Identification Of *Staphylococcus Aureus* Isolated From Different Infections And Study The Ability Of Nuclease Production

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Abstract

This study was conducted for the period from 1/3/2016 to 15/6/2016 in Baquba city in Iraq. Eighty samples were collected from different infections from Baquba General Hospital and AL-Batool Hospital. Twenty isolates (25%) were found to be *Staphylococcus aureus* . The susceptibility test was applied on these isolates against (12) antibiotics. The results revealed that the highest resistances were for Penicillin and Methicillin with 100% for each, while the lowest resistance were for Erythromycin (6.3%) and Vancomycin (9.1%). The results of virulence factors that had *S. aureus* showed possession of all isolates many virulence factors and a high production of which increases the pathoginicity of it. All isolates were able to produce urease (100%), heamolycin (100%), protease (85%), lipase (80%) and gelatinase (100%). Twenty isolates were screening for nuclease production, the isolates SA9 and SA19 were found to be an efficient nuclease production, 30mm the diameter of clear zone around colonies were cultured on DNase agar.

Key wards: *Staphylococcus aureus*, nuclease production, Antibiotics.

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Identification of *Staphylococcus aureus* isolated from different infections and study the ability of nuclease production

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تشخيص بكتريا المكورات العنقودية الذهبية المعزولة من اصابات مختلفة و دراسة قابليتها على انتاج انزيم النيوكلييز

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الخلاصة

اجريت هذه الدراسة للمدة من 2016/3/1 الى 2016/6/15 في مدينة بعقوبة في العراق. جمعت 80 عينة من اصابات مختلفة من مستشفى بعقوبة العام و مستشفى ألبتول عشرون عزلة (25%) منها تعود الى المكورات العنقودية. اجري فحص الحساسية على العزلات ضد (12) مضاد حيوي. كشفت النتائج مقاومة عالية (100%) للمضادين البنسيلين و الميثيسيلين، بينما كانت االمقاومة منخفضة للمضادين الاريثرومايسين (6.3%) و الفانكومايسين (9.1%). اظهرت نتائج عوامل الضراوة التي تمتلكها عزلات المكورات العنقودية ارتفاعا في معدل انتاج هذه العوامل مما يزيد من امراضيتها. جميع العزلات كانت لديها القابلية عل انتاج أنزيم اليوريز (100%)، الهيمولايسين (100%) بينما كانت (85%) من عزلات منتجة لانزيم البروتييز و انزيم الليبيز (80%) و انزيم تمييع الجيلاتين (100%). اختبرت قابلية جميع العزلات على انتاج انزيم النيوكلييز، كانت العزلتين 9 و 19 الافضل في انتاج الانزيم، حيث كان قطر المنطقة الشفافة حول المستعمرات المزروعة على وسط الدنيز 30 مليميتر.

الكلمات المفتاحية: المكورات العنقودية، انتاج انزيم النيوكلييز، المضادات الحيوية

Introduction

The genus *Staphylococcus* is belong to the family *Staphylococcaceae* it has at least 35 species and coagulase activity or coagulase test is usually used to distinguish among *Syaphylococcus spp.* Coagulase producing *Staphylococci* in medical microbiology this term is synonymous with *Staphylococcus aureus* but coagulase – negative Staphylicocci the term is used referring for these species that do not produce coagulase [1,2] . *Staphylococcus aureus* is a gram positive, non-spore forming , non-motile bacterium, usually arranged in grape- like irregular clusters (staph) spherical shape (cccus), facultative anaerobes, chemoorganotrophic and fermentative metabolism [3,4]. *S. aureus* is a major human pathogen that occurs in many different types of

infections of the human body, this due to S. aureus have different virulence factors as producing a wide variety of enzymes and toxins. Nearly all strains of *S. aureus* secret a group of enzymes which includes coagulase, nucleases, proteases, lipases, Dnase and collagenase. The main function of these proteins may be convert local host tissues into nutrients required for bacterial growth[5,6]. Some strains of *S. aureus* produce one or more additional exoprotein (toxins), include; toxic shock syndrome and exofoliative toxin. Neverthelese, *S. aureus* produce enterotoxins and a number of cytotoxic molecules[3]. Infections with *S. aureus* have been difficult to handle due to the development of drug resistance, as methicillin-resistance *S. aureus* (MRSA). In addition, MRSA are resistance to all penicllins[5,7]. The staphylococcal nuclease encoded by the nuc 1 gene, is an important virulence factor *S. aureus* . Deoxyribonucleases are group of enzymes that are capable of hydrolyzing the phosphodiester linkages of nucleic acids. Nucleases play vital role in cellular functions; specifically 4 R's i.e. replication, repair, restriction and recombination. These are also involved in transposition, transcription and topoisomerization, as well as RNA processing, RNA splicing, editing and interference. DNases has wide range of applications both inside and outside the cells. By using nucleases in different ways, it has become easier to recombine DNA, remove harmful genes, and replace single gene on DNA strand; applications include gene therapy for genetic diseases, genetic engineering, DNases has been used in chemotherapeutic and industrial fields also[4,8,9,10].

Materials and Methods

Samples collection:

Eighty different clinical samples were collected from patients and carriers in Baquba General Hospital and Al-Batool Hospital over period from 1/3/2016 to 15/7/2016. The samples were included (30 from urin, 14 from ear swabs, 16 from wound and 20 from burn). The samples were tested for all tests in microbiology unit in Al-Batool Hospital Laboratory.

Isolation and Identification of *Staphylococcus aureus*:

The collected samples were inoculated on the blood agar, incubated at 37ºC for 24 hours. The isolates were examined for their shape, size, colour, pigments, and haemolytic activity. Then

transferred and streaked on mannitol salt agar for detecting the ability of each isolate to ferment mannitol. All plates were incubated at 37ºC for 24 hours then a single pure isolated colony was transferred to Nutrient agar medium for the preservation and to carry out other biochemical tests that confirmed the identification of isolates. The isolates were identified according to the Bergey's Manual [11] and according to [12] as the following: gram stain and biochemical tests which included (catalase test, oxidase test, nitrate reduction test and pigment production test).

Antimicrobial susceptibility test:

The sensitivity and resistance of *S. aureus* to antimicrobials agents was tested by the disc diffusion method on Muellar-Hinton agar using antibiotic discs according to Clinical and Laboratory Standards Institute (CLSI) guidelines [13]. Twelve antibiotics were tested: Vancomycin (30Mg), Amikacin (30µg), Tetracycline (30 µg), Ciprofloxacin (5 µg), Penicillin (10 µg), Amoxicillin (10 µg), Ticarcillin (75 µg), Erythromycin (15 µg), Gentamycim (30 µg), Rifampin (5 µg), Methicillin (5 µg)and Cefotriaxoue (30 µg). Interpretation of inhibition zones was carried out based on the manufactures and CLSI guidelines [13].

Detection of virulence factors:

The *S. aureus* ability to produce some of virulence factors (enzymes and toxins) were recognized and tests were applied on 20 isolates that identified. it included: Haemolysin production, Lipase production, protease production, urease production and gelatin liquefaction[14].

Detection of nuclease production:

Nuclease production was tested by cultured on DNase agar plate following the procedure described by the manufacturer. The plate were incubated at 37C˚ for 24 hours then the plate was observed for the presence of clear zone around the colony which indicated a positive DNase test [15].

Determination of nuclease amount:

The nuclease amount was determined according to the Brad ford method [16] and used Bovine serum albumen (BSA) as standard curve.

Results and Discussion

Isolation and Identification:

Eighty samples were collected from patients and carriers, the samples comprised from (urin, ear swab, wound and burn). Twenty isolates (25%) have the ability to grow on the Mannitol salt agar which considered selective and differential media for genus *Staphylococcus* [17]. All 20 isolates had ability to ferment mannitol and form large golden colonies surrounded by wide yellow zones. Microscopic examination was used to all 20 isolates after staining by gram stain and cells appeared as Gram-positive cocci arranged in grape-like irregular clusters. For further identification some of the biochemical tests was performed on 20 isolates, included: catalase test was all 20 isolated gave positive results. The 20 isolates gave the negative result to the oxidase test. Also all 20 isolates were positive to coagulase test. Additionally, nitrate reduction test was applied for further identification because the S. *aureus* often reduce nitrate to nitrite.

All 20 isolates were tested for their ability to produce pigments on the skimmed-milk agar and the colonies formed deep golden yellow pigment. All these results was agreed with other results reported by other researchers [3,18,19,12].

Susceptibility test of *Staphylococcus aureus*:

The sensitivity of 20 isolates were tested against 12 antibiotics. The susceptibility test was applied according to the Kirby-Baure Method (antibiotic disc diffusion method).

Table (1): Percentage sensitivity and resistance of different antibiotics for *Staphylococcus aureus.*

Antibiotics	(%) of Resistance	(%) of sensitivity
Vancomycin	9.12%	90.88%
Amikacin	25.49%	74.51%
Tetracycline	16.23%	83.77%
Ciprofloxacin	12%	88%
Penicillin	100%	0%
Amoxicillin	25.80%	74.19%
Ticarcillin	16.90%	83.1%
Erythromycin	6.3%	93.7%
Gentamycin	18%	82%
Rifampin	12.5%	87.5%
Methicillin	98%	2%
Cefotriaxone	49.2%	50.8%

The results in table (1) showed that all isolates (100%) were resistance to penicillin antibiotic and this results was agreed with local studies by Hala, M.H.(2011)[2] and Al-Maliki (2009)[20] 100% and 97.8%reported." This increased in penicillin resistance isolates among *Staphylococcus aureus.* strains can be explained in most cases to production of β- Lactamase enzyme that destroyed the β- Lactam ring and inactivated the penicillin and this enzyme was encoded by plasmid that easy to transfer among strains [3]. *Staphylococcus aureus.* was resists to Methicillin (98%), this result agreed with others studies [6,19], this resistance is due to the acquisition of new penicillin-binding protein; PBP2a, which has law affinity to most β-Lactam antibiotics and also, mediates cross-resistance to all these compounds[21]. The resistance of cefotrixone was (49.2%), while *S. aureus* resists Amoxicillin , Amikacin and Gentamycin with 25.8%, 25.4% and 18% respectively. The results showed that resistance to other antibiotics such as Tricarcillin (16.9%), Tetracycline (16.2%) and Rifampin (12.5%) , while resistance of ciproflaxin, vancomycin and erythromycin was (12%), (9.1%) and (6.3%) respectively, this results that constant with other studies[2,19,22]. In general the resistance to different antibiotics may be due to the type of antibiotics and how much that used among the patients in the

community. In addition to that the resistance to ward any antibiotics was depended on the amount of PBP2a or β-Lactamase enzyme that produced by each strain of *S. aureus* . All these reasons could create variations in the rate of resistance.

Detection of some virulence factors

Results in table (2) revealed that the detection rate of some virulence factors of *S. aureus*, it was highest in production of urease(100%), haemolysin (100%), protease (85%), lipase (80%) and gelatinase (100%). These results were agreement with that reported by local researchers [2,22]. Additionally, worldwide studies reported results consistent with present results in highest production of virulence factors of *S. aureus*[6,19,23,24].

Table (2): Some of virulence factors of *Staphylococcus aureus*

Virulence factor	Positive results (%)	Negative results (%)
Urease	100	0
Haemolycin	100	0
Protease	85	15
Lipase	80	20
Gelatinase	100	0

Nuclease production

Table (3): Assay of nuclease production by reported the diameter of zone of DNA hydrolysis

Isolate no.	Diameter of DNA hydrolysis(mm)
SA1	18
SA2	15
SA3	22
SA4	7
SA5	26
SA6	15
SA7	10
SA8	21
SA9	30
SA10	12

SA11	19
SA12	27
SA13	13
SA14	10
SA15	24
SA16	8
SA17	17
SA18	20
SA19	30
SA20	25

Table (3) shows the detection rate of nuclease production, the ability of isolates on DNA hydrolysis was assayed by demonstrated the diameter of clearing zone around colonies.

The results revealed variation in ability of isolates to produce the enzyme, where the ability trading between 7-30mm, the isolates SA9 and SA19 was found to be an efficient nuclease producer (30mm). This result agreed with that reported by local researcher (Esam H. Hameed)[25] . So the present results were consented with the results of the study reported by David. et. al. (2010)[26] and Kamble, et. al. (2011)[8].

Determination of nuclease amount:

Table (4): Determination of nuclease amount for 8 isolates

Isolate no.	Concentration of protein (mg/ml)
SA9	19.2
SA19	18
SA12	10
SA5	8.3
SA20	9.8
SA15	12
SA3	1.6
SA8	2.8

Selected 8 isolated of *S. aurues* was found to be an efficient nuclease production on DNase agar culture for determination of nuclease amount. The results of the present study showed the isolates of SA9 and SA19 was efficient in production of nuclease by the high concentration of nuclease 19.2mg/ml and 18mg/ml. Table(4). This results agreement with other results reported by [8,26].

Conclusions

This study shows a high incidence of virulence factors that have *S. aureus*. A higher of resistance rate to antibiotics by *S. aureus* and Characterize isolates SA9 and SA19 *S. aureus* whith high efficiency on the production of the nuclease with the possibility to exploited for some practical applications such as purification of proteins.

Recommendations

A combination of phenotyping and genotyping tests is recommended for future investigations of the influence of staphylococcal nuclease and a bacteriologic and genetic study integrated on *S. aureus.*

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