PREVALENCE OF PVL GENE AMONG METHICILLIN RESISTANCE S. AUREUS ISOLATES IN BAGHDAD CITY

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ABSTRACT

In the current study eighty-four staphylococcal isolates were obtained from patients who were admitted to Baghdad in hospitals during the period July to November 2013 to aim investigation about prevalence of methicillin resistant S. aureus (MRSA) phenotypically and genotypically, on the other hands to determine prevalence of pvl gene among S. aureus isolates additional to screening about antibiogram profile. There were identified (74 out of 84) isolates as S. aureus isolates, the phenotypic screening method about MRSA has been revealed the percentage of MRSA isolates were (74.32%) while genotypic screening method has been revealed (84.43%) harboring with mecA gene. Also the results have been revealed (6.55%) of MRSA isolates harboring of pvl gene. Antibiogram profile of isolates have been revealed completely resistance of MRSA isolates toward cloxacillin, oxacillin and cefepime while some isolates showed high resistance toward Amoxicillin /Clavulinic acid (98.36%) and Ceftazidime (95.2%). the moderate resistance toward tetracycline (31.14%), the low resistance toward azithromycin (24.6%), vancomycin (16.4%), gentamycin (13.11%), ciprofloxacin (13.11%) and finally the lowest resistance toward imipenem (6.55%).


INTRODUCTION

S. aureus bacteria consider one of the most frequent significant human pathogen which responsible for different infections ranging in severity from mild superficial skin infection to the life threatening bacteremia, the prevalence and distribution of methicillin resistant S. aureus (MRSA) in clinical setting patients have been raised the conception increase the nosocomial infections all over the world[1], some of S. aureus isolates produce a powerful
toxin responsible for rapid spread and increase virulence of MRSA, this toxin composed of two components cytolytic and pore forming toxin, it’s called Panton- Valentine- Leucocidin (PVL).\(^2\) Which encoded by \textit{pvl} gene carried on a temperate bacteriophage, and can transfer from one isolates to other via phage transduction therefore it has ability to transmit not only vertical transmission but also horizontal transmission among clones.\(^3\) Recently has been observed \textit{pvl} gene tightly associated with CA-MRSA isolates.\(^4\) Clinically, CA-MRSA causes necrotizing pneumonia, necrotizing fasciitis, bloodstream infection and septic shock, additional to many reports documented the CA-MRSA isolates are more virulent than HA-MRSA isolates belong to carriage exotoxins.\(^5\) now a day various methods have been reported for genotypic detection of MRSA isolates most of them depending on Molecular techniques rely on PCR amplification to DNA fragments.\(^6\) Detection of \textit{fem}A gene and \textit{meca}A gene consider a golden standard method to detecting and identification MRSA isolates.\(^7\)

**Collection and identification of bacterial isolates**

Eighty four staphylococcal bacterial isolates were obtained from Medical city hospitals, Ibn-Albaladly hospital and Alemam-Ali hospital in Baghdad during the period July 2013 to November 2013. They were isolated from different clinical sites including: Surgical wound from hospitalized patient, Ear infection, midstream urine from patient suffering urinary tract infections, sputum of patients with respiratory tract infections, blood from patient with septicemia beside samples from hospital environment. Each isolate was identified according the morphology, routine biochemical tests according to\(^8\) and confirmed by EPI Staph test.

**Phenotypic detection of MRSA isolates**

It was performed to primary screening about MRSA by using Kirby-Bauer disc diffusion method and cefoxitin antibiotics disc, also to determine sensitivity toward commonly used Antibiotics, The process was carried as recommended in CLSI guide lines.\(^9\)

**Genotypic detection of methicillin resistant \textit{S. aureus} isolates MRSA and \textit{pvl} gene**

The prevalence of MRSA isolates was done by using multiplex PCR with specific primers and amplicon size as list in table (1) to detecting \textit{fem} A (housekeeping gene)\(^10\) and \textit{meca}A gene (responsible for methicillin resistance)\(^11\) Template DNA was prepared by simple boiling methods.\(^12\) Briefly, few isolated colonies of overnight growth bacteria were suspended thoroughly in 5 ml of TE buffer and boiled in water bath for 5 min. after centrifugation the supernatant was separated and applied as template of DNA. PCR mixture
was prepared by adding 12.5µl of GoTaq®Green master Mix (2X) promega, 5µl template DNA, 1.5µl from each forward and reverse primers with final concentration 1 pmol /µl, finally volume was completed to 25µl by adding nuclease free water. PCR condition was illustrated in table (2). Uniplex PCR was used with specific primers to screening for prevalence pvl gene\textsuperscript{[11]} among S. aureus isolates as list in table (1) and PCR condition illustrated in table (2). PCR products were detected in 1 % agarose gel for 1 hr. at 50 V, stained with ethidium bromide and visualized by transilluminator.

### Table 1: primers and amplified PCR products size used in this study

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Primer sequences 5………………..3</th>
<th>Origin</th>
<th>Product size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>femA F</td>
<td>CGATCCCATATTTACCATATCA</td>
<td>OligoData, South Africa</td>
<td>450</td>
<td>Diep et al., 2006</td>
</tr>
<tr>
<td>femAR</td>
<td>ATCACGCTCTTCTGTTAGTT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mecA F</td>
<td>GTAGAAAATGACTGACGTCCGATAA</td>
<td>OligoData, South Africa</td>
<td>314</td>
<td>Cabrera et al., 2010</td>
</tr>
<tr>
<td>mecA R</td>
<td>CCAATCCACATCTTGGGTCTAA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pvl F</td>
<td>ATCATAGTAAAAATGTCTGGACATGA TCA</td>
<td>OligoData, South Africa</td>
<td>433</td>
<td>Cabrera et al., 2010</td>
</tr>
<tr>
<td>pvl R</td>
<td>GCATCAAGTGTATTGGATGAAAGGCA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: PCR condition to genes used in current study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Initial denaturation</th>
<th>Repeated cycles</th>
<th>Denaturation step</th>
<th>Annealing step</th>
<th>Extension step</th>
<th>Final extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>femA</td>
<td>95 °C/30 sec.</td>
<td>35</td>
<td>94 °C/30 sec.</td>
<td>53 °C/30 sec.</td>
<td>72 °C/30 sec.</td>
<td>72 °C/10 min</td>
</tr>
<tr>
<td>mecA</td>
<td>95 °C/30 sec.</td>
<td>35</td>
<td>94 °C/30 sec.</td>
<td>54 °C/30 sec.</td>
<td>72 °C/30 sec.</td>
<td>72 °C/10 min</td>
</tr>
</tbody>
</table>

### Data analysis
- Dendogram and phylogenetic tree were built by suing unweighted pair group method (UPGMA) and genetic distance model Tamura-Nei with cost matrix identity (1.0-0.0) with geneious software,

### RESULTS
- Eighty four isolates of Staphylococcus spp. were collected from different hospitals in Baghdad, seventy –four were confirmed as Staphylococcus aureus isolates.
- Two methods were used to screening about methicillin resistance S. aureus, it was used phenotypic method as disc diffusion method and the results were showed (55) isolates is MRSA (74.32%), (8) isolates is MSSA (10.82%) and some isolates (11) showed intermediate resistance to cefoxitin (14.86%) while in genotypic methods were used multiplex PCR technique with specific primers to housekeeping gene femA and mobile genetic element.
mecA the results were showed all isolates (74) contained fem A (100%) is \textit{S. aureus}, while (61 out of 74) isolates contained mecA (82.43%) is MRSA and (13 out of 74) isolates did not contained mec A (17.57%) is MSSA.

• (4 out of 61) from MRSA isolates harboring with \textit{pvl} gene (6.55%).

• Disc diffusion method have been revealed the completely resistance (100%) toward cloxacillin, oxacillin and cefepime while some isolates showed high resistance toward Amoxicillin /Clavulinic acid (98.36%) and Ceftazidime (95.2%). the moderate resistance toward tetracycline (31.14%), the low resistance toward azithromycin (24.6%), vancomycin (16.4%), gentamycin (13.11%), ciprofloxacin (13.11%) and the lowest resistance appeared toward imipenem (6.55%).

• Dendogram and phylogenetic tree have been revealed (151) node and (76) tips. Also reflected highly diversity resistance antibiogram among isolates addition to high diversity between MRSA and MSSA isolates still found some similarities in antibiogram pattern.

DISCUSSION

\textbf{Bacterial isolation and Identification}

\textit{S. aureus} is a commensal bacterium which almost colonizes the nose of healthy persons. It causes a wide spectrum of infection, beginning from skin and soft tissue infections to invasive diseases. Because \textit{S. aureus} have numerous virulence factors, this will make it have ability to colonize and distribute of different environments. Rapid emergence of MRSA had been observed in the last two dedicate associated with complicated the control of infection.\textsuperscript{[13]}

In this study eighty four isolates of \textit{Staphylococcus spp.} were collected from different hospitals in Baghdad. It was divided between seventy five isolates from clinical samples from patient and nine isolates from hospital environment. The source of clinical isolates distributed as (n=5) isolates from Blood, (n=15) isolate from urinary tract infection, (n=43) skin infection, (n=2) eye swab, (n=5) ear swab, (n=3) nasal swab, one isolate from seminal fluid and lastly one isolated from sputum. As it appear in figure.(1)
According to source and percentage of isolation it could be said that the highest percentage in *S. aureus* clinical isolates goes to skin infection (51.35%) as compared with other infections followed by UTI (16.2%), ear infection (6.75%), Blood infection and nasal infection (4.05%), eye infection (2.70%), and finally sputum and seminal fluid (1.35%), (13) was revealed in their research *S. aureus* isolates was the predominant pathogen recovered from burn wound (33.6%) which is colonized and infected skin tissues more than other bacterial species.

**Screening for Methicillin resistance** *S. aureus* isolates MRSA

**A-Phenotypic method to screening about MRSA**

First one by phenotypic properties, the traditional method used in most of laboratories to detecting MRSA by Disc diffusion method to methicillin, oxacillin and cefoxitin antibiotics. Cefoxitin has recently been investigated as an alternative agent for detection of the resistance by using disc diffusion method and almost studies indicated that test is more reliable and less environmental condition requirements needed.\(^\text{[15]}\) as shown in figure(2).
S. aureus is a bacteria has ability to survive against common antibiotics and therapeutics, therefore till now consider one of the five causes of hospital acquired infection. Furthermore MRSA isolates are more infectious than ordinary S. aureus isolates.

In this study, the results have been showed the seventy four isolates of S. aureus divided to three groups: (55) isolates is MRSA (74.32%) as shown in figure\cite{3,8} isolates is MSSA (10.82%) and some isolates (11) showed intermediate resistance to cefoxitin (14.86%) as shown in figure \cite{3}, the high percentage of MRSA demonstrated increase prevalence and outbreak of MRSA isolates among Iraqi patient sitting in hospitals, one of the important reason which increasing the numbers of patient sitting in hospital over the capacity of hospital belong to high daily percentage of bombs in Baghdad. Also the number of hospitals in Baghdad is very low compared with number of peoples settled in it. The results of this study contradictory to\cite{16} which local study mentioned in their research (21.62%) of S. aureus were MRSA isolates . Unfortunately MRSA become major problem to healthcare and increase the prevalence in last dedicates over all the world, furthermore the variation in values leading to vary in prevalence of MRSA in different communities.\cite{17}
In Disc diffusion method has been found some of *S. aureus* isolates appeared intermediate resistance to cefoxitin, this group of isolates is confused because some researchers consider it resistance isolates and other researchers consider it sensitive isolates, these results belong to challenges face disc diffusion method like inoculum size, incubation period, temperature, salt concentration and pH of the medium therefore disc diffusion method less sensitivity and specificity \[18\] also alteration in existing of PBPs have been reported in MRSA isolated causing to moderately resistance *S. aureus* (MORSA), were shoed low level of resistance and their clinical significance is unclear also low level of resistance may be observed in isolates with producing large amount of penicillinases, however intermediate resistance not frequently reported.\[15\]

**B-Genotypic method to screening about MRSA**

Another way to screening about MRSA has been done by housekeeping gene *femA* and mobile genetic element *mecA* and using polymerase chain reaction PCR, this technique characterized with (93.8 to 100 %) sensitivity and (98.6 to 100) specificity, now a day, clinicians and researchers have been extracted huge genomic information from clinical samples especially in clinical bacteriology leading to major transformation in diagnostic way, belong to the diagnostic in PCR.\[19\]
Figure (4) Agarose gel electrophoresis (1% agarose, 7V/cm, for 90 min) for multiplex PCR products *fem* A (amplified size 450 bp) and *mec* A (amplified size 314 bp) compared with (100 bp) DNA ladder, all lanes represent positive for *fem*A gene, lanes 1,2,3,5,7,8,10,11,13 represents positive for *mec*A gene and lanes 4,6,9,12 represent negative for *mec*A gene.

The results obtained from PCR technique have been revealed all isolates (74) contained *fem* A (100%) is *S. aureus*, while (61 out of 74) isolates contained *mec* A (82.43%) is MRSA and (13 out of 74) isolates did not contained *mec* A (17.57%) is MSSA. Our results were agree with (1) who showed *mec*A prevalence (75.5%) in *S. aureus* isolates, while disagree [18] showed prevalence of *mec* A (100%). Actually the significant of rapid diagnostic pathogen in clinical samples play critical role in improve patient care because the accurate identification of pathogen at species level and antibiogram sensitivity consider the first line in treatment and control on infectious diseases. [20] One of the interest finding some isolates showed intermediate resistance and sensitive to cefoxitin in phenotype while in genotype appear harboring *mec* A gene this maybe belong to low expression of gene or the gene needing inducible factor for expression, otherwise some isolates showed resistance to cefoxitin phenotype and did not appear have *mec*A gene maybe resistant by others mechanism.

**Prevalence of Panton-Valentine leukocidin (pvl) gene among MRSA isolates**

PVL is an exotoxin causing pore-forming consider bi-component (components S and F) so it called a synergohymenotropic toxin, it has been observed some isolates of *S. aureus*
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recovered from patient suffering from necrotizing pneumonia can produce this toxin so it related to pathogenicity of bacteria.\cite{21} In this study we screening about prevalence of \textit{pvl} gene among \textit{S. aureus} isolates and the results have been showed four isolates (MRSA SP1, MRSA N1, MRSA B1 and MRSA S24) harboring with \textit{pvl} gene out of seventy four of \textit{S. aureus} isolates (5.40\%) all of them are MRSA isolates so according to this study the prevalence of \textit{pvl} gene among MRSA isolates is (6.55\%). Our results revealed low prevalence of \textit{pvl} gene so the results closely to local study in Al-Nasiriya city \cite{1} showed (4.4\%) \textit{S. aureus} isolates positive to \textit{pvl} gene While disagree with others local studies mentioned to higher prevalence of \textit{pvl} gene among MRSA isolates in Baghdad city (17.1\%; 27\%) (2; 23) another study was done by \cite{11} in Manila City revealed (83\%) of \textit{S. aureus} isolates harboring with \textit{pvl} gene. Furthermore no more data available in the prevalence of \textit{pvl} gene among Iraqi \textit{S. aureus} isolates. Recently have been appeared several CA-MRSA isolates producing \textit{pvl} toxin increase spreading in the world\cite{23}.\textit{S. aureus} isolates become positive for \textit{pvl} gene by transmission of bacteriophage containing \textit{pvl} encoding gene\cite{24} because of highly distribution of virulence factors in MRSA isolates among different regions, so it is significantly to determine the prevalence of PVL toxin in both MRSA and MSSA especially this toxin associated with more abscesses formation, figure (5) showed \textit{pvl} gene fragment amplified by unpleix PCR reaction.

Figure (5) Agarose gel electrophoresis (1\% agarose, 7V/cm, for 90 min) for \textit{pvl} gene (amplified size 433 bp) compared with (100 bp) DNA ladder. lanes1,2,3,4 represent positive for \textit{pvl} gene lane M ladder and lane 5 represent negative for \textit{pvl} gene.
Diversity in epidemiological factors such as body health status and geographical location can effect on the prevalence of genes in MRSA isolates from one region to another.\textsuperscript{[25]}

**Antimicrobial sensitivity (Agar diffusion method)**

In this study The results of antibiotics susceptibility test have been revealed all MRSA isolates showed high percentage of resistance against different generation of $\beta$-lactam antibiotics (cloxacin, oxacillin, Amoxicillin /Clavulnic acid, Ceftazidine and cefipime) belong to harbouring with $mecA$ gene which encoding to pincillin binding protein 2 enzyme (PBP2a) which is a unique transpeptidase with low affinity for $\beta$-lactam antibiotics.\textsuperscript{[26]} PBP2a enzyme play role in catalyse the transpeptidation reaction that cross-links the peptidoglycan of bacterial cell wall.\textsuperscript{[26]} our results are agree with many local studies were done on MRSA isolates\textsuperscript{[27]} who was founded MRSA isolates in Al-Diwaniya city resistant toward oxacillin (100%) also (2) mentioned to high resistance in MRSA isolates (100%) toward oxacillin in hospitalized patents in Baghdad, while our results partially disagree with\textsuperscript{[28]} who mentioned high resistance of MRSA isolates toward Amoxicillin /Clavulnic acid (93.1%) and low resistance toward oxacillin (29.5%), the main reason belong continuous rising resistance toward $\beta$-lactam antibiotics may attribute to misuse of the antibiotics by the people \textsuperscript{[29]} as shown in table \textsuperscript{[3]} MRSA isolates showed moderate resistance toward tetracycline (31.11%) while high no. isolates showed intermediate resistance (49.18%), the current results disagree with \textsuperscript{[28]} who mentioned the resistance of MRSA isolates (61%) on the other hands agree with\textsuperscript{[31]} who mentioned the resistance (34.9%) and intermediate resistance (15%).the active site for tetracycline is 30S ribosomal subunit, MRSA isolates can raise resistant toward tetracycline by two way chromosomal mutation and by acquired plasmid has $tetK$ and $tetL$ genes which responsible for active efflux pump furthermore $tetM$ and $tetO$ genes responsible for ribosomal protection and modification.\textsuperscript{[30]} MRSA isolates showed resistance (24.60%) toward Azithromycin which is belong macrolides play role in inhibition of protein synthesis, the resistance predominant belong to harbouring MRSA isolates with $ermA$, $ermC$ and $msrA$ genes which encoding to ribosomal methylase enzymes.\textsuperscript{[31]} Our results disagree with\textsuperscript{[32]} showed the resistance and intermediate resistance among MRSA isolates (63.64%, 10.90%) respectively. (16.40%) of MRSA isolates showed resistance to vancomycin VSRRA and intermediate resistance VISA (55.73%) we observed high numbers of intermediate isolates compared with rate of resistance this disagree with local studies \textsuperscript{[28]} showed VRSA (25%) one the hands\textsuperscript{[16]} showed the rate of VRSRA (37.5%) and VIRS (12.5%) this belong to harboring
with plasmid or mobile genetic element Tn1546 carry vanA, B, C, D, E, F and G genes which encoding to six types of phenotypic resistance.

MRSA isolates showed low resistance to gentamycin (13.11%) but relatively high intermediate resistance (73.78%) this results closely to local study[^33] showed the rate of MRSA isolates resistance (13.63%) toward gentamycin but[^29] showed the rate of MRSA resistance (29.3%) and this disagree with us. It observed variant level expression of resistance toward gentamycin among MRSA isolates, this belong to transposon Tn4001 has different chromosomal binding site for integration so low insertion specificity leading to low expression of resistance genes (18). MRSA isolates resistance to ciprofloxacin (13.11%) with low intermediate resistance (1.63%) and high sensitivity (85.26%). Finally the results showed the lowest resistance rate of MRSA isolates toward imipenim (6.55%) with the highest sensitivity rate (91.84%) with low intermediate rate (1.63%). The results of current study was closely with (6) showed MRSA sensitivity toward imipenim (97%) table 3 showed MSSA rate of resistance toward previous antibiotics. The results are reflected the specific variation in antibiotics usage may be exporting the geographical distribution of resistance, so it critical to identify the etiology of infection and antibiotic sensitivity pattern of MRSA because this pattern consider alarming trend that mandating to antibiotics policy to minimize resistance.

### Table 3: Percentage and antibiotics sensitivity for MRSA and MSSA isolates

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Code</th>
<th>Total MRSA No. 61(%)</th>
<th>Total MSSA No. 13(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R</td>
<td>I</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>CX</td>
<td>61(100)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>OX</td>
<td>61(100)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>FEP</td>
<td>61(100)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Amoxicillin /Clavulenic acid</td>
<td>AMC</td>
<td>60(98.4)</td>
<td>1(1.63)</td>
</tr>
<tr>
<td>Ceftazidine</td>
<td>CAZ</td>
<td>58(95.2)</td>
<td>1(1.63)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>TE</td>
<td>19(31.1)</td>
<td>30(49.2)</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>AZM</td>
<td>15(24.6)</td>
<td>14(22.95)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>VA</td>
<td>10(16.4)</td>
<td>34(55.7)</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>CN</td>
<td>8(13.1)</td>
<td>45(73.8)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>CIP</td>
<td>8(13.1)</td>
<td>1(1.63)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>IPM</td>
<td>4(6.55)</td>
<td>1(1.63)</td>
</tr>
</tbody>
</table>

### Dendrogram and phylogenetic analysis

Antibiogram typing was built on comparison of sensitivity among isolates toward many antibiotics, So identification of unusual pattern of antibiotic resistance among isolates from different patients can be indicator for outbreak and epidemiology of pathogenic isolates.[^34] In
the current study all information were obtained from antibiotics sensitivity toward (74) isolates of MRSA and MSSA were participated in phylogenetic tree and dendogram.

Dendogram was built by suing unweighted pair group method (UPGMA) and genetic distance model Tamura-Nei with cost matrix identity (1.0-0.0) and geneious software. Figure (6) showed dendogram and phylogenetic tree have (151) node and (76) tips. as shown in the dendogram the isolates segregated into two major groups A and B also each group has more than one cluster, Group A was divided into two smaller group A1 and A2, the phylogenetic tree was reflected highly diversity resistance antibiogram among isolates also high diversity between MRSA and MSSA isolates still found some similarities in antibiogram pattern as it shown in cluster related to A1a2 MRSA S12 isolate and MRSA EN3 did not have differences in antiobiogram pattern on the other hands the cluster related to A1a1 was segregated in it some isolates not only sharing same antibiogram but also having same genetic markers, MRSA N1 as well as MRSA SP1 have *pvl* gene. These results were reflected the possibility of same clone for circling and spreading to do more than one infection in different site of infection, so it maybe consider this clone as epidemic clone.

Despite *S. aureus* is harmless in healthy individuals as well as health workers, they could become carries with highly risk for spread infection to other patients belong to their dealing and interaction with them.\[35\] Unfortunately in Iraq there is no literature reported specific epidemic clone of MRSA or determined the specific genetic markers pattern to frequent isolates to compare our results with them because this study the first one mention the approach of MRSA typing. Despite of antibiogram is a simple, inexpensive and rapid perform technique in routine microbiology laboratory; however in most circumstances cannot used only typing methods for MRSA belong its poor discriminatory power.\[36\] Because of antibiotic resistance patterns are influenced by selective antibiotic pressure, environment, gained or loss plasmid carrying resistance genes and many other genetic mechanisms.\[9\]
CONCLUSION
The results of current study have been showed S. aureus isolates isolated from skin infection conform the highest percentage of isolation, also the genotypic method is more accurate from phenotypic method to detect MRSA and consider golden standard method to confirm S. aureus to be MRSA. Furthermore critical detecting prevalence of pvl gene in MRSA isolates to determine CA-MRSA and the important of antibiogram pattern to identify the etiology of infection and consider precaution antibiotics policy to minimize resistance.

Figure (6) dendogram of antibiotics and phylogenetic tree
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33. Al-Geobory H A. Comparative study between Methicillin resistant Staphylococcus aureus (MRSA) and Methicillin sensitive Staphylococcus aureus (MSSA), and detect the antimicrobial effects of some plant extracts on them. Msc. Thesis. College of Science/Baghdad University. Iraq. 2011

