Antibiotic Resistance Patterns of Community Acquired Methicillin Resistance *Staphylococcus aureus* (CA-MRSA) in Al-Hilla/ Iraq

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Abstract:

Out of 301 clinical samples, a total of 46 (15.2%) *Staphylococcus aureus* isolates were recovered, of which (84.7) were isolated from skin samples, (13%) from both ear and urine, and (2.1%) from sputum.44 (95%) isolates were identified as β-lactam resistant. Out of these isolates, 32(72%) were found in skin samples. All 44 β-lactam resistant isolates were subjected to disk diffusion test (DDT) for detectionthe susceptibility to 17 antibiotics used in the present study. The study showed that *S. aureus* isolates were resistant to most β-lactam antibiotics, but they were sensitive to imipenem, meropenem and rifampin.The susceptibility tovancomycin was tested using Two-fold agar dilution method. 33(75%) of *S. aureus* isolates showed sensitivity to vancomycin while 11(25%) showed reduced susceptibility to vancomycin and were identified as Vancomycin intermediate resistant *S. aureus* (VISA). Out of 44 β-lactam resistant isolates 13(29.5%) isolates were found to be resistant to oxacillin and identified as MRSA as they were resistant to both oxacillin and cefoxitin.Out of 13 MRSA isolates, three isolates were classified as MDR-MRSA, and ten isolates as NMDR-MRSA. All MDR and NMDR were sensitive to vancomycin.

أنماط مقاومة المضادات الحيوية للمكورات العنقودية المقاومة للمثيسيلين المكتسبة من المجتمع في مدينة الحلة/ العراق

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المفتاح: المكورات العنقودية المقاومة للمثيسيلين المكتسبة من المجتمع، المكورات العنقودية المقاومة للمثيسيلين

الخلاصة:

تم عزل 46عزلة (15.2%) تابعة للمكورات العنقودية الذهبية من 301 عينة سريرية وكانت 84.7% من هذه العزلات ماخوذة من الجلد، 13% من الاذن والادرار.و 2.1% من البلغم.اظهرت النتائج ان غالبية العزلات (95%) كانت مقاومة للبيتالاكتام وان 72% منها كانت من عينات الجلد.عرضت جميع العزلات المقاومة للبيتالاكتام لفحص انتشارالقرصفي الاكار لمعرفة حساسيتهاتجاه 17مضاد حيوي المستخدمة في هذه الدراسة.اظهرت الدراسة ان المكورات العنقودية الذهبية كانت مقاومة لغالبية مضادات البيتالاكتام لكنها كانت حساسة للفانكومايسين،الاميبينيم، الميروبينيم والريفامبين. تم استخدام طريقة التخفافيف في الوسط الصلب حيث كانت 75% من العزلات حساسة للفانكومايسين و(25%)منها اظهرت حساسية متوسطة للفانكومايسين.وجد ان 13 (29.5%) عزلة كانت مقاومة للاوكساسيلين واعتبرت المكورات العنقودية مقاومة للميثيسلين كونها مقاومة للاوكساسيلين و سيفوكسيتين.بينت هذه الدراسة ان 3 عزلات من اصل 13 مكورة عنقودية مقاومة للمثيسيلين صنفت كعزلات غير متعددة المقاومة وجميعها كانت حساسة للفانكومايس.

Introduction:-

The coordinated expression of *S. aureus*virulence factors is regulated by a complex network including the quorum-sensing system *agr*and the well characterized virulence gene regulators. The specific role and/or importance of a virulence factor in *S. aureus*pathogenesis may vary from one infection type to another. When considering that a high number of *S. aureus*strains are more difficult to be treated due to their multiple resistance to antibiotics, the elucidation of *S. aureus*pathogenesis at the molecular level becomes imperative in the fight against this important human pathogen, for the purpose of finding new therapeutic strategies (1, 2).

The aim of this study was to determine the prevalence of CA-MRSA isolates obtained from clinical samples, andstudying theantimicrobial susceptibility in Hilla/Iraq.

Materials and methods:-

Bacterial isolates:-

Fourtysix *S. aureus* isolates were obtained from clinical samples in Al-Hilla/Iraq during the period from March to June 2011 . Clinical samples were collected from the main three hospitals in Al-Hillacity (HillaTeaching hospital, MarganTeaching hospital, Childhood and gynecology hospital), in addition to some private clinic. clinical isolates were as follows: ear (3), burn (2), skin infections (32), wound (5), sputum (1), urine (3). These bacterial isolates were identified as *S. aureus*based on their morphology, Gram-staining, catalase properties. Coagulase test was performed to identify *S. aureus*isolates.

Antimicrobial Susceptibility

The antimicrobial susceptibility patterns ofisolates to different antimicrobial agents was determined and interpreted according to CLSI (2012). Disk diffusion test was used against 12 antibiotics,the following antimicrobial agents were obtained (from Oxoid, U.K) as standard reference disks as known potency for laboratory use: ampicillin (AMP, l0 mg), amoxicillin (Amx, 25mg), amoxicillin-clavulanate (20/10mg), oxacillin (OX,1µg),Imipenem (IPM,10mg), meropenem (MEM, 10mg), gentamycin (GN, l0mg), rifampin (R, 30mg), erythromycin (E, 15 mg), chloramphenicol (C, 30mg), doxycycline (DO, 30mg) tetracycline (TE, 30 mg).

The susceptibility to vancomycin was determined usingtwo fold agar dilution method,HiComb test was performed to determine oxacillin resistance.

Results and Discussion:-

Isolation and Identification of *Staphylococcus aureus* isolates:

Out of 301 clinical samples, 46 (15.2%) *Staphylococcus aureus* isolates were recovered during this study,. Most of the isolates (69.5%) were isolated from skin samples, but no isolatewas isolated from CSF and vaginal swabs (Table 1).

According to the fact that, there is a close correlation between these specimens, including (SSTs), wound, and burns. So the results of isolation of *S. aureus* from these specimens were not separated. However, a high percentage of isolation rate was detected in these samples (84.7%) (Table3-1). Humans seem to have little resistance to surface *S. aureus*colonization, so the bacteria are easily able to colonize in the nose and on the skin. These surface bacteria almost never invade the body further to cause a serious infection in healthy people. The isolation rate of *S. aureus* from skin depends on several factors like virulence of isolates, health status of patients and effect of environmental conditions. This result might be due to the fact that breaks in the skin from wounds, surgery, burns or lesions by insect bites with the impairment of host defenses and production of different types of toxins in these cases. This might be related to the inhibitory effect of serum exuding from broken skin on liolenic acid. Linolenic acid is an essential free fatty acid normally present on intact skin, which is responsible for inhibition of *S. aureus* colonization (3).

This is similar to the result obtained by (4), but it was more than (5) who found that (42.9%) of skin infections were caused by *S. aureus*.The existence of *S. aureus* isolates for ear and urine was (6.5%, 6.5%) respectively. This is due to the fact that *S. aureus* arecomensals of mucosa of upper respiratory tract and mucosal surfaces of urogenital tract (6). Also it's ability to adhere to tissue using adhesions like clfB, clfA (that facilitate adherence to keratinocytes and desquamated epithelial cells) suggesting that these adhesion factors play an important role in colonization (7). This low percentage may be due to different climatic conditions, personal hygiene, and severity of infection. In a local study (8), found that *S. aureus* in ear infection was16%.

S. aureus known to be a cause of UTI among catheterized patients (9). In our study, all patients were not catheterized, this is the justification for this percentage. While (10), found that the percentage of *S. aureus*isolates in urine samples was14.8% from a total of 212 urine samples in Al-Nasyria / Iraq. She found high percentage, may be due to large number of collected samples in comparison with the present study. (Table 1) showed low percentage (2.1%) of *S. aureus*isolates in sputum, this may be due to the natural habitat in upper respiratory tract rather than lower part, the relation between influenza and secondary staphylococcal pneumonia has long been recognized.In Iraq, (12) found the isolation rate of *S. aureus*isolateswas 22%, this may be due to differences in source and numbers of samples.

As shown in (Table 1), *S. aureus*isolates was not detected in vaginal and CSF samples. This may be due to small number of collected samples or due to *S. aureus*isolates rarely found in CSF. However (10) found, that high percentage *S. aureus*isolates was detected in vaginal swab and no *S. aureus*isolates were detected in CSF. The variation may be differences in personal hygiene, environmental conditions and health status.

Antibiotic susceptibility by disk diffusion test (DD test):

In this study 17 antibiotics performed to all staphylococcal isolates for testing their susceptibility to identify the most effective one against *S. aureus* particularly MRSA, because indiscriminate using of multiple broad spectrum antibiotic may associated with increased risk of MRSA infection (11).However, the overwhelmed *S. aureus* isolates that recovered from clinical samples were highly resistant to most antibiotics that used in present study (Figure 1). This can be attributed to the fact that, antibiotics may have revolutionized the treatment of common bacterial infections (12).

The results revealed that all bacterial isolates showed high resistance (100%), to amoxicillin and ampicillin. The resistance to amoxicillin-clavulanic acid in the present study were 93.1%, the interpretation for this variation may be due to the addition of clavulanic acid which can inhibit the action of β-lactamases enzyme (13).The mechanism of this resistance is mostly due to either production of β-lactamases that hydrolyze β-lactam ring which is controlled by plasmid or chromosomal regulation, or lack of penicillins receptors on cell wall and/or alteration in their permeability to β-lactam antibiotics preventing the uptaking of them (14).

Table(1): Numbers and percentages of *S .aureus*isolates recovered from different sources of infection

Source of samples	Total No. of samples	No. of *S. aureus* isolates	Percentage
Ear swabs	37	3	6.5%
Burns	12	2	4.4%
SSTIs*	65	32	69.5%
Wound	25	5	10.9%
Sputum	23	1	2.1%
Urine	70	3	6.5%
Vaginal swabs	4	0	0
CSF	65	0	0
Total number	301	46	100%

*SSTs=skin and soft tissue infections

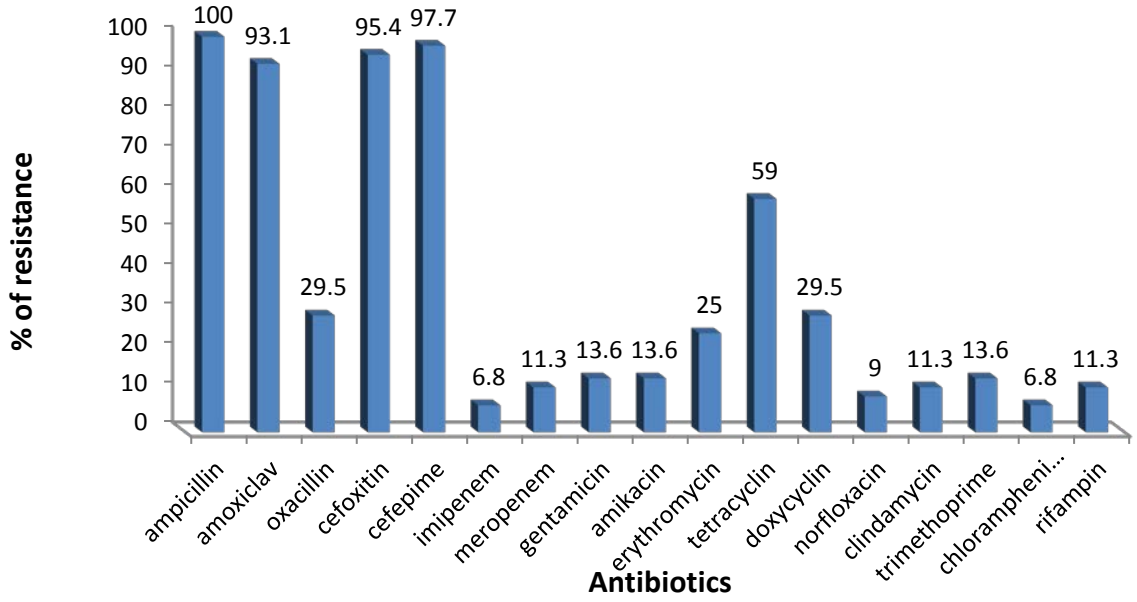
In the present study, oxacillin resistance was 29.5%. Oxacillin replace methicillin as Oxacillin is stable under storage conditions, and methicillin actually is an excellent inducer of the *mec*Agene. However, methicillin is not the agent of choice for MRSA recognition and its not preferred to evaluate methicillin resistance, so it should be replaced by oxacillin and cefoxitinfor detection of MRSA isolates (15). Staphylococcal resistance to either oxacillin or methicillin occurs when the organism including an altered PBP (PBP2A) that is encoded by the *mec*Agene. This result was in accordance with result obtained by (16), who found that the oxacillin resistance rate was 3o%. But more than the result of (6), which was 22.5% in Najaf/Iraq.

Cephalosporins, cefepime(4^{th}generation) and cefoxitin showed that percentages of *S. aureus* isolates resistance was (95.4%, 97.7%) respectively (Figure1).This can be explained by the fact that all Staphylococcal strains produce β- lactamase which destroys the β-lactam ring resulting in inactive products (17).Furthermore; β-lactamase produced by staphylococci excreted into the surrounding environment by which the hyper production of β-lactamase will give longer validity and surviving to this bacterium, because the hydrolysis of β-lactams takes place before the drug can bind to PBPs in the cell membrane (18).

In the present study all bacterial isolates exhibited high sensitivity to carbapenems (imipenem and meropenem) (Figure1).Imipenem and meropenem are broad-spectrum carbapenems antibiotics. Beta-lactam rings of these antibiotics are resistant to hydrolysis by most β-lactamases (19). However, the result was in accordance with those results being reported by (20). High ratio of resistance (59%, 29.5%) for tetracycline and doxycycline was found respectively, due to differences in source of samples, as most of samples were from skin infection and due to empirical prescription of these drugs by physicians and overuse by patients. In the present study, the percent of resistance to erythromycin was (25%) which is lessthan that obtained by (6), (81.5%) who isolated erythromycin resistance of *S. aureus* from hospital infections in European countries. However, the

majority of isolates in present study were isolated from outpatient that showed more susceptibility to the antibiotics.

Clindamycin in the present study showed resistance rate (11.3%). This result was less than reported by (21) (23%) and (22) (35%). This is due to some isolates have virulence factors more than other isolates, also differences in source of samples , conditions of tests used and type of techniques. All these factors may lead to differences in resistance levels (23).



Figure(1): Percentages of resistance of 44 *Staphylococcus aureus* isolates to different antibioticsby DDT

As shown in (Figure 1), the percentage of resistance for gentamycin and amikacin were (13%) for each.This result was more than the result obtained by (24), had resistance rate 10% .The resistance rate to trimethoprim was13.6%, This antibiotic has moderate therapeutic effect, and could be used for the treatment of infections caused by*S. aureus* that resist to different types of antibiotics.Trimethoprim was approved for the treatment of skin ang soft tissue infections caused by *S. aureus*(25).Variations in sensitivity are related to the frequency of usage of the individual antibiotics in hospitals compared to outpatients. This result was not agreed with results obtained by (6), who found resistance rates(52.5%) from hospitalized patients.

Resistance to Rifampin has been detected, and the percentage of *S. aureus* resistance to this antibiotic was found to be (11.3%) (Figure 1).This low resistance can be assigned to the ability of the pathogens to develop resistance to rifampin may required long period of time for organism to evolve resistance at a high rate of cell division (26). This percentage may be reduced by combination with other agent, because rifampin should not be used alone for antimicrobial therapy (15).

S. aureus represented resistance rate(11.3%) to Norfloxacin (Figure 1). Mitscher, (2004), has stated that norfloxacin is effective against *S. aureus* isolates, and this type of antibiotic inhibits bacterial growth by effecting DNA maintenance, therefore, *S. aureus*is sensitive to this type of antibiotics.

Detection of methicillin resistant *S. aureus* (MRSA):

Disk diffusion test of MRSA isolates:

For this test both oxacillin and cefoxitin were used, other than methicillin, for detection of MRSA isolates. Results revealed that out of 44 *S. aureus* isolates, 13 (29.5%) isolates were resistant to both of these antibiotics in disk diffusion test. Oxacillin is stable under storage conditions, and cefoxitin actually is an excellent inducer of the *mec*A gene (27). According to that, oxacillinand cefoxitin resistant isolates were initially interpreted as MRSA.

In the present study, MRSA isolates represent 2(4.5%) of urine samples (Table 2). This result is less than the percentage that obtained by (28,5), they found that (20%, 8.16%) of UTI were caused by MRSA respectively. This result can be attributed to the ability of these bacteria to form biofilm and variable types of adhesions.

Results also revealed that MRSA isolates were 11(25%) of all clinical isolates recovered from different skin infections (Table 2). The ability of these isolates to cause infectionmay be due to the fact that *S. aureus* is frequent commensal bacteria on the human skin and mucous surfaces (29). Unfortunately, most purulent skin infections are not routinely cultured. Rather, they are frequently treated empirically with antibiotics, which are ineffective against MRSA and can compound antibiotic resistance.

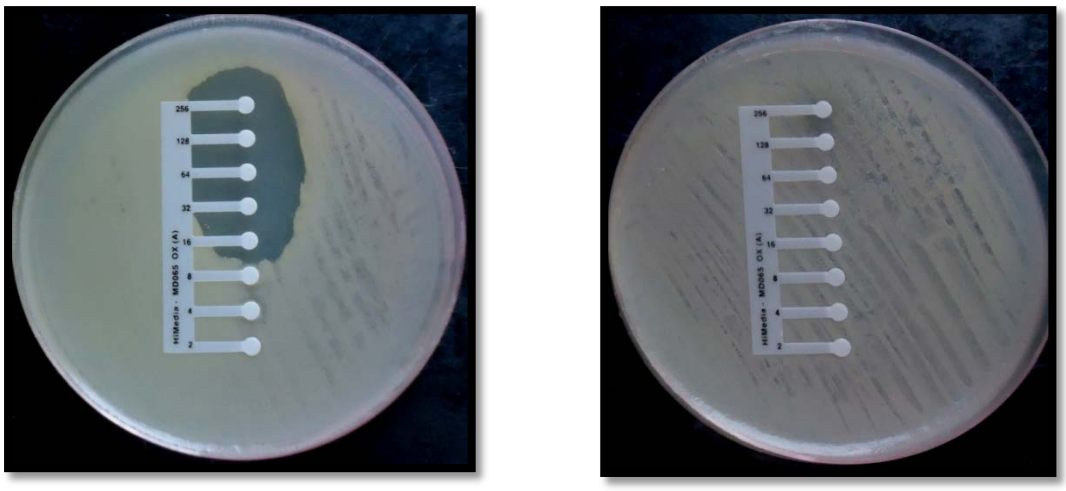
Although sometimes neglected, appropriate drainage is the definitive management of many skin and soft tissue infections and is always an important adjunct to antibiotic therapy in deeper, closed-space infections. MRSA cases conducted in Minnesota in 2000, community acquired MRSA (CA-MRSA) accounted for 75% of all SSTI in that population. Surgical Site Infection (SSI) are the most common adverse events affecting hospitalized patients with SSTIs who have undergone surgery(30).

Table (2): Numbers and Percentages of 13 MRSA isolates detected by disk diffusion test (DDT)

Source of MRSA isolates	No. of MRSA isolates	% of Resistance*
Urine	2	4.5
Abscesses, boils, burn, wound	11	25
Total No.(44)	13	29.5

MIC of MRSA isolates :

HiComb MIC test was used for 13 MRSA isolates against oxacillin (Figure2). This test was carried out for all MRSA isolates that showed resistance for both oxacillin and cefoxitin in DDT(Table 3). Results showed that MIC values of all MRSA isolates were resistant to oxacillin and the MIC values ranged between 16 - ≥ 256 µg/ml for oxacillin(Table 3).Results revealed that there was a variation in the degree of susceptibility of the isolates to the oxacillin. The MIC values for two isolates(isolates U 175 and W79) reached to ≥ 256 µg/ml, and that can be attributed to the characteristics of these isolates by their ability to resist more than 83.3% of the antibiotics used in the present study in DDT.



Figure(2): Determination of MIC of MRSA isolates by HiComb test: Right, Isolate U175 (MIC ≥256µg/ml). Left, isolate S28(MIC 16 µg/ml).

The Isolates; (U173), (U175) and (W79), are MRSA isolates and considered as multidrug resistant (MDR), that resist (88.8%), (83.3%) and (83.3%) of antibiotics respectively, these *S. aureus* isolates occupied the primacy in the list of antibiotic resistance that used in the present study. This resistance can be attributed to the trait of some other mechanisms of MRSA regarding to cell wall thickening that makes penetration by antibiotics difficult, conferring resistance to multiple antibiotics not just β-lactams (31). These oxacillinMIC values were less than values obtained by(32) who found that the MIC values for oxacillinresistant isolates were in range of 4-512 µg/ml, and this variation may regarding to the regional specifications like the source, number of samples and virulence characteristics of thecollected isolates of his study.The range of MIC values of MRSA isolates was more than the MIC values obtained by (5), who found that MIC value for oxacillin resistant isolates were in range of 4-32 µg/ml, this is may be due to the type of test(HiComb test) used in present study, which is fast, more accurate, and advanced technique used for detection of MRSA.Results shown in (Table 3) indicates that 38.4% of the isolates have given MIC value 16 µg/ml. This result was less than the results obtained by other researchers (6,5) who found that the predominance value of MIC belonging to oxacillin was 16µg/ml among MRSA isolates.

Antibiotic Profiles ofMRSA:

Resistance of MRSA isolates to β-lactam antibiotics:

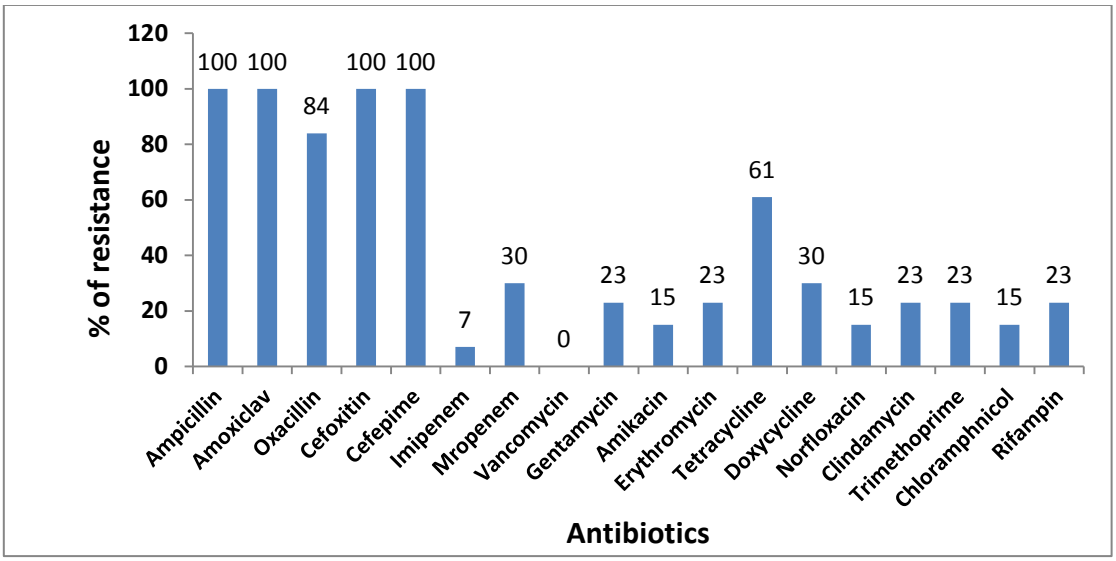
In the present study, of MRSA isolates that resist to β-lactam antibioticswas shown in Figure (3), the results revealed that all MRSA isolates were fully resistant 100%. to ampicillin, amoxyclav, cefepime, cefoxitin , While 11 isolates (84%) were highly resistant to oxacillin. Meropenem had resistant rate (23%) while imipenem show low resistance rate (7%).MRSA is resistantto practically all β-lactam antibiotics, a class of antibioticsrepresented by penicillins and cephalosporins (15,33). Resistance due to β-lactamases and PBPs mediated *mec*A gene is an emerging global problem that needs to be monitored. Resistant organisms are increasing in number and causing more severe infections because of their continuous mutation. MRSA strains are resistant to the β-lactamase-resistant penicillins, by virtue of changes in the penicillin-binding protein in their cell membrane (34). In this study MRSA isolates exhibited high resistance rate toward penicillins and Cephalosporins (Figure 3). This result was in line with (35) and that could be attributed to the

structural gene for Penicillin-binding protein which is responsible for the intrinsic resistance of MRSA. This protein called PBP2A that have low affinity for binding to β-lactam antibiotics (57), this impairment binding affinity disabling the ability of disruption the cell wall synthesis, and rendering the drug ineffective.

Table (3): Antibiotic resistance of MRSA isolates detected by DDT and MIC tests

Isolate Designation	Disk Diffusion test		Oxacillin MIC (≥ 4 µg/ml) *	Vancomycin MIC (≥ 32µg/ml)*
	oxacillin 1 µ g ≤10 mm) *(	cefoxitin 30µg (≤21 mm) *		
S22	8	10	64	0.12
S23	9	11	128	0.12
S26	10	12	64	0.24
S29	9	13	32	4
S57	10	12	16	0.96
S59	9	10	32	0.24
UL60	10	10	16	2
W79	0	0	256≥	8
B95	10	14	16	8
U175	9	10	256≥	8
S2	11	15	128	4
S28	11	19	16	4
U173	6	10	16	8

*Numbers between brackets refer to the breakpoints recommended by CLSI (2010)



Figure(3): Percentages of antibiotic resistance of 13 CA-MRSA isolates by DDT

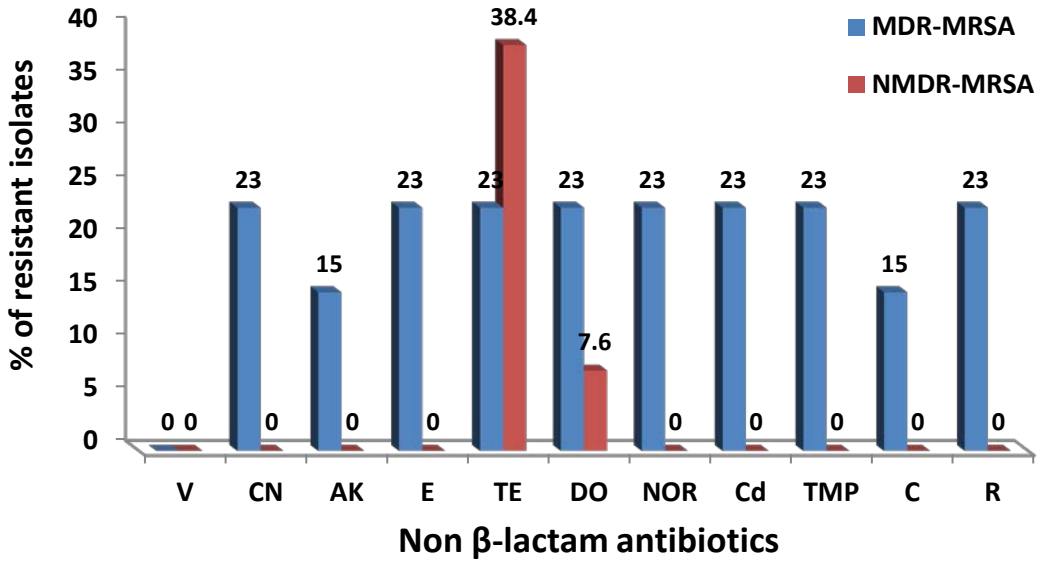
Furthermore; expression of PBP2A on a Staphylococcus organism confers resistance to all penicillins and cephalosporins (37). Although MRSA intimate that resistance of the PBPs to Penicillins and cephalosporins is chromosomally mediated by the *mec*A gene (38).

Resistance of MRSA isolates to Non β-lactams:

The antibiotic profiles of all MRSA isolates was determined. The MRSA isolates examined were subdivided into twogroups according to their antibiotic profiles and comprised10 (76.9%) non multidrug resistant NMDR- MRSA isolates, resistant to lessthan two (< 2) non ß-lactam antibiotics as (Figure 4) and 3 (23%) multidrug resistantMDR-MRSA isolates, resistant to three or more (≥ 3) non ß-lactamantibiotics.

High resistance rate (61%) was detected to tetracycline, of which 3 isolates (23%) in MDR-MRSA and 5 isolates (38.4%) in NMDR-MRSA, while resistance rate to doxycycline was (30%) of which 3 isolates (23%) in MDR-MRSA and one isolate (7.6%) in NMDR-MRSA. NMDR-MRSA showed variable resistance to tetracyclines, as the majority of isolates in the present study were recovered from skin infections that treated by these agents as empirical therapy. However, In a local study (5), found that 40% of MRSA isolates were resistant to tetracycline. Also results of tetracycline resistance in the present study were more than that obtained by (39), who found that the resistance rate for tetracycline was 37.8% in MRSA isolates recovered from outpatient clinic in Riyadh/Saudi Arabia. the resistance rate for doxycycline in the present study was more than the result obtained by other researchers. (5),who found that all this MRSA isolates were susceptible to doxycycline. The resistance to these antibiotics are plasmid mediated (40) and usually inserted in mobile genetic elements called Staphylococcus Cassette Chromosome type IV(SCC *mec* IV) that carry the *mec*A gene which is responsible for methicillin resistance that is widely spread in the community.

Figure(4), showed that resistance rate of MDR-MRSA was (23%) for each: Gentamycin, erythromycin, clindamycin, trimethoprim and rifampin. A study conducted on MRSA isolates between 2000 and 2002 in Japan showed that 47% of the isolates were gentamicin resistant (41). Clindamycin resulted in 23% resistance (Figure 4). This supports experts' recommendations of avoiding empirical therapy with clindamycin when local rates of clindamycin resistance exceed 10-15% among MRSA isolates causing skin and soft tissue infections (42). In the present study rifampin showed resistance rate(23%) which was higher than the result obtained by (43), who found no resistance rate to rifampin. This study identified that rifampinwas susceptible only 77% of the time; because of this, it is recommended that rifampin primarily be prescribed in combination with another antibiotic known to be effective against CA-MRSA isolates. In the present study, Amikacin, norfloxacin, chloramphenicol had the same resistance rate (15%) in MDR-MRSA isolates. The resistance rate of MRSA isolates to norfloxacin was less than that obtained by Alborzi*et al*.,(2000), who mentioned that norfloxacin has anti Staphylococcal activity with resistance rate 40% for MRSA isolates. Sub inhibitory levels of norfloxacin results in the induction of fibronectin-binding proteins and associated with increase in adhesion to fibronectin-coated surfaces (44).Therefore, isolates that are initially susceptible may become resistant within three to four days after initiation of therapy (15). This suggests that exposure of MRSA to norfloxacin*in vivo* may result in increase bacterial virulence by augment binding to fibronectin (45). For chloramphenicol resistance rate (15%),it was less than that obtained by (39), who found that MRSA isolates recovered from outpatient clinic in Riyadh/Saudi Arabia had a resistance rate (45.9%) to chloramphnicol. The differences in resistance rate may be due to different test conditions, environmental factors and health status of patients.



Figure(4): Antibiotic resistance of MDR-MRSA and NMDR-MRSA isolates to non β-lactam antibiotics using DDTV: vancomycin, CN:gentamycin, AK:amikacin, DO:doycyclinine, NOR:norfloxacin, Cd:clindamycin, TMP:trimethoprime, C:chloramphenicol, R:rifampin

The results revealed that all (No.13) MRSA isolates were fully susceptible (100%) to vancomycin, of which 4 isolates (30.7%) represented intermediate susceptibility to vancomycin (VISA) (MIC values 8-16µg/ml), and 9 isolates (69.2%) showed full sensitivity to vancomycin (0.24-4µg/ml). No isolate was found to be VRSA. Two isolates (S2, S28) showed sensitivity to vancomycin and oxacillin (Table 3). Some studies demonstrated *in vitro* a synergism between Oxacillin and vancomycin against many MRSA isolates. In a local study (46) found that MRSA was susceptible to vancomycin when mixed with oxacillin.

In MRSA stains, an increase in the amount of peptidoglycan results in affinity trapping of vancomycin, preventing the antibiotic from penetrating deeper into the cell wall (27). Moreover, MRSA has reported to possess genes encoding to protein mode thicker cell wall which will cause more vancomycin molecules to be trapped in the peptidoglycan layer before reaching the cytoplasmic membrane where peptidoglycan synthesis occurs resulting in a thickened cell wall of VRSA and VISA strains (47).

All NMDR-MRSA were susceptible to erythromycin, norfloxacin, chloramphenicol, rifampin, and trimethoprim, tetracycline, doxycycline clindamycin, gentamycin, amikacin. Isolation of NMDR-MRSA has been reported with increasedfrequency in worldwide (48). And despite universalguidelines published by the CLSI for the susceptibility testingof *S. aureus*tomethicillin (15). In conclusionrapid detection of MRSA confers benefit to patients, especially when testing can be conducted on clinical samples for disease validation.The prevalence of CA-MRSA might be underestimated partly because skin infection samples are not routinely cultured. Moreover, resistance to oxacillin is heterogenous and can easily be missed by suboptimal screening methods.

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