

Comparison between phenotype and molecular resistance characteristic in *Staphylococcus epidermidis* isolates from wound infections in Al-Basrah province, Iraq

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ABSTRACT

Background: *Staphylococcus epidermidis* is considered the upper respiratory tract's human skin flora and mucosal membrane and displays low pathogenic capacity in healthy individuals. Drug-resistant strains can be identified as a natural result of the microflora through antibiotic therapy and are a possible cause of pathogenic strain resistance genes.

Methods: Culture, biochemical analysis and Vitek[®]2 Utilizing for identified the One hundred and fifty swab sample was collected from different wounds infected. *S. epidermidis* strain's ability to resist antibiotics was tested using a disk diffusion method. Result of antibiotic sensitivity test was confirmed and supported by Vitek[®]2 system. Also, the PCR antibiotic resistance gene was detected.

Results: Out of 150 swab samples, twelve were positive for *S. epidermidis*. Disc method was shown the 75%,66.7%,83.3% and 58.3% harboured highest prevalence of antimicrobial resistance against penicillin, oxacillin, cefoxitin and erythromycin respectively. While the moderate prevalence 50.7%,41.7% and 33.3% of resistance against tetracycline,clandomycin and ciprofloxacin respectively. Furthermore, lowest incidence was shown the 25% for both of resistance against rifampin, and gentamycin. The Vitek[®]2 system was confirmed and support antibiotic sensitivity test. A most frequently found antibiotic resistance genes amongst *S. epidermidis* strains, according to the findings, were *mecA* (91.7%), *blaZ* (91.7) , *ermA* (16.7%),*ermB* (25%),*ermC* (25%), *tetM* (25%), *tetK* (33.3 %) and *aacA-aphD* (41.7%) respectively. All *S. epidermidis* strain doesn't have ,*vanA* and *vanB* antibiotic resistance gene.

Conclusion: Frequency of resistance to antibiotic should be detected more than one method , and used the Vitek[®]2 system detected the antibiotic resistance gave better support for result. Additional PCR technique, actually very important to detect antibiotic resistance genes of *S. epidermidis* strains.

Keywords: *Staphylococcus epidermidis*; antibiotic resistant characteristic; resistant genes; wound infection..

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1. Introduction

Staphylococci are popular bacterial colonizers of human and other mammalian skin and mucosal membranes. It major Gram-positive opportunistic bacteria that have a variety of pathogenic species [1-2]. As essential nosocomial pathogens, coagulase-negative staphylococci have been increasingly recognized [3]. Primarily *Staphylococcus epidermidis*, are associated with human skin flora and upper respiratory tract mucosal membranes, and have in stable individuals, commensals have a low pathogenic risk [4].

Even then, in immunocompromised patients, *S. epidermidis* can be responsible for some serious infection [5]. Furthermore, drug-resistant pathogens can be selected as a normal part of the microflora during antibiotic therapy and are a possible reservoir for pathogenic strains resistance genes [6-7-8-9]. Infections of the prosthetic bone, artery graft, surgical site, central nervous system shunt, operation wounds, and heart system, among other things, have been linked to *S. epidermidis* [2].

There is no clear genetic difference between pathogenic and commensal *S. epidermidis* strains, and nosocomial strains are rich in virulence and antibiotic resistance genes. It has been indicated that such genes are mobilized between and within species inside the companion genome pool [10-11]. There is few available information on the molecular epidemiology of *S. epidermidis* strains existing in Iraq. Therefore, The current study looked into the incidence rate, antimicrobial resistance properties, and the distribution of *S. epidermidis* virulence factors. Isolated wound infection strains from Al-Basrah province, Iraq.

2. Materials and Methods

Collection of specimens

One hundred and fifty swab sample was collected from surgical wounds, gunshot wound wounds, burn wounds, puncture wounds, sliced wounds, lacerated wounds, and diabetic foot infection wounds through October-2018 to December-2018 in Al-Basrah province, Iraq.

Isolation and identification

Positive swab cultured was purification by repeating sub-cultivation to acquire single colonies in pure culture, After that Gram-stain, catalase, slide coagulase test, motility test, mannitol salt agar, blood agar hemolysis, nitrate reductase and urease test was used to identifying the bacterial isolates [12]. The identification of isolates was confirmed by Vitek[®]2 system.

Antibiotic sensitivity test

Susceptibility test was carried out using the standard disk diffusion screening was performed according to CLSI guidelines [13]. Penicillin, oxacillin, cefoxitin, erythromycin, clandomycin, tetracycline, gentamycin, rifampin, vancomycine and ciprofloxacin (10µg), (1µg), (30µg), (15µg), (2µg), (30µg), (15µg), (5 µg), (30µg) and (5µg) respectively. Also the result of disk test was confirmed and supported by Vitek[®]2 system.

DNA Extraction

The DNA Presto Mini g DNA Bacteria kit (Geneaid, USA) was used for genomic DNA extraction. The DNA sample was regarded pure, when the rate between 1.8 - 2.0 ng.

PCR screening of antibiotic resistance

PCR analysis was used to examine the emergence of antibiotic resistance genes in bacterial isolates table(1).

Table 1. Resistance genes that used in the present study

Gene	Product sizes	Reference
<i>blaZ</i>	639 bp	[14]
<i>mecA</i>	310 bp	[15]
<i>erm(A)</i>	421 bp	
<i>erm(B)</i>	359 bp	
<i>erm(C)</i>	572 bp	
<i>vanA</i>	713 bp	[16]
<i>vanB</i>	430 bp	[17]
<i>tet(M)</i>	158 bp	[15]
<i>tet(K)</i>	360 bp	
<i>aacA-aphD</i>	227 bp	[18]

3. Results and Discussion

Nosocomial *S.epidermidis* strains have been known for their high susceptibility to a variety of antibiotics [19-20-21]. In this study, Just 66 (44%) of 150 clinical swabs sample was obtained from different wounds infection from October to December 2018 were positive for bacterial growth., in which 15(22.72%), 7(10.61%), 9(13.63%), 6(9.1%), 8(12.12%), 8(12.12%) and 13(19.7%), surgical wounds, gunshot wound wounds, burn wounds, puncture wounds, sliced wounds, lacerated wounds, and diabetic foot infection wounds respectively..The higher percentages isolate were *Pseudomonas aeruginosa* 29 (44%), *Staphylococcus aureus*

14(21.21%) and *Staphylococcus epidermidis* 12(18.18%), followed by *Klebsiella pneumoniae* 5(7.58%), *Escherichia coli* 4(6.06%), *Burkholderia cepacia* 1(1.52%) and *Acinetobacteia baumannii* 1 (1.52%) figure (1).

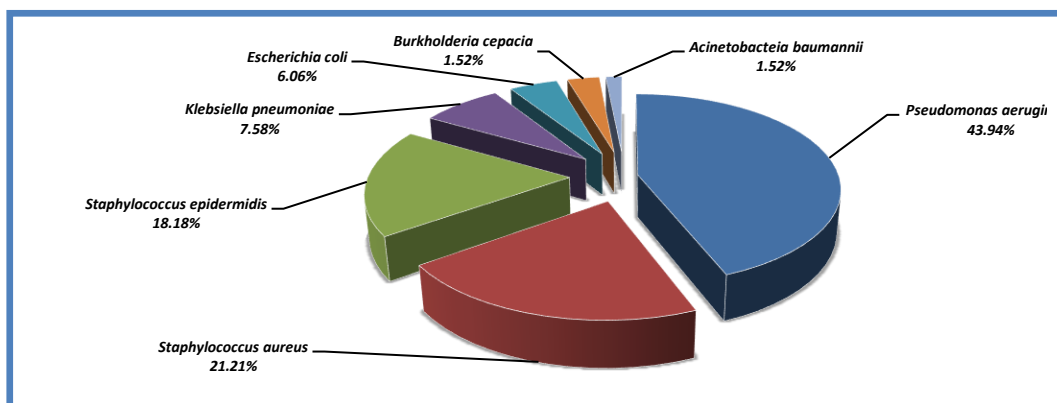


Figure 1. The bacterial species isolated frequency from studies group

The *S. epidermidis* strains were shown to be present in 12(18.18%) of wound infection samples figure (1).The result of antibiotic sensitivity test by using an antibiotic disc method was shown the 75%,66.7%,83.3% and 58.3% harboured The highest spread of antimicrobial resistance penicillin, oxacillin, cefoxitin and erythromycin respectively.While the moderate prevalence was shown the 50.7%,41.7% and 33.3% of resistance against tetracycline,clandomycin and ciprofloxacin respectively. Furthermore, lowest incidence was shown the 25% for both of resistance against rifampin, and gentamycin. Whereas the all *S.epidermidis* strain was shown a sensitive characterization against vancomycine table (1). The result of the Vitek®2 system that's used for detecting the antibiotic sensitivity test confirm and support the result of antibiotic disc method and also was shown approximately the same result table (2).

Table 2. Pattern of antibiotic resistance for *S. epidermidis* strains isolated in the current study

Positive samples for <i>S. epidermidis</i>	Antibiotic resistance pattern (%)									
	Pen. *	Oxa.	Cef.	Ery.	Clin.	Rif.	Tet.	Cip.	Gen.	Van
Surgical wound (4)	3(75)	3(75)	4(100)	2(50)	2(50)	1(25)	2(50)	1(25)	2(50)	0
Burn wound (3)	2(66.7)	2(66.7)	2(66.7)	2(66.7)	1(33.3)	1(33.3)	2(66.7)	1(33.3)	0	0
Diabetic foot wound (3)	2(66.7)	2(66.7)	2(66.7)	2(66.7)	1(33.3)	0	1(33.3)	1(33.3)	0	0
Lacerated wound (2)	2(100)	1(50)	2(100)	1(50)	1(50)	1(50)	1(50)	1(50)	1(50)	0

*Pen: Penicillin (10 µg/disk), Oxa:Oxacillin(1µg),Cef: Cefoxitin (30µg), Ery:Erythromycin (15µg),Clin: Clandomycin (2µg),Rif: Rifampin (5 µg),Tet: Tetracycline(30µg), Cip:Ciprofloxacin (5µg) , Gen: Gentamycin (15µg), and Van:Vancomycine (30µg).

Table 3. Vitek® 2 pattern of antibiotic resistance for *S. epidermidis* strains isolated in the current study

Positive samples for <i>S. epidermidis</i>	Antibiotic resistance pattern by Vitek® 2 (%)								
	β.lactmase	Oxa.*	Cef.	Ery.	Clin.	Tet.	Rif	Gen.	Van
Surgical wound (4)	4(100)	4(100)	4(100)	2(50)	2(50)	2(50)	1(25)	2(50)	0
Burn wound (3)	2(66.7)	2(66.7)	2(66.7)	2(66.7)	1(33.3)	2(66.7)	1(33.3)	0	0
Diabetic foot wound (3)	3(100)	2(66.7)	3(100)	1(33.3)	2(66.7)	1(33.3)	0	0	0

Positive samples for <i>S. epidermidis</i>	Antibiotic resistance pattern by Vitek® 2 (%)								
	β .lactmase	Oxa.*	Cef.	Ery.	Clin.	Tet.	Rif	Gen.	Van
lacerated wound (2)	2(100)	1(50)	1(50)	1(50)	1(50)	1(50)	1(50)	1(50)	0

*Oxa:Oxacillin, Cef:Cefoxitin, Ery:Erythromycin, Clin:Clandomycin, Rif: Rifampin, Tet:Tetracycline, Gen: Gentamycin, and Van:Vancomycine.

A high expansion of people who are multi-drug resistance a part from that *S. epidermidis* was also found during their investigation. *S. epidermidis* strains have emerged in Mexico with similar increases in antibiotic resistance [22]. Antibiotic resistance prevalence in the coagulase-negative staphylococci strains were 94.20%, 89.50%, 79.10%, 53.70%, 59.50%, 58.50%, 52.80%, 39.10%, 29.50%, 26.70%, 2.30, 18.40%, and 0%, reported in the study of [23] versus penicillin, erythromycin, oxacillin, clindamycin, tetracycline trimethoprim-sulfamethoxazole, ciprofloxacin, chloramphenicol, gentamicin, ceftizoxime, teicoplanin, rifampin, and vancomycin respectively. While antibiotics has been confirmed to be 100%, 100%, 0%, 37%, 33%, 0%, 16%, 80%, 80%, 0% and 0%, reported in the study of [24] to the ciprofloxacin, amoxicillin-clavulanic acid, erythromycin, clindamycin, gentamicin, mupirocin, levofloxacin, oxacillin, tetracycline, rifampin and trimethoprim-sulfamethoxazole respectively. Furthermore, resistance of the *S. epidermidis* strains in Belgium were recorded a similar patterns [25], Spain [26], Ireland [27] and Iran [28,21].

The current research also focuses into how resistance genes grow in *S. epidermidis* strains. According to the results, one of the most commonly identified *S. epidermidis* strains resistance genes were *mecA* (91.7%), *blaZ* (91.7), *ermA* (16.7%), *ermB* (25%), *ermC* (25%), *tetM* (25%), *tetK* (33.3%) and *aacA-aphD* (41.7%) respectively. While all *S. epidermidis* strain doesn't have, *vanA* and *vanB* antibiotic resistance gene table(4).

Table 4. Genes pattern of antibiotic resistance in *S. epidermidis* strains isolated in the current study

Positive samples for <i>S. epidermidis</i>	Antibiotic resistance genes (%)									
	<i>mecA</i>	<i>blaZ</i>	<i>ermA</i>	<i>ermB</i>	<i>ermC</i>	<i>tetM</i>	<i>tetK</i>	<i>vanA</i>	<i>vanB</i>	<i>aacA-aphD</i>
Surgical wound (4)	4(100)	4(100)	1(25)	0	1(25)	1(25)	1(25)	0	0	3(75)
Burn wound (3)	3(100)	3(100)	0	1(33.3)	2(66.7)	1(66.7)	1(33.3)	0	0	1(33.3)
Diabetic foot wound (3)	3(100)	3(100)	0	2(66.7)	0	1(33.3)	1(33.3)	0	0	1(33.3)
Lacerated wound (2)	1(50)	1(50)	1(50)	0	0	0	1(50)	0	0	0

The study of [29] was found the spread of antibiotic resistance genes *mecA*, *ermA*, *ermB*, *ermC*, *femA*, *tetK*, *tetM*, *blaZ* and *msrA* are 29.60 %, 33.10 %, 5.80 %, 21.60 %, 7.50 %, 13.70 %, 28.80 %, 93.50 % and 9.40 %, among coagulas-negative Staphylococci. *S. epidermidis* isolated from clinical infection samples showed a wide allocation genes for antibiotic resistance, particularly *aacA-aphD*, *tetK*, *mecA*, and *tetM*. Clindamycin resistance was found in a wide range of *S. epidermidis* strains [30].

The *ermC* gene was found in some strains of *S. epidermidis*. The aminoglycosides resistance is more common encoded by *aacA-aphD* (69.59 %). Due to the fact that gene normally widely distributed amongst staphylococci human origin [31]. According to [32], the *ermA*, *tetK*, *ermC*, *vatA*, *vatB*, *vatC* and *tetM*, *aacA-aphD* were found in 30.90 %, 76.40 %, 74.50 %, 74.50 %, 1.80 %, 0 %, 5.50 % and 16.40 %, of staphylococci strains isolated from human infection. The frequency of resistance to much more seven types of antibiotics was found to be 17.39 % in a study of [21].

4. Conclusion

Frequency of resistance to antibiotic should be detected more than one method , and used the Vitek®2 system detected the antibiotic resistance gave better support for result. Additional PCR technique, actually very important to detect antibiotic resistance genes of *S. epidermidis* strains.

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