Identification of Methicillin Resistance *Staphylococcus aureus* in Immunocompromised Host Using PCR Techinque and Detection of Its Sensitivity to Antibiotics

Noor M. Abdullah
Amna N. Jassim
Abaas A. Alani
University of Baghdad,
College of Science for women

Abstract

Two hundred and twenty clinical specimens were collected from were collected from patients in Medical City Hospitals (Baghdad /Iraq).During the period From November 2013 to April 2014.The specimens were included (54) ear swab, (56) blood, (70) burn swab, and (40) urine sample. It was found that (49.03%) of the 108 isolates were belong to Staphylococcus spp., were diagnosed as coagulase-positive staphylococci (COPS), where 112 (50.9%) of clinical samples were coagulase negative (CONS). From the collected clinical samples, 85(78.7%) were MRSA according to sensitivity test and vitek system, and the rest were MSSA 23(21.9%).

In the present study, ceftaroline is drug of choice, because of it activity against Methicillin-resistant *S. aureus* .The effectiveness of the ceftaroline antibiotic are being tested on 85 isolate of Methicillin-resistant *S. aureus* (MRSA), all isolate were sensitive to ceftaroline.

Keywords: *S. aureus.* MRSA, Ceftaroline, Susceptibility test.

Introduction

The *S. aureus* bacterium is a serious human pathogen that causes life-threatening nosocomial and community associated infections (1). Staphylococcal infection can affect many sites and organs of the human body. Invasion of the skin cause impetigo, cellulitis. In the lungs abscesses and pneumonia are the result. Infection of the heart leads to endocarditis. Meningitis and abscess formation can be the result of infection to the central nervous system as well as, keratitis can be the result of eye infection (2).

S. aureus are grouped into two major classes: Methicillin Sensitive *S. aureus* (MSSA) and Methicllin Resistant *S. aureus* (MRSA). MRSA strains have been associated with nosocomial or hospital acquired infections world over and have also emerged as an important cause of community acquired infections (3). Resistance to methicillin is mostly determined by the presence of mecA gene encoding altered penicillin binding protein which shows low affinity to β-lactam antibiotics (4).The mecA gene, which originates from a mobile genetic element named the staphylococcal cassette chromosome mec [SCCmec] invariably inserted into the orfX gene of methicillin-resistant staphylococci, is the genetic basis of methicillin resistance (5, 6).

Vancomycin has been the cornerstone of treatment of patients with serious MRSA infections. Consequently, vancomycin use has been increasing since the mid-1980's, resulting in the emergence of MRSA with reduced susceptibility to vancomycin (7). However many researchers have been noted MRSA that resistant to vancomycin (8,9).

The cephalosporin class of antimicrobial agents is known for its broad spectrum of activity, proven efficacy and favourable safety profile, making it the most commonly prescribed class of antimicrobials (10).Ceftaroline fosamil is a cephalosporin prodrug whose active principle, ceftatoline (CPT), is active against MRSA and drug-resistant *S. pneumoniae* (11). This compound has the distinction of being the first anti-MRS β-lactam to be marketed in the USA (2010), where it received FDA approval for treatment of acute bacterial skin and skin structure infections (SSSI) and for community-acquired pneumonia (CAP) (12). Ceftaroline shows good clinical efficacy against (MRSA) due to its ability to bind to PBP2A (13)

Materials and Methods

Staphylococcus aureus isolation and identification

Specimen's collection: From November 2013 to April 2014, Two hundred and twenty specimens were collected from patients in Medical City Hospitals (Baghdad /Iraq). The specimens were included (54) ear swab, (56) blood, (70) burn swab, and (40) urine sample.

Isolation: The collected specimens were inoculated on the blood agar, incubated at 37ºC for 24 hours.The isolates were examined for their haemolytic activity. Then transferred and streaked on mannitol salt agar, and to detecting the ability of each isolate to ferment mannitol. All plates were incubated at 37ºC for 24 hours, then transfer a single pure isolated

colony to nutrient agar medium for the preservation and to Completion other biochemical tests that confirmed the identification of isolates.

Identification of *Staphylococcus aureus*

1- Gram's stain: The isolates were stained by Gram stain to detect their grape-like clusters of blue color under microscopic examination (14).**2-Cultural characteristics:** Different cultural characteristics (color, shape, edge, and size) of *S. aureus* was studied on the growth media (mannitol salt agar and blood agar) (15).**3-Biochemical tests** (Catalase test, Tube coagulase test, Oxidase test, and Mannitol (fermentation test) **.4-Susceptibility test. 5- Vitak II system.**

DNA Extraction

DNA Extraction from Bacterial isolates

DNA extracted and purifed by using Wizard® genomic DNA purification kit (promega, Madison, WI, USA).

Specific primers: *MecA* primer

Sequences of oligonucleotide primers and their location in the *mecA* gene (16)

Primer	Primer sequence	Product length (basepairs)	Location (nucleotide numbers)
mecA	Mec-A1 (+)AAAATCGATGGTAAAGGTTGGC	533	1282-1303
mecA	Mec-A2 (-) AGTTCTGCAGTACCGGATTTGC	533	1739-1814

Polymerase Chain Reaction (PCR) Technique

Target DNA amplification with a pair of primers, resulting in several copies of the target sequence (17).

Preparation disks of ceftaroline

Ceftaroline stock solution prepared by dissolving 600 mg in 20 ml of D.W.The reconstituted solution is a pale yellow solution that is free of any particles. Each disk should contain 30 Mg of ceftaroline, were prepared according to this equation: **C1 * V1= C1 * V2**

Statistical Analysis

The Statistical Analysis System- SAS (2012)(18) was used to effect of different factors in study parameters. Chi-square test was used to significant compare between percentage in this study.

Results and Discussion:

Two hundred and twenty clinical samples were collected from different patients, the samples included burn swab, ear swab, urine, and blood table (1).108 (49.03%) of clinical samples were *S. aureus* giving

positive result in coagulase test ,catalase test ,and biochemical test ,where 112 (50.9%) of clinical samples were coagulase negative.

Table (1) Prevalence of *S. aureus* isolates among different specimens

Source of sample	Total No.	No. of *S. aureus* isolates	Percentage (%)
Ear swab	54	23	42.59 %
Blood	56	20	35.7 1%
Urine	40	16	40 %
Burn	70	49	70 %
Total	220	108	49.09%

The highest percentage of MRSA isolates, was from burn samples, it was 93.87% table (2).This high percentage may attributed to that the skin which represent the first line of defense and an example of innate immunity is damaged and the burned parts of patient remain exposed to air, that increase opportunity of infection with pathogenic bacteria .Burns remain considerable serious problem of public health associated with morbidity and mortality ,our Explanation close to the explan of Othman and Kendrich, (2011)(19). Burn patients become susceptible to infection due to the loss of the protective barrier and decreased cellular and humoral immunity (20).Infection remains a major complication in burn patients after initial period of shock and the chance of infection persist until complete wound healing (21).

From the collected clinical samples, 85 (78.7%) where MRSA according to sensitivity test and vitek system, and the rest were MSSA 23(21.9%).Present results were agree with agree with the results of a local study by Al-Maliki, (2009)(22) who showed that the percentage of MRSA to the MSSA were (80.3%, 16.4%) respectively.As well as, Al- Hasani, (2011)(23) reported that the ratio of MRSA was (83.70%). On the other hand, Al-Geobory, (2011)(24) showed that the ratio of MRSA was (90.90%). On the other hand, current results did not agree with the results of peck et al., (2009)(25) which showed that only (51.4%) of isolates were methicillin resistant. Also, the finding of AL-alem, (2008)(26) showed that the ratio of MRSA strain was (56%). These observed differences may due to the variation in the geographic area, sources of clinical specimens, genetic background and the collection site of isolates.

Table (2) Prevalence of MRSA isolates among *S. aureus* isolates

Source of sample	No. of *S. aureus* isolates	No. of MRSA isolates	Percentage (%)
Ear swab	23	17	73.91 %
Blood	20	14	70 %
Urine	16	8	50 %
Burn	49	46	93.87 %
Total	108	85	78.7 %

Susceptibility of *S. aureus* isolates was detected against (10) types of antibiotics, the tested pathogenic isolates were found to exhibit obvious level of resistance against the used antibiotics and the susceptibility pattern for these clinical *S. aureus* isolates are shown in table (3). In this study the results demonstrated that out of (108) tested *S. aureus* isolates that were isolated from(4) sources, about (85) isolates showed a high level of resistance to Penicillin G , Chloramphenicol, Cefoxitin, Oxacillin , and Methicillin respectively table(3) .

Table (3) Resistance & Sensitive to Difference Antibiotic Used for Sensitivity Test of *S. aureus* Isolates

Antibiotic	Resistance	Intermediate	Sensitive
Gentamycin	60 (55.56%)	12 (11.11%)	36 (33.33%)
Erythromycin	70 (64.81%)	8 (7.41%)	30 (27.78%)
Clindamycin	81 (75.00%)	18 (16.67%)	9 (8.33%)
Cefoxitin	89 (82.41%)	2 (1.85%)	17 (15.74%)
Chloramphenicol	90 (83.33%)	0 (0.00%)	18 (16.67%)
Penicillin G	90 (83.33%)	0 (0.00%)	18 (16.67%)
Oxacillin	86 (79.63%)	0 (0.00%)	22 (20.37%)
Vancomycin	24 (22.22%)	0 (0.00%)	84 (77.78%)
Tetracyclin	82 (75.92%)	6 (5.56%)	20 (18.52%)
Methicillin	85 (78.70%)	3 (2.78%)	20 (18.52%)

The vitak- 2 Compact was used to confirm the identification and typing of MRSA isolates which previously identified by conventional biochemical tests, the result from vitak- 2 were closely with those obtained from sensitivity test for same antibiotics that used in both tests.

A confirmatory test was carried out for the selected isolates using PCR technique for further characterization up to the species level by the amplification of (mecA) gene ,and all the isolates are found to be positive for the presence of (mecA) gene figure(1).

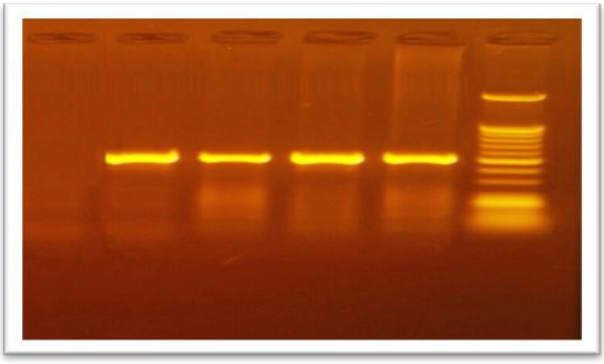

Figure (1) Agarose gel electrophoresis of PCR product amplified from mecA genes. These genes from MRSA isolates. M = DNA marker fragments. Lane 1, 2, 3 & 4 indicate the mecA positive samples, 5 negative control. The DNA fragments of 533 bp were amplified from mecA gene.

In the present study, ceftaroline is drug of choice, because of it activity against MRSA .The effectiveness of the ceftaroline antibiotic are being tested on 85 isolate of MRSA, 39 (45.88%) isolates gave 32 mm dimmter inhibition zone, 18 (21.17%) isolates gave 30 mm dimmter inhibition zone, 8(9.4%) isolates gave 28 mm dimmter inhibition zone, 7 (8.2%) isolates gave 27 mm dimmter inhibition zone, and 13 (15.29%) isolates gave 26 mm dimmter inhibition zone figure (2). All 85 isolate of (MRSA) are sensitive to ceftaroline, so ceftaroline has demonstrated bactericidal in vitro activity against MRSA, and this conclusion agree with (27,28)

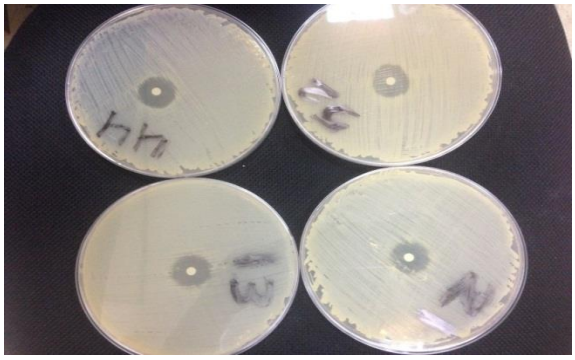

Figure (2) The effect of ceftaroline on MRSA with bactericidal in vitro activity

In the present study the pattern of minimum inhibitory concentration of *S.aureus* isolates to ceftaroline, was determined using MIC method; the results revealed that MIC range from (0.5 µg/ml - 0.12 µg/ml), for 81 (95.29%) MRSA isolates the MIC (0.5 µg/ml), 3 (3.52%) MRSA isolates the MIC (0.25 µg/ml), and 1(1.17%) MRSA isolate the MIC (0.12 µg/ml).MIC results for the tested clinical isolates are summarized in table (4).

Table (4) MIC Values of Ceftaroline

MIC	No. of isolates	Percentage (%)
0.5 µg/ml	81	95.29%
0.25 µg/ml	3	3.52%
0.12 µg/ml	1	1.17%

Ceftaroline demonstrated good activity against MRSA, It in vitro activity against MRSA is related to its high affinity for PBP2a, (29).Different ceftaroline MICs value depending on geographical location (30), and inverse correlation between PBP2a binding affinity and ceftaroline MIC, which is expected because the anti-MRSA activity of ceftaroline reflects binding and inhibition of PBP2a (31). Data from case series suggest that ceftaroline is safe and effective for severe MRSA infections with success rates in over 70% of cases (32,33), and Polenakovik

and Pleiman, 2013). To date, clinical trials have demonstrated efficacy of ceftaroline similar to that of comparator agents in the treatment of ABSSSI and CABP. Clinical trials suggest that ceftaroline is well tolerated common to the cephalosporin class. Overall, the most common adverse events occurring in more than 2% of patients in clinical trials were diarrhea, nausea, and rash (34).

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التعرف على بكتريا العنقوديات الذهبية المقاومة للميثيسيلين في الاشخاص المثبطين مناعيا بأستخدام تفاعل البلمرة المتسلسل والتحري عن حساسيتها للمضادات الحيويه

نور ماجد عبد الله أ.د. امنه نصيف جاسم أ.د. عباس عبد المعيد مصطفى

الخلاصة

شملت هذه الدراسة جمع (206) عينة من مرضى يعانون من امراض ضعف المناعة في مستشفى مدينة الطب (العراق/ بغداد)، خلال الفترة من شهر تشرين الثاني /2013 الى شهرنيسان/ 2014. تضمنت العينات (70) مسحة من الحروق ، (56) عينة دم،(54) مسحة من الاذن، (40) عينة ادرار. اظهرت الدراسة ان (49.03%) 108 من العزلات تعود الى جنس *S.aureus* حيث اعطت نتيجة موجبة لفحص الانزيم المخثر للبلازما COPS (Coagulase-Positive Staphylococci)،في حين اعطت 112 (50.9%)من العزلات نتيجة سالبة لفحص الانزيم المخثر للبلازما-Coagulase Negative Staphylococci)CONS.من بين 108 عزلة بكتيرية من COPS شخصت85 (78.7%) عزلة بكتيرية على انها بكتريا العنقوديات الذهبية المقاومة للميثسلين MRSA ( ) methicillin resistance *Staphylococcus aureus* طبقا لاختبار الحساسية للمضادات الحياتية وجهاز الفايتكvitek 2 system،بينما شخصت 23 (21.9%) عزلة بكتيرية على انها بكتريا العنقوديات الذهبية الحساسة للميثسلين methicillin sensitive (MSSA *Staphylococcus aureus).*