 56%] respectively sensitive to Synercid and [44%, 52%] respectively sensitive to Linezolid. MRSA isolates showed high level [100%] as resistance to antibiotic groups of β -lactams. **Conclusions:** This study highlights the need for continuous surveillance of antibiotic susceptibility pattern of Methicillin resistant and sensitive *Staphylococcus aureus* with a view for selecting appropriate therapy.

Key words: In vitro Antibiotic susceptibility pattern, MRSA, MSSA.

INTRODUCTION

Staphylococcus aureus is a coagulase-positive *Staphylococcus*. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a variant of *Staphylococcus aureus* which is resistant to flucloxacillin and all other β -lactams (penicillins, cephalosporins and carbapenems), MRSA varies in its sensitivity to other antibiotics but is almost always sensitive to glycopeptides, (vancomycin or teicoplanin)⁽²⁾.

MRSA is an organism responsible for several difficult to treat infections in humans and is especially troublesome in hospitals where patients with open wounds, invasive devices and weakened immune systems are at greater risk of infection than the general public⁽³⁾.

According to Centers for Disease Control and Prevention (CDC), multidrug resistant organisms (MDROs) are defined as microorganisms, that are resistant to one or more classes of antimicrobial agents⁽⁴⁾.

MRSA problem is not unique to one country. As of early 2005, the number of deaths in the United Kingdom attributed to MRSA has been

estimated by various sources to lie in the area of 3,000 per year. *Staphylococcus* bacteria account for almost half of all UK hospital infections⁽⁵⁾.

MRSA strains are particularly important because they are a leading cause of health care associated infections worldwide and have also emerged as a major cause of community associated infections⁽⁶⁾.

This study was carried out to determine and compare the antibiotic susceptibility patterns in methicillin resistant and sensitive *Staphylococcus aureus* isolates (MRSA, MSSA).

MATERIALS & METHODS

Three hundred ninety five (395) clinical samples were collected from National Cancer Institute (NCI), Cairo University, Egypt and immediately transferred to the microbiological laboratory for bacteriological assay.

Isolation and purification of bacteria:

Samples were cultured on broth media, blood agar, mannitol salt agar (MSA) and incubated at 37 °C for 24-48 hrs. and 1-5 day

according to sample sources as described by Sridhar 2008⁽¹⁴⁾. After incubation period isolated bacteria were identified.

Identification of bacterial isolates:

All isolates were identified by colony morphology, gram stain and manual standard biochemical testes including Catalase, Coagulase, Mannitol fermentation testes and Oxidase test. *Staphylococci* formed golden yellow colonies with complete haemolysis on blood agar. They were gram positive cocci arranged in groups and were catalase positive, coagulase positive, fermented manitole and oxidase negative.

Identification of isolates was confirmed using Microscan auto identification system (Microscan WalkAway 9696).

MRSA was identified by being resistant to cefoxitin in the manual test and oxacillin with microscan system.

Antimicrobial susceptibility tests of *Staphylococcus* isolates by Microscan system:-

Microscan Dried Gram Positive Bacteria BreakPoint Combo Panel Type 20 (PBP C20) (Dade Behring, West Sacramento, (USA), based upon (30) different antimicrobial agent as in **table (1)**, susceptibility tests dosed and dried into the Microscan panel then rehydrated with 115ul of pure isolated of gram positive suspension.

Table (1) Antimicrobial agents used for gram positive bacteria

Antibiotic group	Antibiotic	Symbol	MIC(ug/ml)	
			Resist.	Senst.
Aminoglycosides	Gentamycin	CN	≥8	≤4
Carbapenam	Imipenam=Tienam	IPM	≥8	≤4
Cephalosporins	Cefazolin[G1]	CFZ	≥16	≤8
	Cefepime= Maxipim[G4]	CPE	≥16	≤8
	Cefotaxime= Claforan[G3]	CTX	≥32	≤8
	Ceftriaxone=Rocephin[G3]	CRO	≥32	≤8
	Cephalothin	CF	≥16	≤8
	Moxifloxacin=Moxlactam	MOX	≥4	≤2
Chloramphenicol	Chloramphenicol=Thiomphenicol	C4	≥16	≤8
Glycopeptides	Vancomycin	VA	≥4	≤2
Lincosamide	Clindamycin=Dalacin	CL	≥4	≤0.5
Macrolides	Azeithromycin	AZI	≥4	≤2
	Erythromycin	E	≥4	0.5
Oxazolidinone	Linezolid	LZD	≥4	≤2
Penicillins	Amoxicillin/Clavulanic acid=Augmanten	AMC	≥8/2	≤4/2
	Ampicillin	AMP	≥8	0.25
	Ampicillin/Sulbctame=Unasyn	SAM	≥16/8	≤8/4
	Oxacillin	OX	≥4	≤2
	Pencillin	P	≥16	≤8
	Pipracillin/Tazobactam=Tazocin	TZP	≥64/4	≤16/4
Quinolones	Ciprofloxacin=Ciprobay[G2]	CIP	≥2	≤1
	Gatifloxacin[G4]	GAT	≥4	≤2
	Levofloxacin=Tavanic[G3]	LEV	≥4	≤2
	Norfloxacin[G2]	NOR	≥8	≤4
	Ofloxacin=Tarived[G2]	OFX	≥4	≤2
Rifampin	Rifampin=Remactan	RD	≥2	≤1
Strepto-gramins, [quinupristin/dalfopristin]	Synercid	SYN	≥2	≤1
Trimethoprim & Sulphonamide	Sutrim= Sulfamethoxazol	SXT	≥4/76	≤2/38
Tetracycline	Tetracycline	TE	≥8	≤4
Nitrofurantoin	Nitrofurantoin	FD	≥64	≤32

Key :- (MIC) = Minimum of inhibitory concentration by ug/ml, (≥) = More than and equal, (≤) = Less than and equal.

Manual method for antimicrobial susceptibility tests (Discs diffusion method):-

Where some antibiotic discs as in **table (2)**, placed on the inoculated Mueller Hinton agar plate, the sensitivity was measured by visible clear zone of inhibition produced around

inserted discs after 24 hours of incubation at 37°C according to the Kirby-Bauer discs diffusion method and the interpretation of the results was done using Clinical and Laboratory Standard Institute guidelines⁽¹⁾.

Table (2) Antimicrobial agents used in disc-diffusion test

Antibiotic group	Antibiotic name	Symbol	Disc conc. (ug)	Diameter of inhibition zone (mm)	
				Resist.	Sent.
Aminoglycosides	Netilmicin	NET	30	≤12	≥15
Cephalosporin	Cefoperazon=Cfobid[G3]	CFP	75	≤15	≥21
	Cefoxitin[G2]	FOX	30	≤24	≥25
	Ceftizoxime[G3]	CZ	30	≤14	≥20
	Cefoperazon/Sulbactam= Sulperazon[G2]	SCF	75/30	≤15	≥21
Chloramphenicol	Chloramphenicol= Thiomphenicol	C4	30	≤12	≥18
Quinolones	Norfloxacin	NOR	10	≤12	≥17
Tetracycline	Vibramycin=Doxycyclin	DO	30	≤12	≥16
Nitrofurantoin	Nitrofurantoin	FD	300	≤14	≥17

RESULTS

Out of identified 408 isolates, 43 isolates were found to be *Staphylococcus aureus*, 16 (37.2%) were *MRSA* and 27 (62.8%) were *MSSA*.

The percentages of multidrug resistant (MDR) isolates were 37/43 (86%) in all *Staphylococcus aureus* isolates, 16/16 (100%) were MDR in *MRSA* isolates and 21/27 (77.8%) were MDR in *MSSA* isolates as shown in **table (3)**.

Table (3) Percentage of multidrug resistant (MDR)

<i>Staphylococcus aureus</i> strains	Multidrug resistant		Total isolates
	No.	%	
<i>Methicillin resistant staph. aureus [MRSA]</i>	16	100%	16
<i>Methicillin sensitive staph. aureus [MSSA]</i>	21	77.8%	27
<i>All Staphylococcus aureus</i> isolates	37	86%	43

Percentage of sensitivity of different *Staphylococcus aureus* strains according to commonly used antibiotics and efficacy of different antibiotics on different *Staphylococcus aureus* strains, *MRSA* were found to be 100% sensitive to vancomycin, 44% to linezolid and

38% to synercid. *MSSA* were 100% sensitive to vancomycin, 56% sensitive to synercid and 52% sensitive to linezolid. *MRSA* isolates showed high level (100%) as resistant to β -lactams antibiotic groups as shown in **table (4)**.

Table (4) Percentage of sensitivity of different *Staphylococcus aureus* isolates according to commonly used antibiotics

Groups of Antibiotic	Antibiotics	MRSA	MSSA	Total
		Total isolates		
		16	27	43
Aminoglycosides	Gentamycin (CN)	0%	33%	21%
Carbapenam	Impenam (IMP)	0%	19%	12%
Cephalosporin	Cefazolin (CFZ)	0%	7%	5%
	Cefepime (CPE)	0%	19%	12%
	Cefotaxime (CTX)	0%	19%	12%
	Cefoxitin (FOX)	0%	22%	14%
	Ceftiaxone (CRO)	0%	19%	12%
	Cephacolin (CF)	0%	7%	5%
	Moxifloxacin (MOX)	0%	15%	9%
	Cefoperazone/Sulb. (SCF)	0%	7%	5%
Chloramphenicol	Chloramphenicol (C4)	6%	26%	19%
Glycopeptide	Vancomycin (VA)	100%	100%	100%
Lincosamide	Clindamycin (CL)	6%	33%	23%
Macrolides	Azethromycin (AZI)	0%	11%	7%
	Erythromycin (E)	13%	26%	21%
Oxazolidinon	Linolid (LZD)	44%	52%	49%
Penicillin	Augmantin (AMC)	0%	7%	5%
	Amicillin (AMP)	0%	0%	0%
	Unasyn (SAM)	0%	11%	7%
	Oxacillin (OX)	0%	22%	14%
	Penicillin (P)	0%	0%	0%
	Tazocin (TZP)	25%	26%	26%
Quinolons	Ciprofloxacin (CIP)	0%	33%	21%
	Gatifloxacin (GAT)	6%	37%	26%
	Levofloxacin (LEV)	6%	30%	21%
	Ofloxacin (OFX)	0%	22%	14%
Rifampin	Rifampin (RD)	13%	52%	37%
Streptogramin	Synercid (SYN)	38%	56%	49%
Sulphmeth./Trimethobrem	Sutrim (SXT)	0%	56%	35%
Tetracycline	Tetracycline (TE)	0%	11%	7%
	Vibramycin (DO)	0%	4%	2%

DISCUSSION

Among the *Staphylococcus aureus* isolates (N=43) in this study, MRSA were 16/43 (37.2%) and MSSA were 27/43 (62.8%).

This agrees with Fateh *et al.*, 2013 in Iran⁽⁸⁾, who reported that the frequency of MRSA were 216 (29.7%) among 726 isolates of *Staph. aureus*, Tekalign and Ketema 2013 in Ethiopia⁽⁹⁾, found that MRSA =39/169 (23.08%) of total *Staph. aureus* isolates were resistant to methicillin and Cefoxitin.

Also in this study, the percentage of multidrug resistant [MDR] isolates was detected of all *Staphylococcus aureus* isolates were 37/43 (86%), MRSA were 16/16 (100%) MDR and MSSA were 21/27 (77.8%) MDR.

The same trend was obtained by Jean *et al.*, 2011⁽¹⁰⁾ who found that 50% of lung cancer infections were multidrug resistant.

On the other hand Fateh *et al.*, 2013 in Iran⁽⁸⁾, reported that MRSA and MSSA isolates were determined 9% and 5% respectively multidrug resistance patterns, Tekalign and Ketema 2013 in Ethiopia (9), found the *Staph. aureus* isolates showed (2.37%) multiple drug resistance patterns (MDR).

Also in this study, the percentage of sensitivity of different *Staphylococcus aureus* isolates according to commonly used antibiotics. Found all *Staphylococcus aureus* isolates were sensitivity to VA=100%, (LZD, SYN) =49%, (RD, SXT)=35% and (CN, C4, CL, E, TZP, CIP, GAT, LEV)=19% to 26%, also reported that MRSA strains were sensitivity

to VA= 100%, LZD=44%, SYN=38%, TZP=25% and (E, RD) = 13% and *MSSA* strains were sensitivity to VA=100%, (SYN, SXT)=56%, (RD, LZD)=52%, (CN, CL, CIP, GAT, LEV)=30% to 37% and (IPM, CPE, CTX, FOX, CRO, C4, E, OX, TZP, OFX) = 19% to 26%.

The same results were obtained by Nuala *et al.*, 2009 in Ireland⁽¹¹⁾, who Reported that the sensitivity of *Staphylococcus aureus* isolates were 100% sensitive to [VA], 0.0% sensitive to [P], while *MRSA* strains sensitivity to [VA]= 100%, [E]= 20%, [LEV]= 6% and [AMC, P]= 0%. Also inagree with Fateh *et al.*, 2013 in Iran⁽⁸⁾, reported that all *Staph. aureus* isolates the highest antibiotic resistance was [P, CL and TE] respectively and less than 30% resistance to [CN] and 100% sensitive to [VA], also found in *MRSA* strains 100% were resistant to [OX], 75% were resistant to [CL], 68% were resistant to [RD] and 59% were resistant to [CN], none (0%) were resistant to [VA], also 98% of *MSSA* strains were resistant to [P], 100% were susceptible to [VA]. Also inagree with Onoh *et al.*, at 2013 in Nigeria⁽¹²⁾, found the *Staph. aureus* sensitivity to [C4,TE] was less than 50%. Also inagree with Kaushal *et al.*, 2012 in India⁽¹³⁾, reported that *Staph. aureus* sensitivity to [VA]=100%, [P]=0% (100% resistant). Also inagree with Tekalign and Ketema 2013 in Ethiopia⁽⁹⁾, found a total of 169 *Staph. aureus* isolates were subjected to antibiotic susceptibility test were the majority= 164 (97%) were sensitive to [VA]. However, no isolates (0.0%) was sensitive to [P], accordingly, the highest resistance= 169 (100%) were observed for [P], followed by [AMP]= 129 (76.3%) and [C4]= 111 (65.7%), the least resistance was observed for [VA] with only 3% resistance recorded, which represent *MRSA* strains, on the other hand, all the 39 (100%) strains of *MRSA* were resistant to [AMP, FOX and P] Similarly, the highest susceptibility were observed for [VA]= 34 (87.2%), but the total of 130 *MSSA* strains, the highest resistance were observed for [P] =130 (100%), followed by [AMP]= 90 (69.2%) and [C4]= 88 (67.7%). Also inagree with Mukhtar and Saeed 2011 in Sudan⁽¹⁴⁾, found the resistance rates of *Staph. aureus* was 83.3% to [P]. Also inagree with Chugh *et al.*, 2011 in India⁽¹⁵⁾, found the Sensitivity pattern of *Staphylococcus aureus* (N = 24) to various antimicrobial agent were to [LZD] = 24 (100%), [TE]= 4 (14.67%), [VA]= 24 (100%).

Also in this study, the percentage of efficacy of commonly used antibiotic on different *Staphylococcus aureus* isolates were found [CN] antibiotic efficacy on [*Staph.*

aureus isolates]= 21%, [*MRSA* strains]= 0%, [*MSSA* strains]= 33%, among [IPM, CPE, CTX, CRO] antibiotics efficacy on [*Staph. aureus* isolates] =12%, [*MRSA* strains]= 0%, [*MSSA* strains]=19%, among [FOX] antibiotic efficacy on [*Staph. aureus* isolates] = 14%, [*MRSA* strains]= 0%, [*MSSA* strains]= 22%, among [C4] antibiotic efficacy on [*Staph. aureus* isolates]=19%, [*MRSA* strains]=6%, [*MSSA* strains]=26%, among [VA] antibiotic efficacy on [*MRSA, MSSA* strains]= 100%, among [CL] antibiotic efficacy on [*Staph. aureus* isolates] =6%, [*MRSA* strains]=23%, [*MSSA* strains]= 33%, among [E] antibiotic efficacy on [*Staph. aureus* isolates] =21%, [*MRSA* strains]= 13%, [*MSSA* strains]=26%, among [LZD] antibiotic efficacy on [*Staph. aureus* isolates]= 49%, [*MRSA* strains]= 44%, [*MSSA* strains]= 52%, among [OX] antibiotic efficacy on [*Staph. aureus* isolates]= 14%, [*MRSA* strains]=0%, [*MSSA* strains]= 22%, among [P] antibiotic efficacy on [*MRSA, MSSA* strains]= 0.0%, among [TZP] antibiotic efficacy on [*MRSA* strains]= 25%, [*Staph. aureus* isolates, *MSSA* strains]= 26%, among [CIP] antibiotic efficacy on [*Staph. aureus* isolates] =21%, [*MRSA* strains]=0%, [*MSSA* strains]=33%, among [GAT] antibiotic efficacy on [*Staph. aureus* isolates]= 26%, [*MRSA* strains]= 6%, [*MSSA* strains]=37%, among [LEV] antibiotic efficacy on [*Staph. aureus* isolates] = 21%, [*MRSA* strains]=6%, [*MSSA* strains]=30%, among [OFX] antibiotic efficacy on [*Staph. aureus* isolates] = 14%, [*MRSA* strains]= 0%, [*MSSA* strains]=22%, among [RD] antibiotic efficacy on [*Staph. aureus* isolates]= 37%, [*MRSA* strains]=13%, [*MSSA* strains]= 52%, among [SYN] antibiotic efficacy on [*All Staph. aureus* strains]= 49%, [*MRSA* strains]=38%, [*MSSA* strains]= 56%, among [SXT] antibiotic efficacy on [*Staph. aureus* isolates] =35%, [*MRSA* strains]=0%, [*MSSA* strains]=56%.

This is inagree with Nuala *et al.*, 2009 in Ireland⁽¹¹⁾, reported that the efficacy of commonly used antibiotic on different isolates. Where found [VA] antibiotic efficacy on *Staph. sp.* isolates=100%, among [E] antibiotic efficacy on *MRSA*= 20%, *MSSA*= 33%, while [LEV] antibiotic efficacy on *MRSA*= 6%. Also in agree with Syeda *et al.*, 2010 in Oman⁽¹⁶⁾, Showed that the efficacy of [CIP] = 21.95% resistant to *Staphylococcus aureus*. Also inagree with Jharna *et al.*, 2012 in India⁽¹⁷⁾, found [CRO] resistance were seen on *Staphylococcus* =109 (65.6%). Also inagree with Kaushal *et al.*, 2012 in India⁽¹³⁾ reported that *Staph. aureus* were 100% sensitive to [VA]. Also inagree with

Rabia et al., 2013 in Pakistan⁽¹⁸⁾, found that 55% of *Staphylococcus aureus* were sensitive to [LEV].

Conclusions

This study highlights the need for continuous surveillance of antibiotic susceptibility pattern of methicillin resistant and sensitive *Staphylococcus aureus* with a view for selecting appropriate therapy.

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دراسات معملية علي حساسية عزالات من الميكروبات *Staphylococcus aureus* المقاومة والحساسة للميثاسيلين للمضادات الحيوية

الميكروبات *Staphylococcus aureus* المقاومة للميثاسيلين هي أحد سلالات الميكروبات *Staphylococcus aureus* المقاومة لمجموعات المضادات الحيوية من النوع البيتا لاكتام وانتشار العدوى بها على نطاق واسع ما يزيد من صعوبة علاجها. وهذه الدراسة تستهدف معرفة المضادات الحيوية المناسبة لعلاجها , فقد تم جمع ٣٩٥ عينة من مرضي معهد الأورام القومي جامعة القاهرة وقد تم عزل الميكروبات وتعريفها ودراسة المضادات الحيوية المناسبة لعلاجها باستخدام الطرق الحديثة المميكنة المستخدم بها نظام الميكروسكان بالإضافة للوسائل اليدوية البسيطة للاختبارات البيكميماوية واختبارات الحساسية للمضادات الحيوية باستخدام اقراص محتوية للمضادات الحيوية وذلك وفقا للمبادئ التوجيهية الحديثة من معهد المعايير الاكلينيكية والمعملية , وقد تم تحديد تلك السلالة المقاومة للميثاسيلين بمقاومتها للمضادات الحيوية من نوع السيفوكستين والاوكتاسيلين.

فقد تم الحصول علي ٤٠٨ عزلة بكتيرية من العينات المشار اليها سابقا فوجد منهم ٤٣ عزلة تنتمي للميكروبات *Staphylococcus aureus* تشمل ١٦ عزلة بما يعادل ٣٧,٢% من السلالة المقاومة للميثاسيلين و ٢٧ عزلة بما يعادل ٦٢,٨% من المقاومة للميثاسيلين وكان كل السلالة المقاومة للميثاسيلين مقاومة لمجموعات متعددة من المضادات الحيوية بنسبة ١٠٠% , أما الميكروبات الحساسة للميثاسيلين كانت مقاومة لمجموعات متعددة من المضادات الحيوية بنسبة ٧٧,٨% وكان أيضا كل الميكروبات *Staphylococcus aureus* (الحساسة والمقاومة للميثاسيلين) حساسة بنسبة ١٠٠% للمضاد الحيوي الفانكوميسين , وكذلك كل الميكروبات *Staphylococcus aureus* المقاومة للميثاسيلين كانت مقاومة لكل المضادات الحيوية من النوع البيتا لاكتام . وهذه الدراسة تلقي الضوء علي ضرورة استمرار دراسة تأثير المضادات الحيوية علي تلك السلالات لاختيار أفضلها في العلاج .