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Thesis Title:

Investigation of molecular characteristics of *Staphylococcus aureus* strains isolated from bacteremia patients

Ethical code: IR.SBMU.MSP.REC.1398.894

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1399-1400

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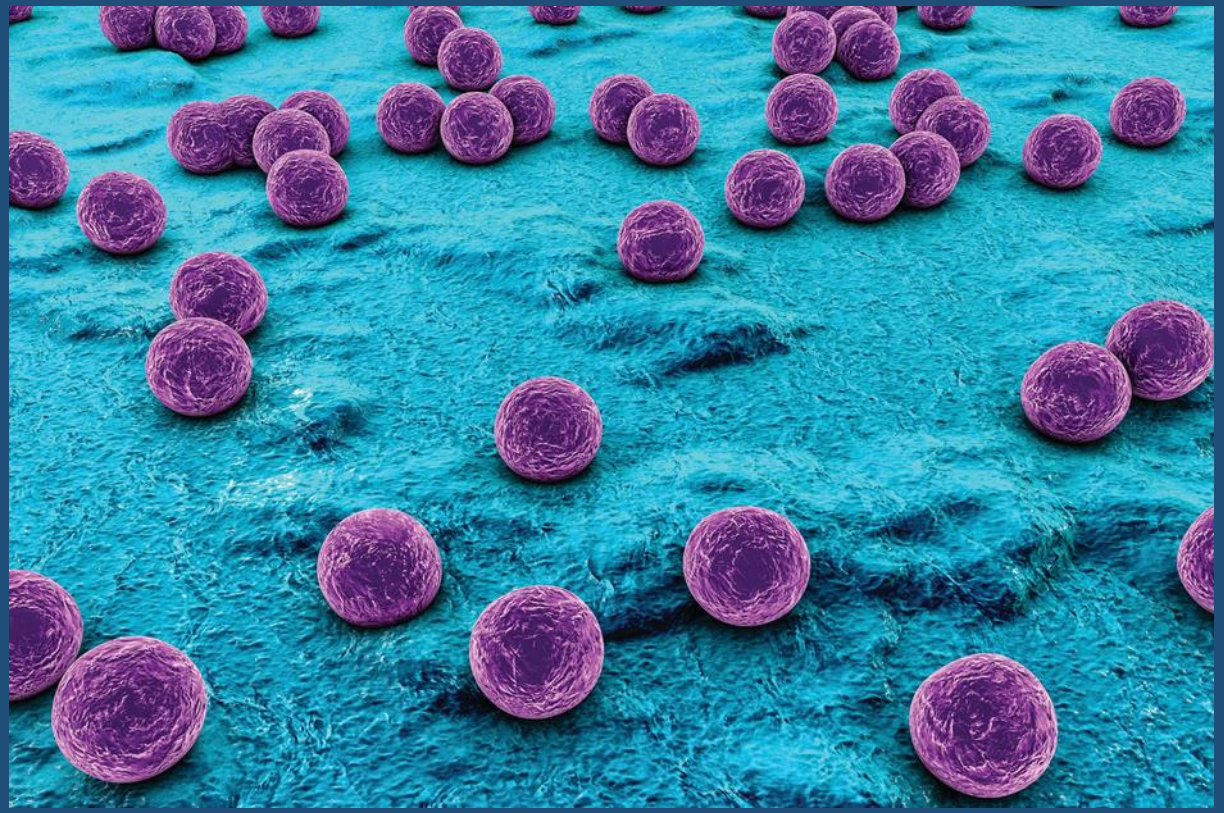
Introduction



Staphylococcus aureus

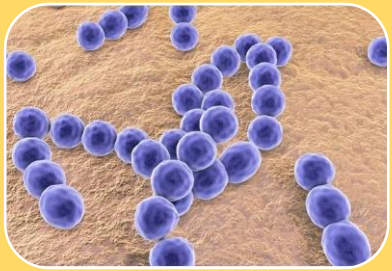
Staphylococcus aureus is both a commensal bacterium and a human pathogen.

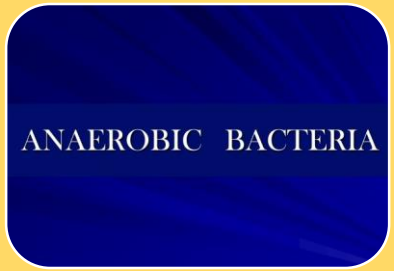
- Causes a wide array of infections, from a simple skin infection to more dangerous situations such as bacteremia, endocarditis, pneumonia & many others that may jeopardize the life of patient.

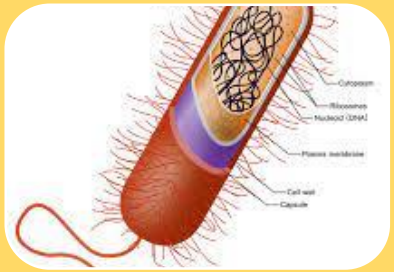
Taxonomy

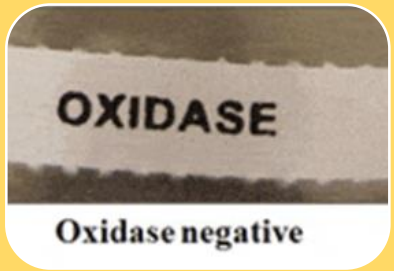
Phylum	• **Firmicutes**
Class	• **Bacilli**
Order	• **Bacillales**
Family	• **Staphylococcaceae**
Genus	• ***Staphylococcus***
Species	• ***aureus***

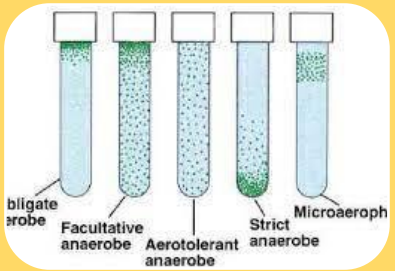
General Characteristics





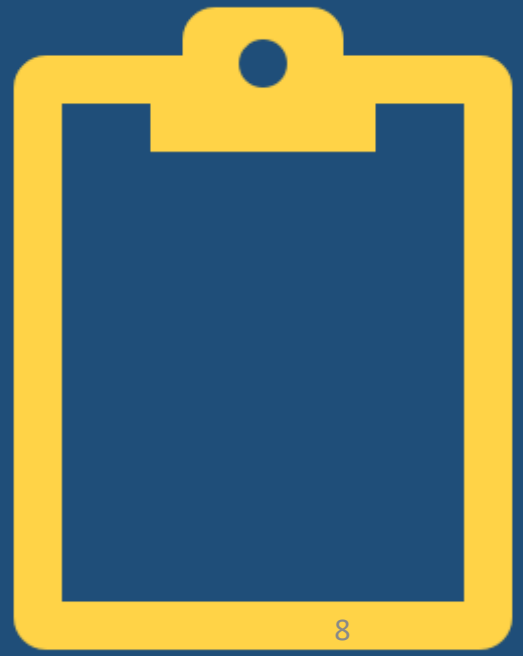




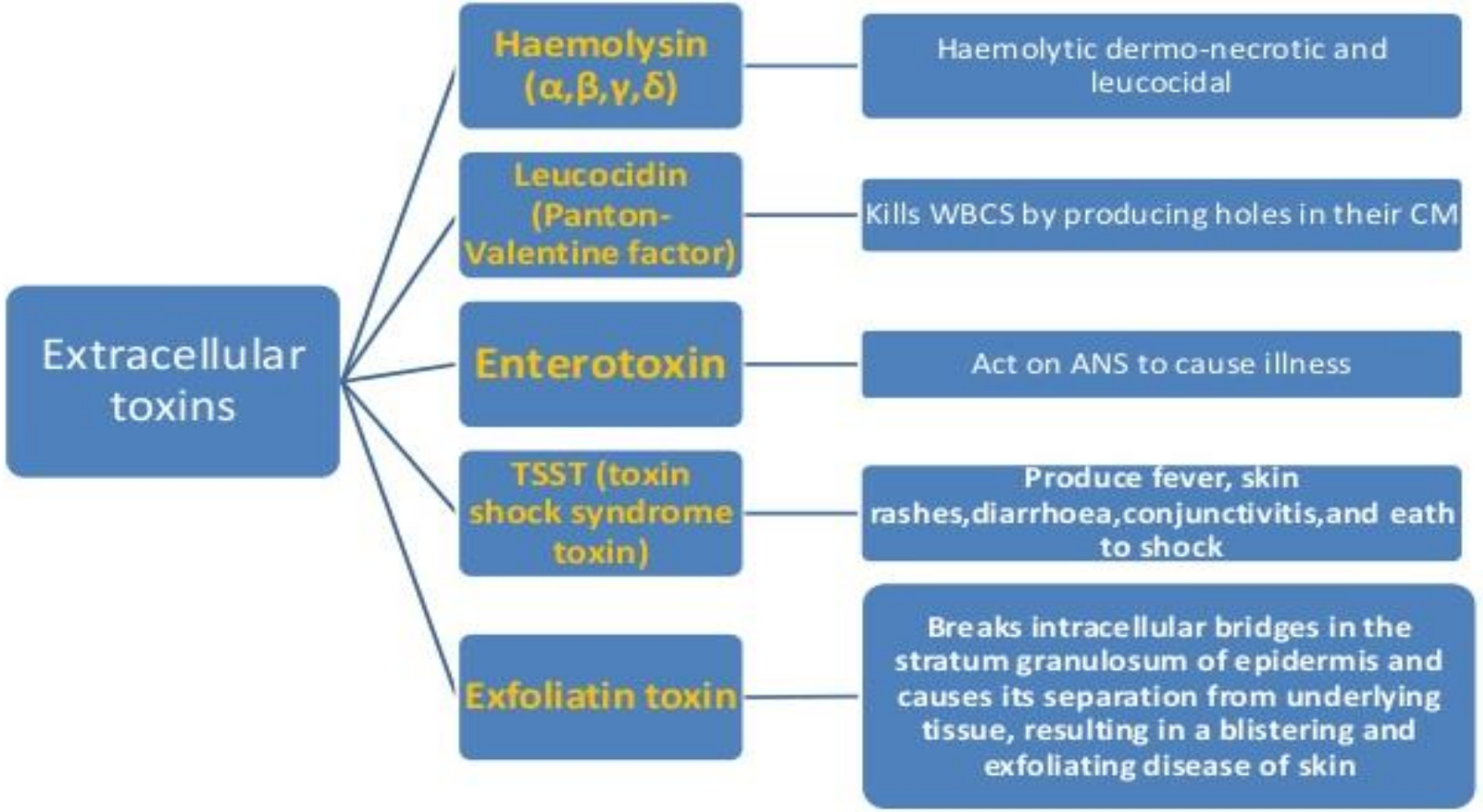


Virulence Factors

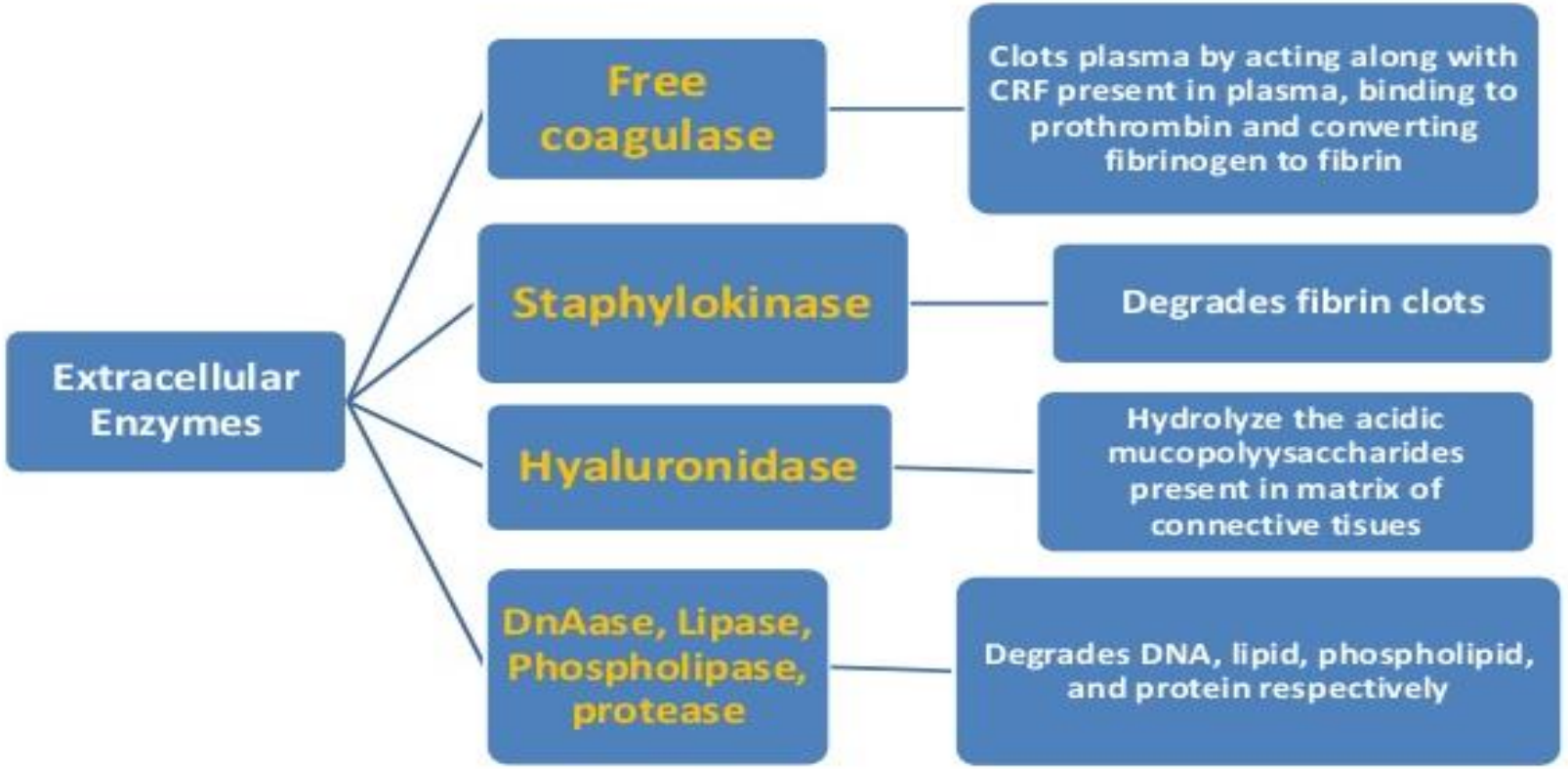
Cellwall associated structures	Extracellular toxins	Coagulase
• Peptidoglycan • Capsule • proteinA • Clumping factor (bound coagulase)	• Haemolysin • Leukocidin • Enterotoxin • TSST • Exfoliatin toxin	• staphylokinase • DNAase • Phosphatase • lipase • Phospholipase • hyaluronidase • serokinase • protease



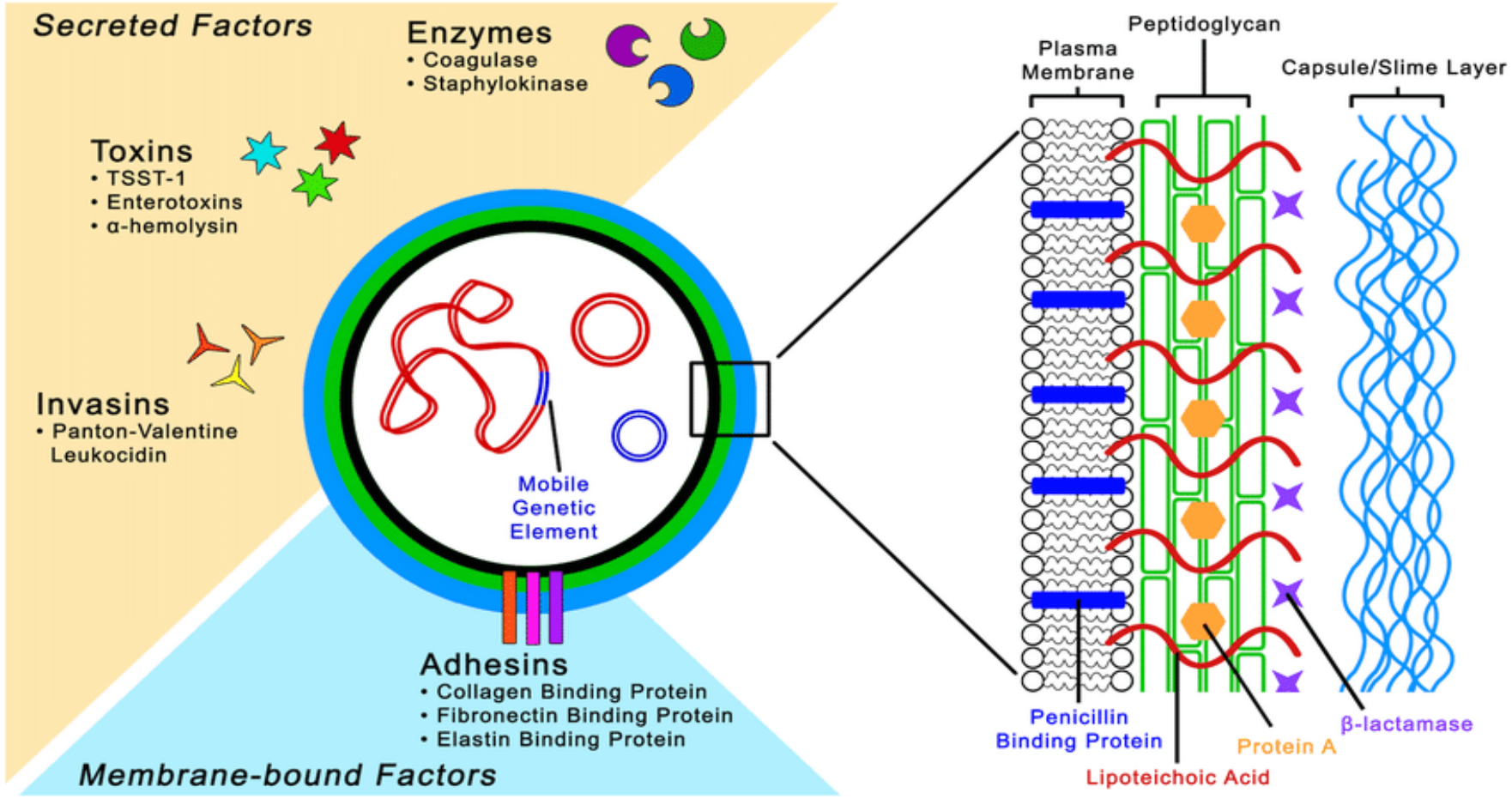
Virulence Factors(….contd)



Virulence Factors(….contd)



Virulence Factors(….contd)



Clinical Syndromes

1. **Cutaneous infections**
   - Folliculitis
   - Boils/furuncles
   - Carbuncle
   - Impetigo
   - Wound infections
2. **Deep infections**
   - Osteomyelitis
   - Periostitis
   - endocarditis
3. **Exfoliative diseases**
4. **Toxin shock syndrome**
5. **Staphylococcal food intoxication**

Virulence Factors(….cont'd)

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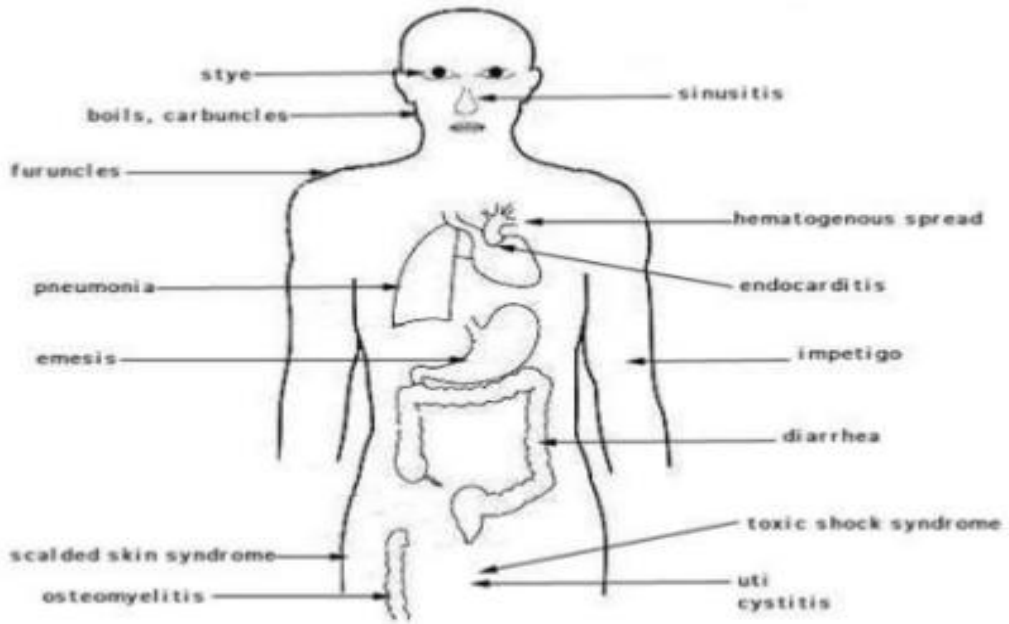


Toxic shock syndrome

- Caused when Toxin shock syndrome toxin (TSST) liberated by S.aureus enters bloodstream
- It is a multisystem illness, characterized by:

High Fever

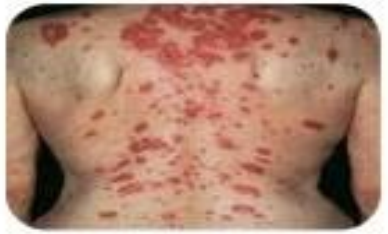
Headache

Vomiting

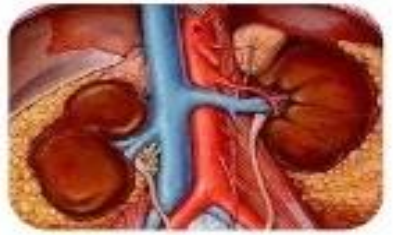
Diarrhoea

Conjunctival reddening

Hypotension



Skin rashes



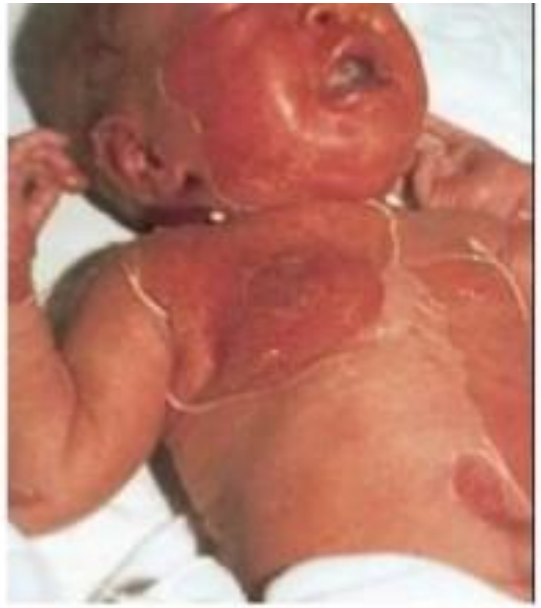
Kidney failure

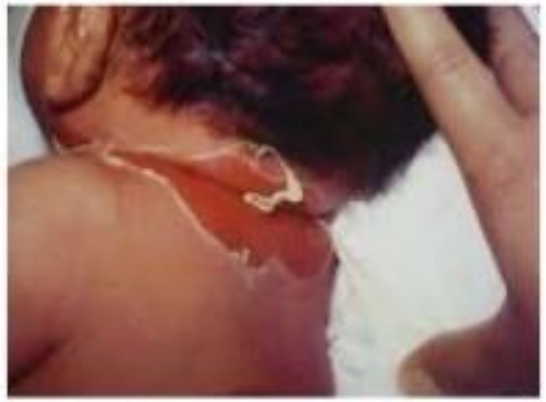
Exfoliative Disease

- (Exfoliate= scaling off tissues in layers)
- Also known as **'Staphylococcal skin scalded syndrome'**

- previously called dermatitis exfoliativa, pemphigus neonatorum, Lyell's disease and Ritter's disease

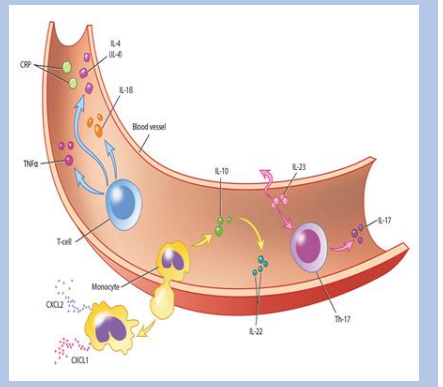
- Epidermal toxin produced by *S.aureus* at skin and is carried by bloodstream to epidermis , where it causes a split in a cellular layer i.e., this toxin separates outer layer of epidermis from underlying tissue





Bacteremia

- Bacteremia is defined as the presence of bacteria in normally sterile blood.
- *S. aureus* bacteremia is associated with higher morbidity and mortality, compared with bacteremia caused by other pathogens.
- There are many risk factors associated with bacteremia.



- The incidence of *S. aureus* bacteremia and its complications has increased sharply in recent years.

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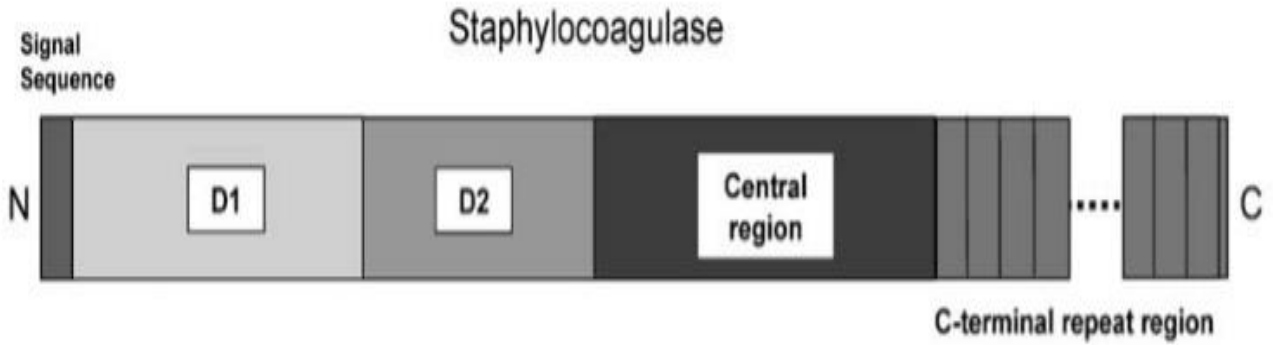
Coa typing

- *Coa* gene typing is one of the most successful methods for differentiating *S. aureus* isolates taken from different areas.
- Provides simple, accurate and reproducible results compared to other methods.
- The coagulase enzyme, an extracellular protein produced by all *S. aureus* isolates, is genetically diverse and has been considered to be a hallmark for typing these strains.

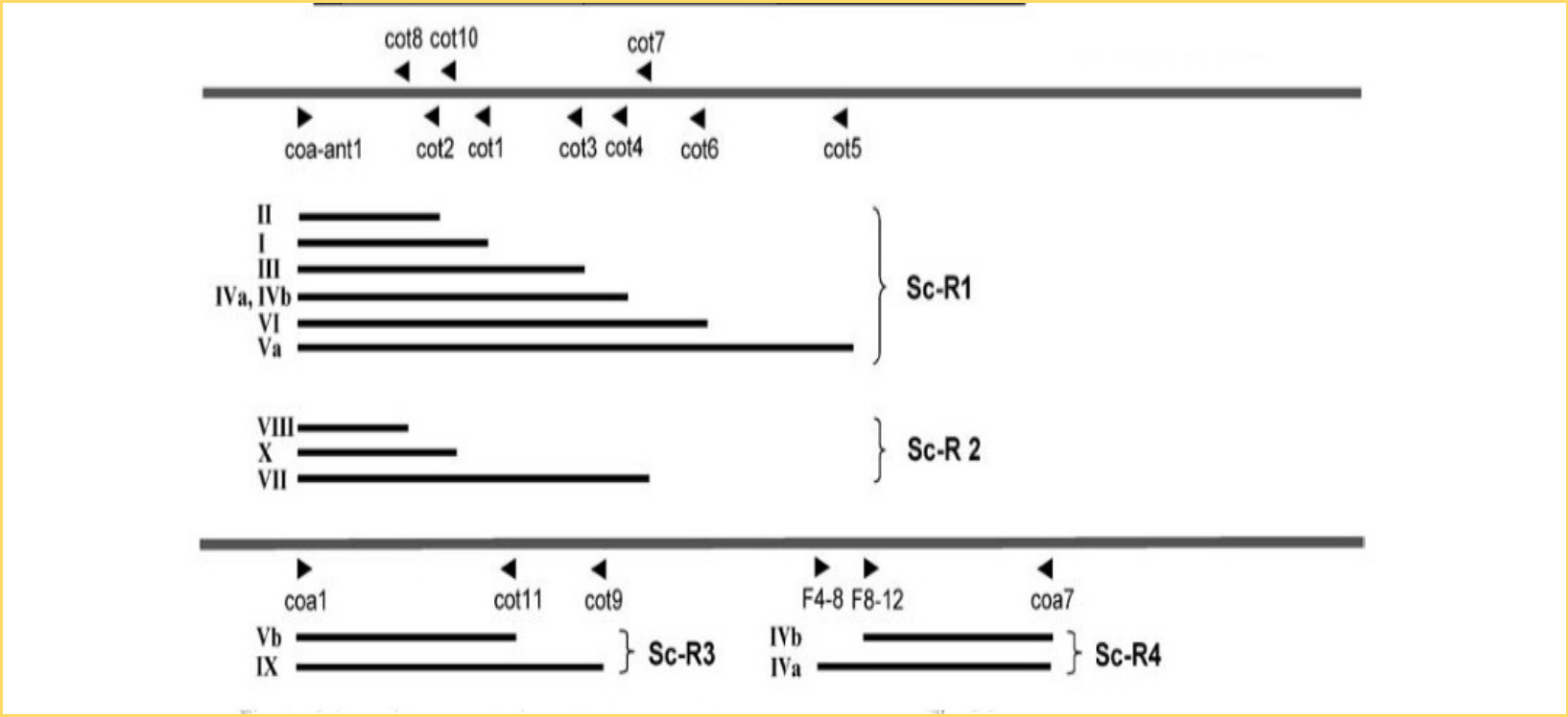
Coa typing

- Structural analysis of *coa* revealed six major regions including:



Coa typing

- According to the sequence variability in *coa* region, ten different types of coagulase (I-X) have been described.



Aim of study

- Determine the *coa* gene polymorphism.
- Investigate the prevalence of toxin genes.
- Resistance pattern in *S. aureus* Strains.

Methodology

Basic bacterial investigation

Isloates:

120 *s.aureus* from bacteremia patients.

Shahid Beheshti University medical sciences hospitals

Confirmation:

Standard biochemical tests(growth on MSA, Gram staining, Coagulase in tube), *nucA* gen

Methods & Materials

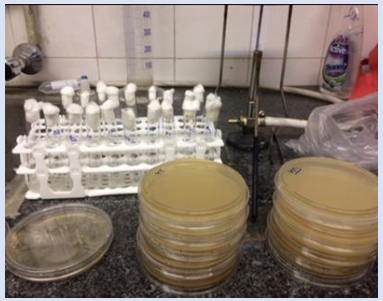
Antibiotic resistance of the strains was assessed by disc diffusion

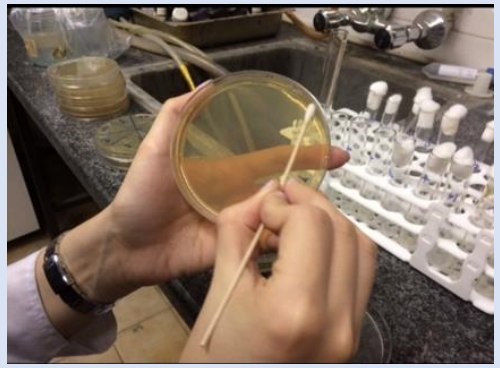
Identification of some toxin genes were performed by PCR

Coagulase typing of *S. aureus* strains was performed by Multiplex PCR

Antibiotic susceptibility

- Susceptibility to Amikacin, Gentamycin, Kanamycin, Erythromycin, Linezolid, Teicoplanin, Rifampicin, Clindamycin, TMP/SMX, Chloramphenicol, Ciprofloxacin, Cefazolin, Ampicillin & Tetracycline were tested.
- Guideline: CLSI 2019
- ATCC25923





DNA preparation

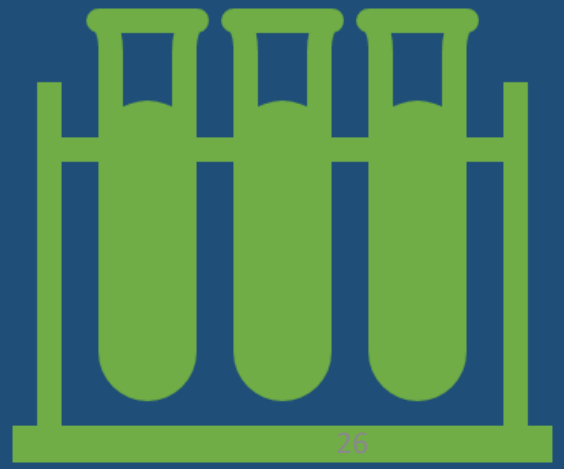
- Genomic DNA from 24–hour cultures of *S. aureus* isolates were extracted using boiling method.

- After the extraction, the purity of DNA was assessed using a nanometer.

Detection of toxin encoding genes

- Polymerase chain reaction (PCR) performed to determine the presence of toxins (eta, etb, tst, pvl) encoding genes.

Product-length(bp)	Primer sequence	gen
		toxins
560	5′CAGGAGGTAATGGTTCATTT 3′ 5′GCATCAAGTGTATTGGATAGCAAAAGC3′	pvl
350	5TGCAAAAGCATCTACAAACGA3′ 5′TGTGGATCCGTCATTCATTG3′	tst
119	5′CTAGTGCATTTGTTATTCAA 3′ 5′TGCATTGACACCATAGTACT3′	eta
200	5′ACGGTATATACATTCAATT 3′ 5′TCCATCGATAATATACCTAA3′	etb

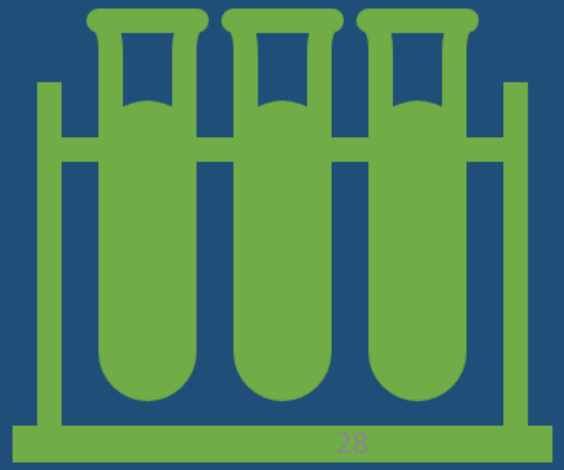


PCR

Factor	Temperature(ºC)				Time			
Gene Step	eta	etb	tsst	pvl	eta	etb	tsst	pvl
Initial denaturation	94	94	94	94	5 min	5 min	5 min	5 min
Denaturation	94	94	94	94	45 s	45 s	45 s	45 s
Annealing	51	51	46	57	45 s	45 s	45 s	45 s
Extension	72	72	72	72	45 s	45 s	45 s	45 s
Final extension	72	72	72	72	5min	5min	5min	5min
Cycle	30	30	30	30	----------			

Coa typing

- 4 sets of multiplex PCR reactions were used for assigning Sc types (I-X) according to the procedure of Hirose et al.
- Set A contained primers for identifying Sc types I, II, III, IVa, Ivb, Va and VI, while Set B contained primers for identifying Sc types VII, VIII, and V.
- Set 3 was used for identifying Sc types IX & Vb.
- Sc types IVa & IVb were distinguished using set 4 primers.

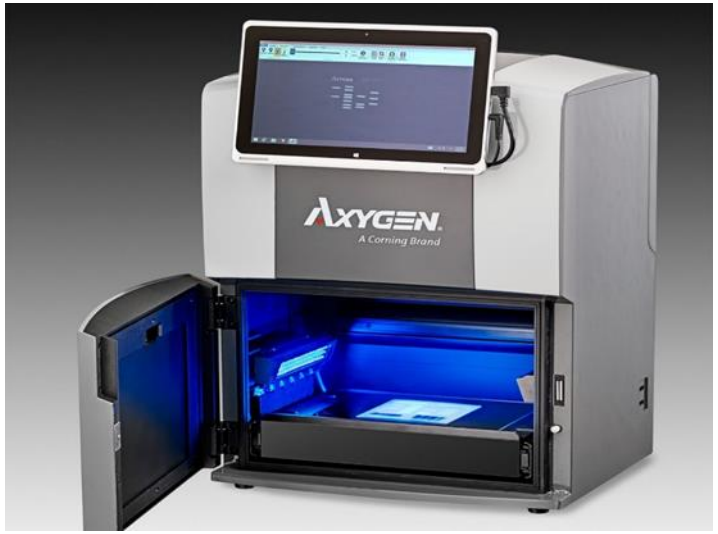


Coa typing

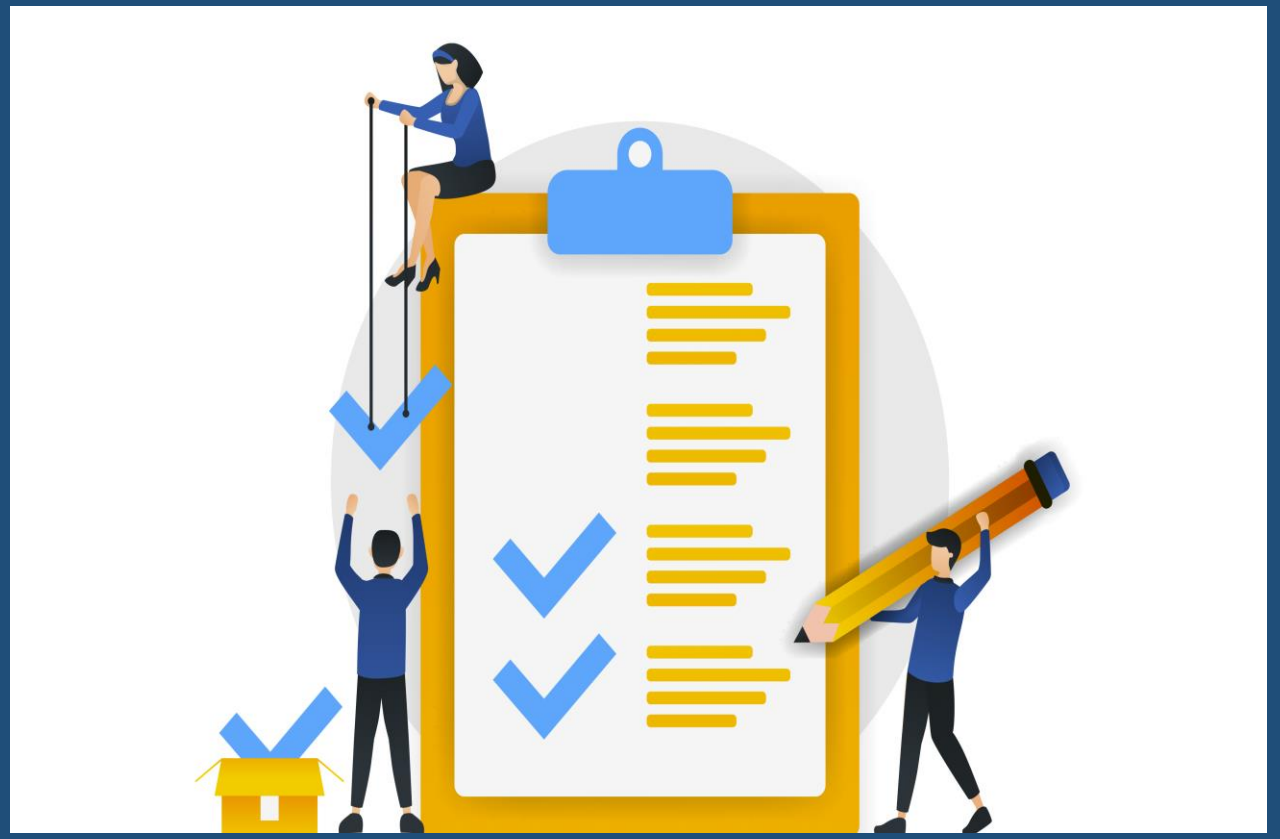
Gen	Primer	SC type	Primer sequence	Product length PCR
Sc-R1	Coa-ant1	Common	GGGCAATTACATTTTGGAGGA	---
	coa7	Common	TGTTCCATCGTTGTATTCACG	---
	cot1	I	ATTTTTTGTATTCCTCATACTGCA	bp 368
	cot2	II	CTTTCGCTTCTTTATAGATAGGATC	bp 288
	cot3	III	TCAAGTCTGAATTCTTATCC	bp 549
	cot4	Iva, IVb	AGCATTATGACCATATTGGC	bp 665
	cot5	Va	TTACCTTGAGTCCCAATTTG	bp 1105
	cot6	VI	CTATAATCATGCTTATCCCA	bp 850
Sc-R2	cot7	VII	TCAAATCAATTTTCGCCCTA	bp 693
	cot8	VIII	GATTTTTTATTACTCCCCAGTAATA	bp 210
	cot10	X	ACTTAATATCCTTGTCATTAGTTG	bp 314
	cot9	IX	ATATACCGTTAGTTACACGC	bp 591
Sc-R3	Cot1	Vb	AATCATAAAATTTCACCGGGC	bp 411
	F4-8	IVa	TTACAGTTGGTACAACTGAAGAAGC	bp 455
Sc-R4	F8-12	IVb	GCCAAAATACCCAACGATGGAACAG	bp 415

Multiplex PCR

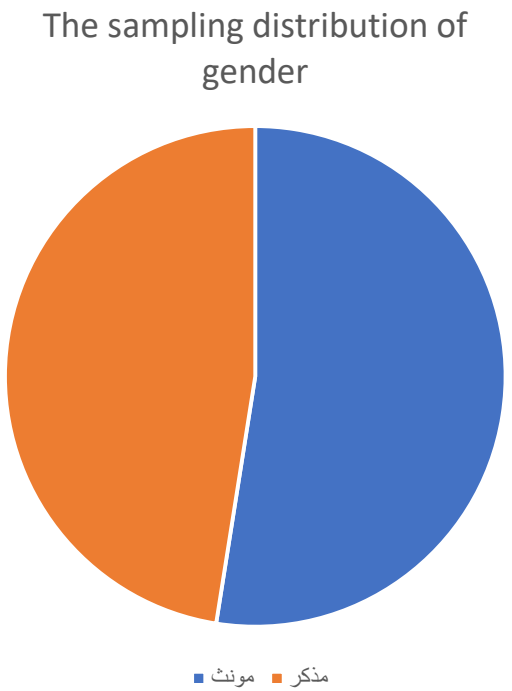
Factor	Tempreture	Time
Initial Denaturation	95	5 min
Denaturation	95	35 s
Annealing	57	40 s
Extension	72	45 s
Final Extension	72	5 min
Cycle	30	----------

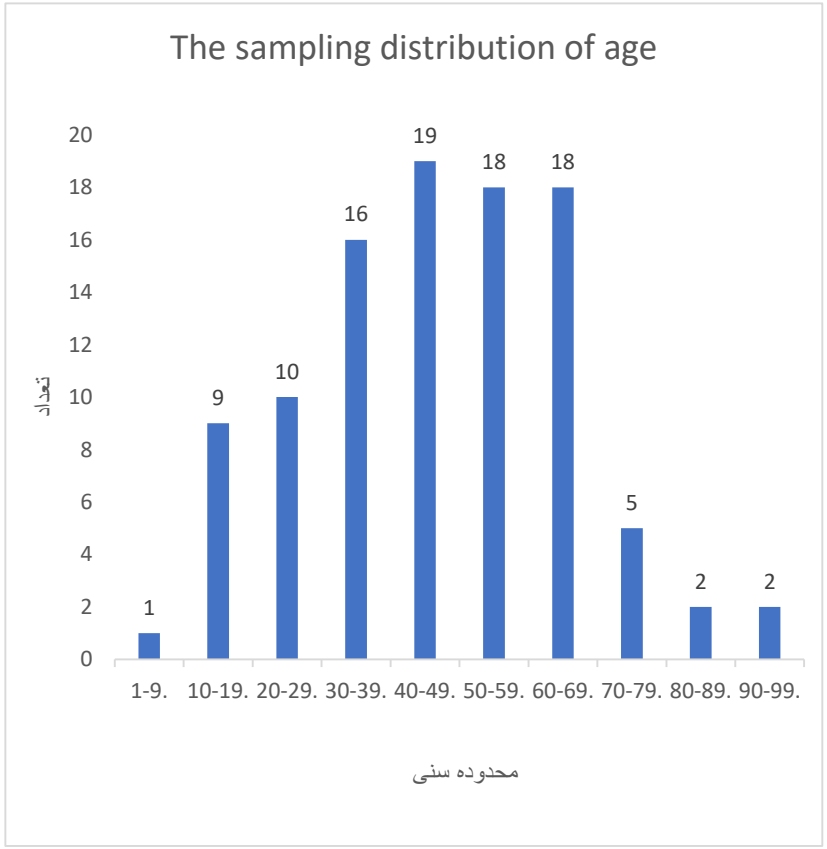


Results Analyses

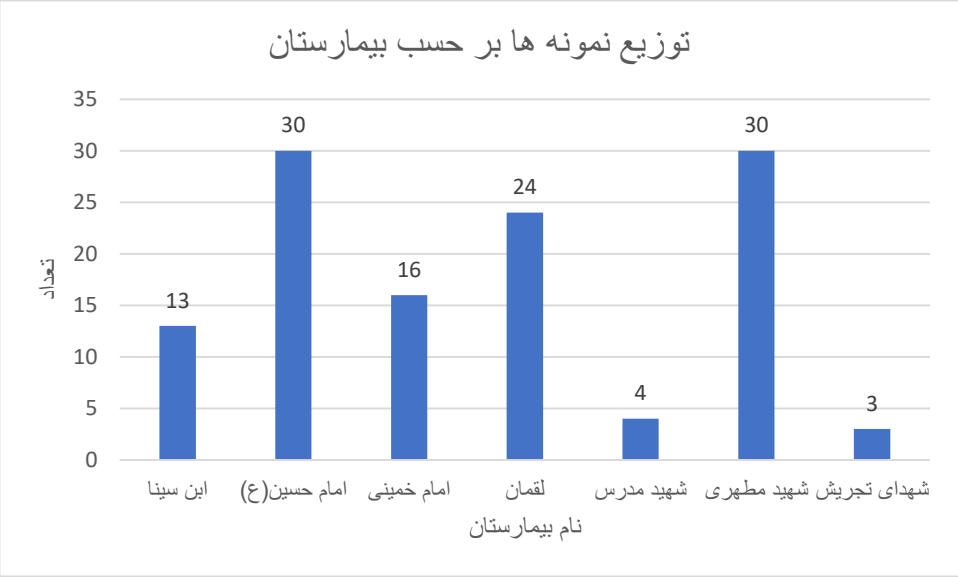


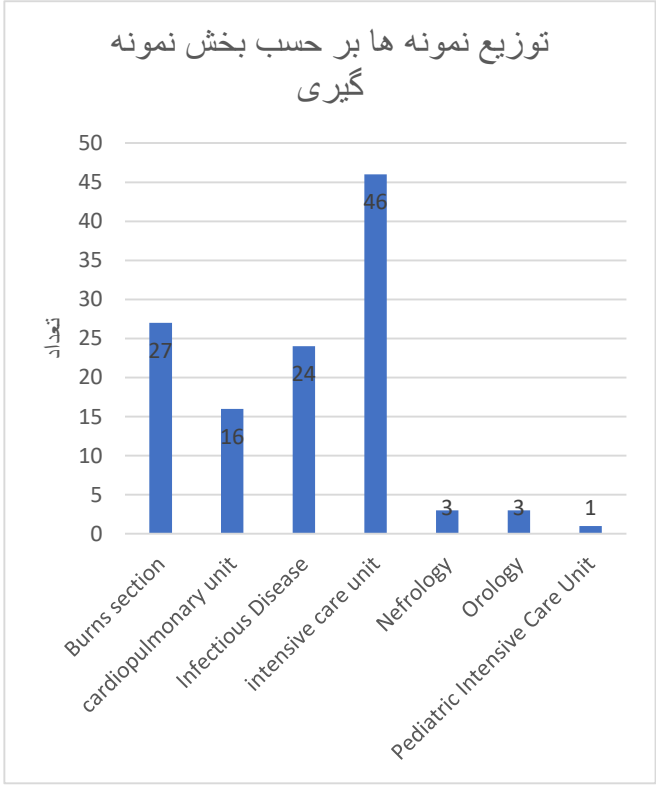
Sample collection results





Sample collection results

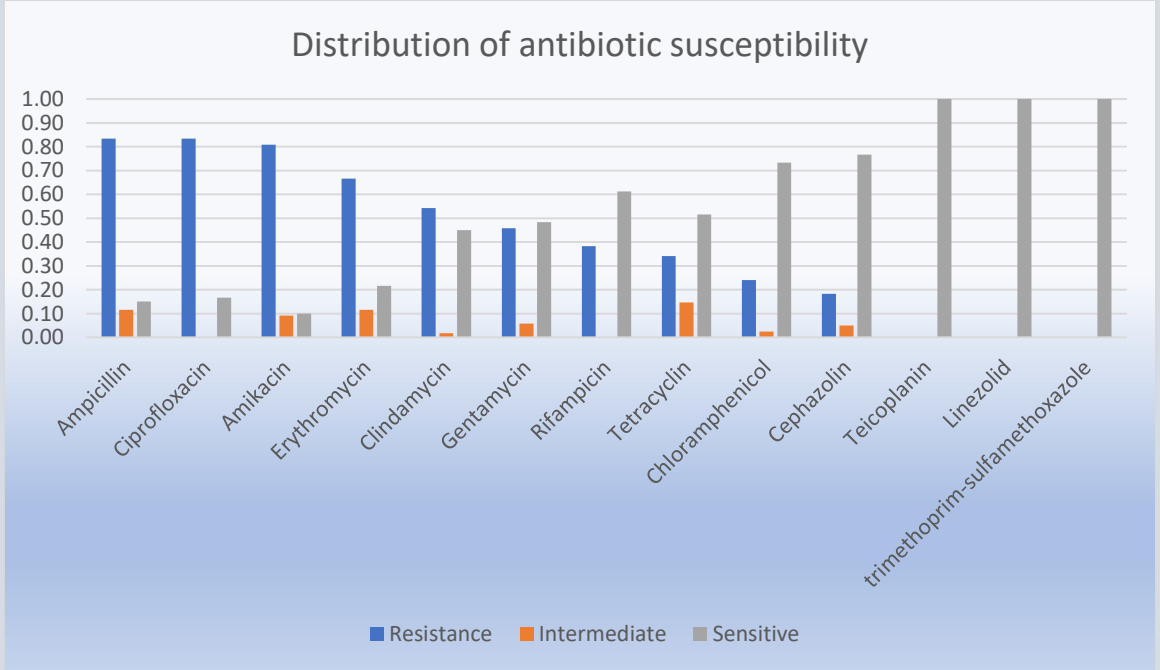




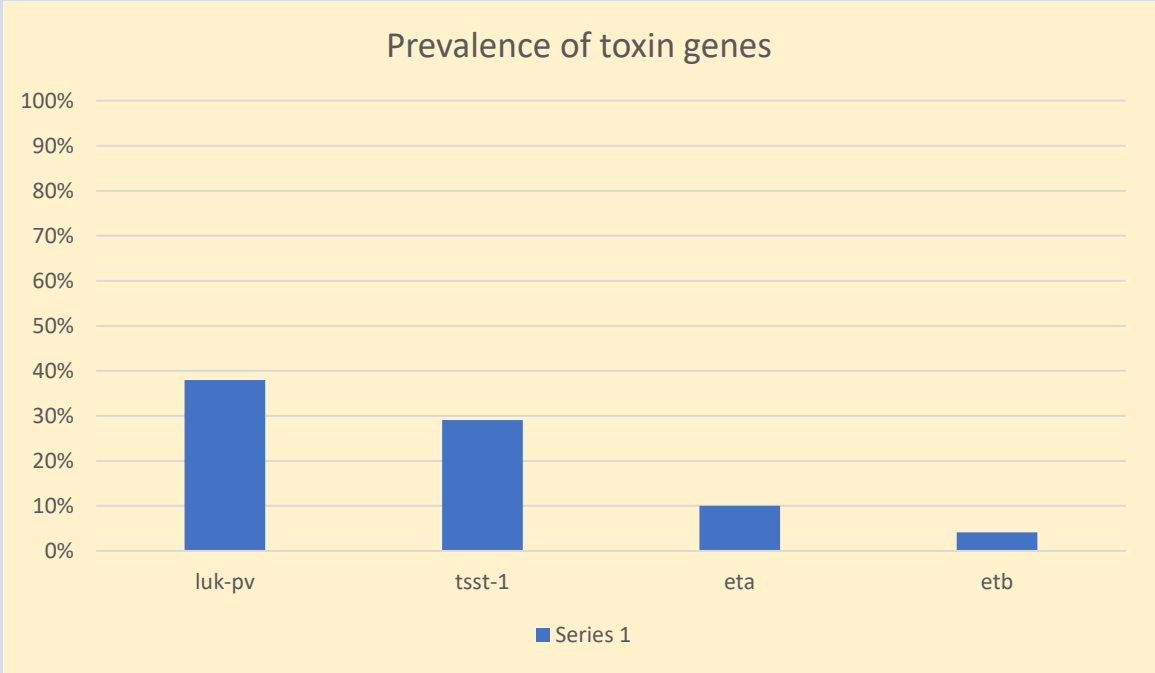
Antibiotic susceptibility

Antibiotic	Resistance(%)	Intermediate(%)	Susceptibility(%)
Ampicillin	83.3	1.6	15.1
Amikacin	80.8	9.1	10
Erythromycin	66.6	11.6	21.6
Ciprofloxacin	83.3	0	16.7
TMP/SMX	0	0	100
Linezolid	0	0	100
Teicoplanin	0	0	100
Gentamycin	45.8	5.8	48.3
Clindamycin	54.2	1.8	45
Chloramphenicol	24.1	2.5	73.3
Rifampicin	38.3	0	61.2
Kanamycin	58.3	2.5	39.2
Tetracycline	34.16	14.6	51.6
Cefazolin	18.3	5	76.6

Antibiotic susceptibility



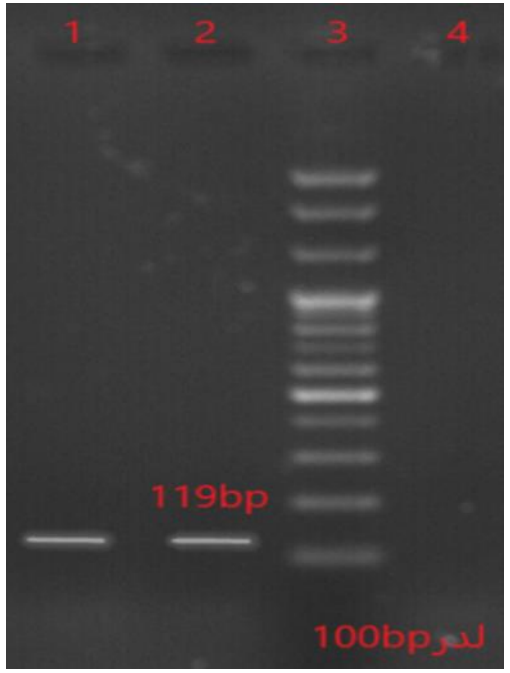
Toxin genes


Prevalence of toxin genes
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
luk-pv
tsst-1
eta
etb
Series 1

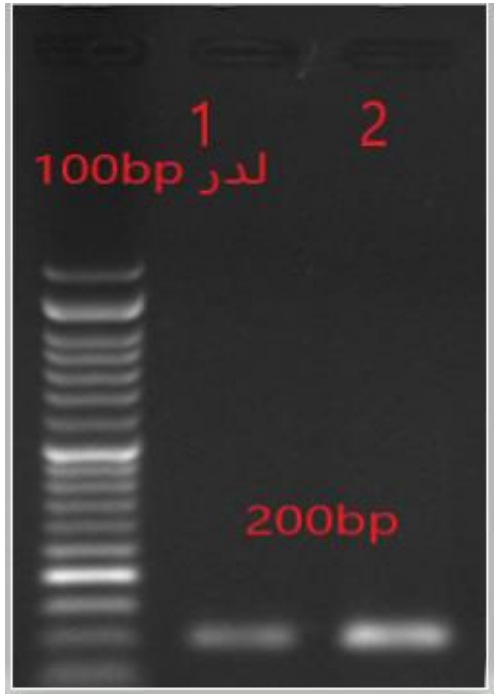
Toxin genes

Toxins	percentage
eta	10%
etb	4/1%
Luk-pv	29/1%
Tsst-1	38%

Toxin genes



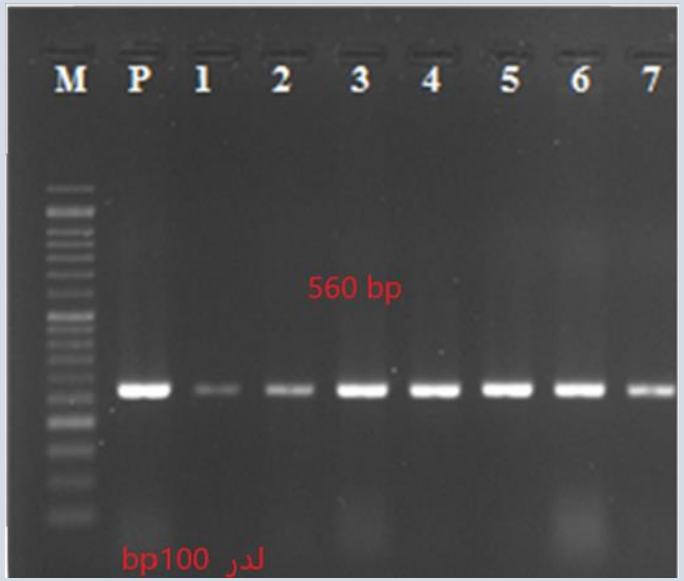
eta(10%) positive



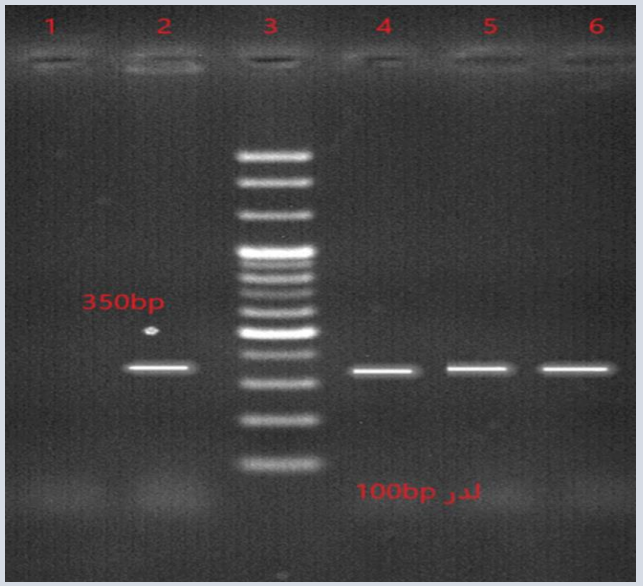
etb(4/1%) positive

Results

- Toxin genes



Luk-pv (29 %)



Tsst-1(38%)

Sc types

SC type	MRSA n= 55	MSSA n= 65	Total n= 120
I	6	4	10
II	22	17	39
III	17	25	42
IVa	3	5	8
VI	3	8	11
VIII	3	3	6
X	1	3	4

Coa typing

Discussion

Discussion

- *Staphylococcus aureus* is responsible for a wide range of diseases, from minor skin infections to fatal necrotic pneumonia.

- Although staphylococci are a part of the normal flora in humans and are therefore part of coexisting microorganisms, they can also in some cases act as an opportunistic pathogen and cause a wide range of diseases because they adapt more quickly to pressures.

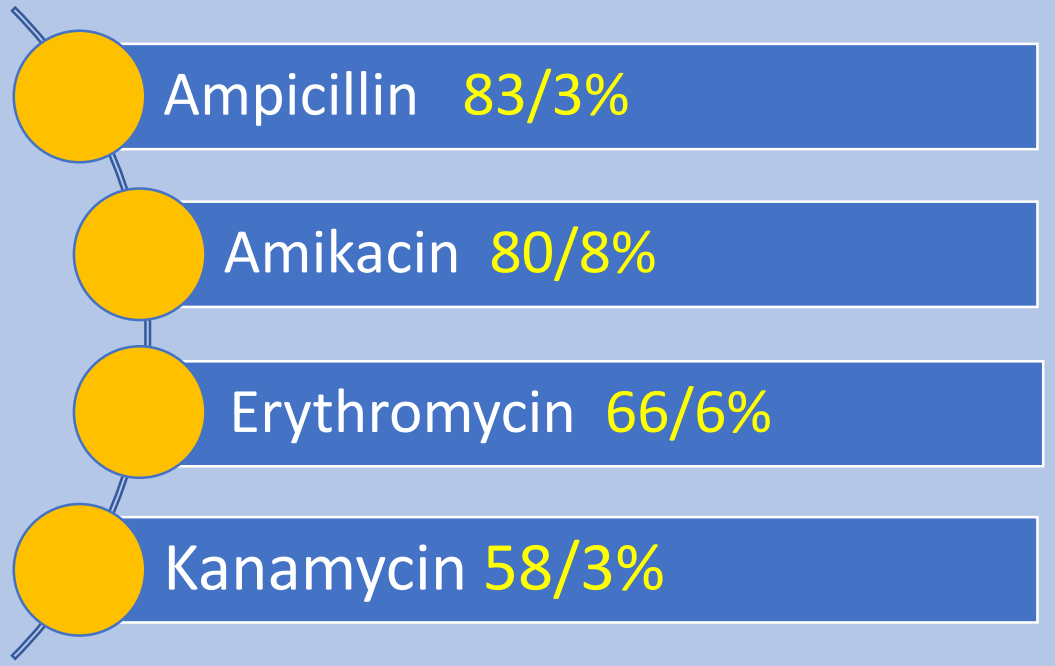
Discussion

- The emergence of antibiotic resistance is a serious threat to the health care system today.

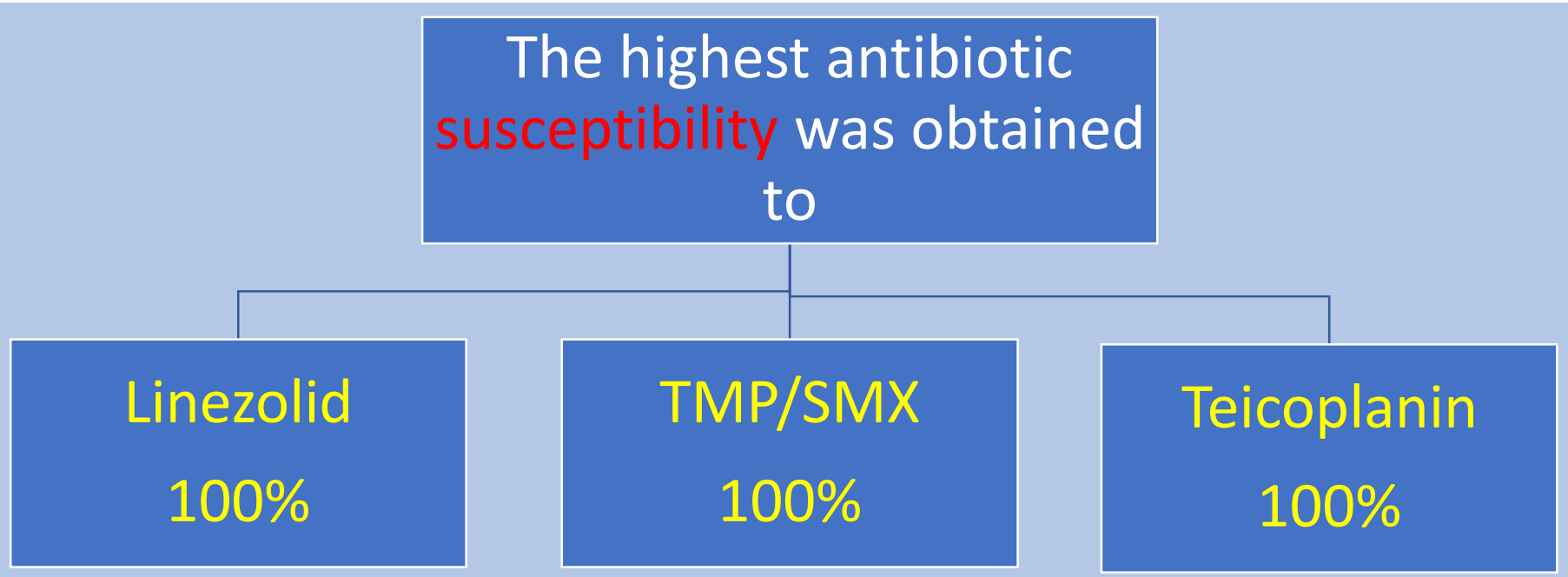
- Several studies have been revealed different findings of resistance rate of *S.aureus* isolated from bacteremia patients which may be linked to various bacterial detection methods.

Discussion

Antimicrobial susceptibility testing revealed a high prevalence rate of resistance to



Discussion


The highest antibiotic susceptibility was obtained to
Linezolid
100%
TMP/SMX
100%
Teicoplanin
100%

Discussion

Shokoohi et al

2000 *S.aureus* strains

- Resistance to erythromycin 56%
- Resistance to Clindamycin 56%

Ranjbar et al

- Resistance to Ciprofloxacin 67%
- Resistance to Erythromycin 63%
- Resistance to chloramphenicol 45%
- Resistance to Clindamycin 24%
- Resistance to Gentamycin 57%

Tahbaz SV et al

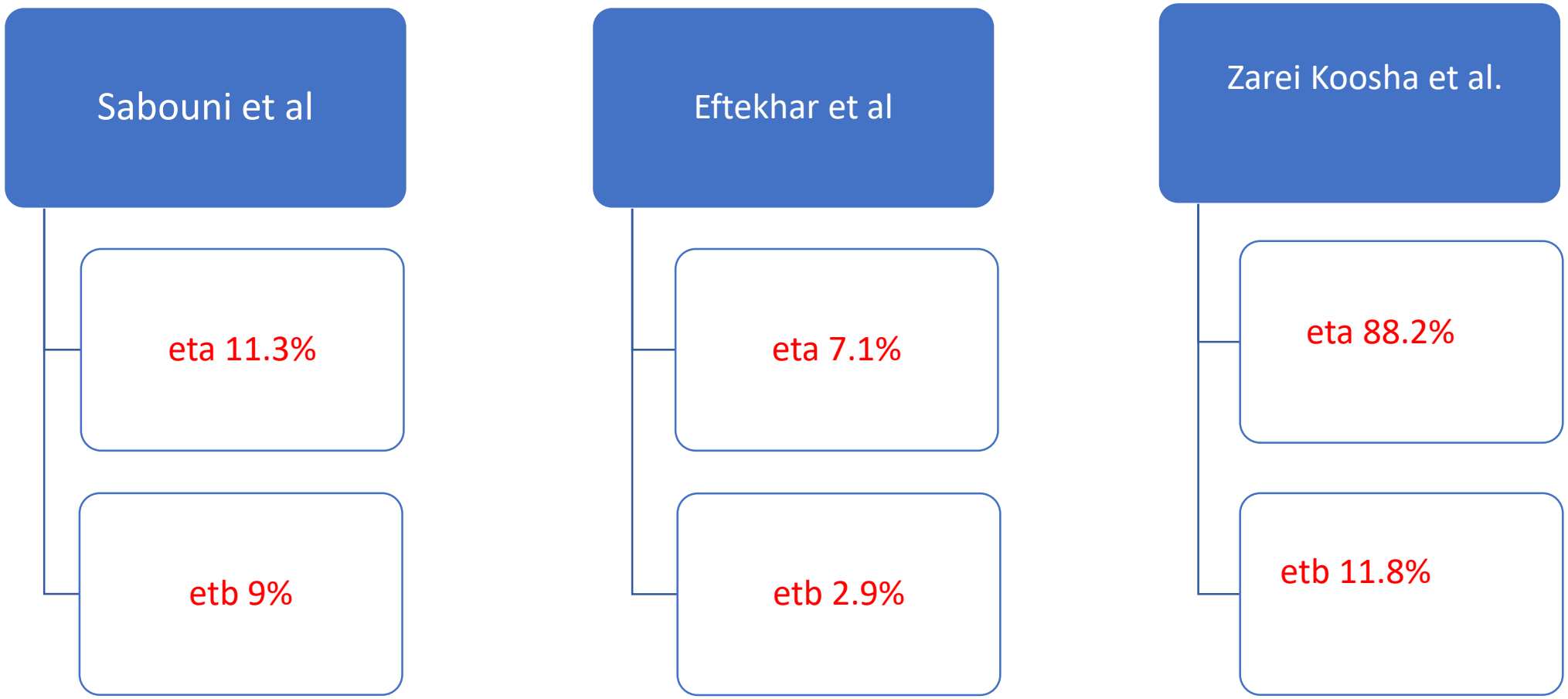
- Resistance to Erythromycin 41%
- Resistance to Ciprofloxacin 58%
- Resistance to Clindamycin 64%
- Susceptibility to Linezolid 100%

Exfoliative toxins

- *Exfoliative toxins A (eta) and B (etb) have been linked to Impetiginous and Scalded skin syndrome.*

- The frequency distribution of eta and etb genes in the present study was obtained with a frequency of 10% and 4.1%, respectively.

Prevalence of exfoliative toxins

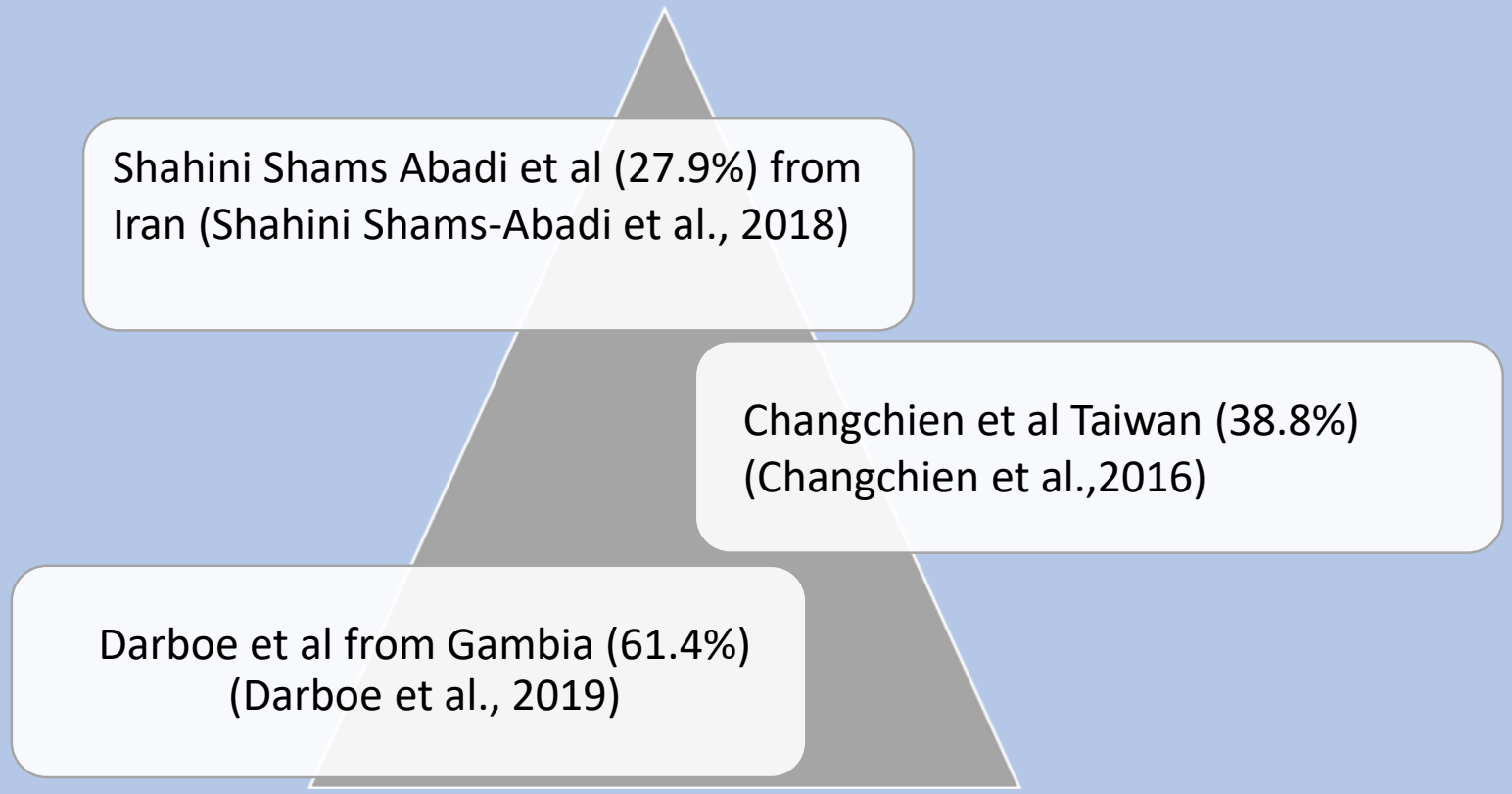


Panton-Valentine **leukocidin**

- *Staphylococcus aureus* has the ability to produce a variety of toxins and can produce alpha, beta, gamma, delta, and leukocidin toxins.
- PVL toxin is a cellular toxin that causes leukocytes to leak and tissue necrosis to increase by cell membrane permeability.
- Leukosidine can be used as a marker of virulence by destroying leukocytes and eventually reducing the number of leukocytes in the host body.

Prevalnce of *luk-pv*

- In this survey, the prevalence of *pvl S. aureus* strains was found to be 29.1%.



toxic shock syndrome

- Toxic shock syndrome is a disorder that affects many parts of the body.
- Its incidence in the United States is about 0.5 per 100,000 (107). The main cause of toxic shock syndrome is the toxin (TSST-1) secreted by *Staphylococcus aureus.*
- Most isolates of *Staphylococcus aureus* isolated from patients with toxic shock produced the toxin TSST (1), which causes fever, hypotension, involvement of various organs, and skin rashes.
- Numerous studies have been used to identify *Staphylococcus aureus* toxins.

Prevalence of *tst*

Yuan et al	China-2014	34.4%
Motamedifar et al	Iran-2015	29.7%
Norozi et al	Iran-2016	66.2%
Shahini Shams Abadi et al	Iran-2018	21.3%
Our study	Iran-2021	38%

Coa typing

- Typing based on coagulase gene polymorphism (coa) is a simple, highly reliable, reproducible, easy-to-interpret, and differentiating method for typing *Staphylococcus aureus* isolates.

- In the present study, 7 coa types were identified among the strains with relatively high heterogeneity.

Coa typing

Hirose et al study from Japan (2010)

325 clinical isolates of *S.aureus*

7 coa types

type II was dominant

Jpn. J. Infect. Dis., 63, 257-263, 2010

Original Article

Identification of Staphylocoagulase Genotypes I–X and Discrimination of Type IV and V Subtypes by Multiplex PCR Assay for Clinical Isolates of *Staphylococcus aureus*

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(Received March 24, 2010. Accepted June 3, 2010)

SUMMARY: Staphylocoagulase (SC) is a major phenotypic determinant of *Staphylococcus aureus*. Antigenic specificity of SC (SC serotype) has been classified into at least 10 types and employed as an epidemiological marker. In the present study, from the sequence information of SC genes, a novel multiplex PCR assay was developed to determine SC genotypes (SC types) I–X corresponding to SC serotypes I–X, respectively, and to discriminate two subtypes of SC types IV and V. Two PCR reactions (Sc-R1 and Sc-R2) for a single isolate were designed for assigning common SC types in *S. aureus* isolates from humans (I–VIII and X). When amplicon was not produced, an additional PCR (Sc-R3) was performed to assign SC type IX or subtype Vb. Subtypes IVa and IVb were discriminated by an additional PCR (Sc-R4). SC types were discriminated successfully for the *S. aureus* strains with established SC types I–X including two subtypes of IV and V. The multiplex PCR assay established in this study could assign SC types for *S. aureus* clinical isolates at a high determination rate, providing more accurate information on incidence of SC types, and was considered to be useful for epidemiologic characterization of *S. aureus*.

INTRODUCTION

Staphylococcus aureus causes a wide spectrum of diseases in humans, and is recognized as a major etiologic agent of nosocomial and community-acquired infections. *S. aureus* produces staphylocoagulase (SC) as an extracellular protein, which has been employed for discriminating *S. aureus* from most of the lesser virulent staphylococci, i.e., coagulase-negative staphylococci, although only a few staphylococcal species (e.g., *S. intermedius*, *S. hyicus*, *S. delphini*, etc.) produce this protein (1–3). SC is one of the virulence factors of *S. aureus* and causes coagulation of plasma in many mammalian species. The SC binds to prothrombin and activates it through a non-proteolytic process, causing spe-

types (I–X) have been classified, among which nine types (I–VIII and X) are found in clinical isolates from humans, while coagulase type IX was detected only in a bovine isolate (14,15).

Sequence analysis and crystal structural analysis of SC revealed that this protein consists of six regions, i.e., signal sequence, N-terminal D1 region and D2 region, central region, 27-amino acid-repeat region, and C-terminal sequence (15,16). The D1 and D2 regions are involved in binding and activation of prothrombin, while C-terminal 27-amino acid-tandem repeat region is associated with adherence of SC to fibrinogen (17). The C-terminal repeat regions have been known to be highly polymorphic in the repeat number irrespective of SC serotype (14,18).

Coa typing

Demir *et al.*

From Turkey

(2011)

120 S. aureus strains isolated from clinical isolates

4 different coa genotype patterns

Turk J Med Sci
2011; 41 (2): 343-352
© TÜBİTAK
E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-1003-657

Original Article

Investigation of toxin genes in *Staphylococcus aureus* strains isolated in Mustafa Kemal University Hospital

Cemil DEMİR[1], Özkan ASLANTAŞ[2], Nizami DURAN[1], Sabahattin OCAK[3], Burçin ÖZER[1]

Aim: The aim of this study was to investigate the presence of genes encoding staphylococcal enterotoxins (SEs), exfoliative toxins (ETAs, ETBs), and toxic shock syndrome toxin-1 (TSST-1) by polymerase chain reaction (PCR) in *Staphylococcus aureus* strains isolated from various clinical samples from the Mustafa Kemal University Hospital. In addition, PCR-based restriction fragment length polymorphism (RFLP) analysis of the coa gene was employed to genotype the isolates.

Materials and methods: A total of 120 *S. aureus* strains isolated from various clinical samples (blood, wounds, urine, conjuctival swabs, and tracheal aspirate) over a 1 year period, 2007-2008, were used in this study.

Results: Almost 65.8% of the isolates possessed at least one toxin gene. The genes most frequently found were *seg-sei* (40.8%), followed by *sea* (30%) and *eta* (19.2%). Overall, 35 toxin genotypes were observed, among which the genotypes *seg-sei, sea-seg-sei,* and *sea-see* predominated at the rate of 8.3%, 5.8%, and 5%, respectively. Four coagulase genotype patterns were observed, with molecular sizes ranging from 570 to 970 bp. *Coa*-based RFLP analysis revealed 7 different patterns using *Alu*I.

Conclusion: Our results have revealed that toxin genes were very prevalent among *S. aureus* isolates, and the toxigenic isolates were independent of the genotypes obtained by PCR-RFLP of the coa gene ($P > 0.05$).

Key words: *Staphylococcus aureus*, toxin genes, PCR-RFLP, coagulase

Coa typing

Afrogh *et al.* (2013)

157 clinical strains

reported 6 coa type with the majority of the pattern I C 1

KOWSAR

Jundishapur Journal of Microbiology

www.jjmicrobiol.com

Molecular Investigation of *Staphylococcus aureus*, *coa* and *spa* Genes in Ahvaz Hospitals, Staff Nose Compared With Patients Clinical Samples

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A B S T R A C T

Background: *Staphylococcus aureus* is one of the important human pathogens which are mainly isolated from wound, skin and contaminated respiratory excretions. Because many of hospital staff and patients carry this pathogen in their nose or skin, close contacts and touching have special role in spreading the infection in hospitals. Also, antibiotic resistant *S. aureus*, especially Methicillin Resistant *S. aureus* (MRSA) have been seen among subjects. Thus, there should be an investigation for Bacteria colonization in nose of hospital staff and patients. Furthermore, investigation of antibiotic resistance pattern and examination of genotyping properties of resistant strains have a high efficacy in control and recognition of infection origin.
Objective: The current study aimed to determine the characteristics of *S. aureus* isolated from patients and staff in hospitals and compare them based on *coa* and *spa* typing methods.
Materials and Methods: In the current study, 157 clinical specimens were collected from patients who were treated at the Ahvaz medical university hospitals including 79 specimens (50.3%) from Sina hospital, 34 specimens (21.7%) from Imam Khomeini hospital, and 44 specimens (28%) from Golestan hospital and 157 nose swab specimens from the staff of these hospitals were collected during 2010. *coa*, *spa* genes of isolated Bacteria were amplified using PCR.
Result: PCR results showed seven different patterns for staff and five different patterns for patients based on *spa* gene, and for *coa* gene five and six different patterns respectively. In addition, the prevalence of MRSA was 52.5 in staff and 83.7 in patients' specimens. Comparison of genetic diversity of *spa*, and *coa* genes in Ahvaz university hospitals doesn't show significant difference (Chi-square and fisher's exact test).
Concloutions: The outcome of this study show that spa and coa typing are suitable methods for MRSA isolates typing because it is easy to use and interpret them, and that these methods can be useful in infection source detection and its control especially in epidemic situations

Keywords: Staphylococcus aureus; ProA, Coagulase

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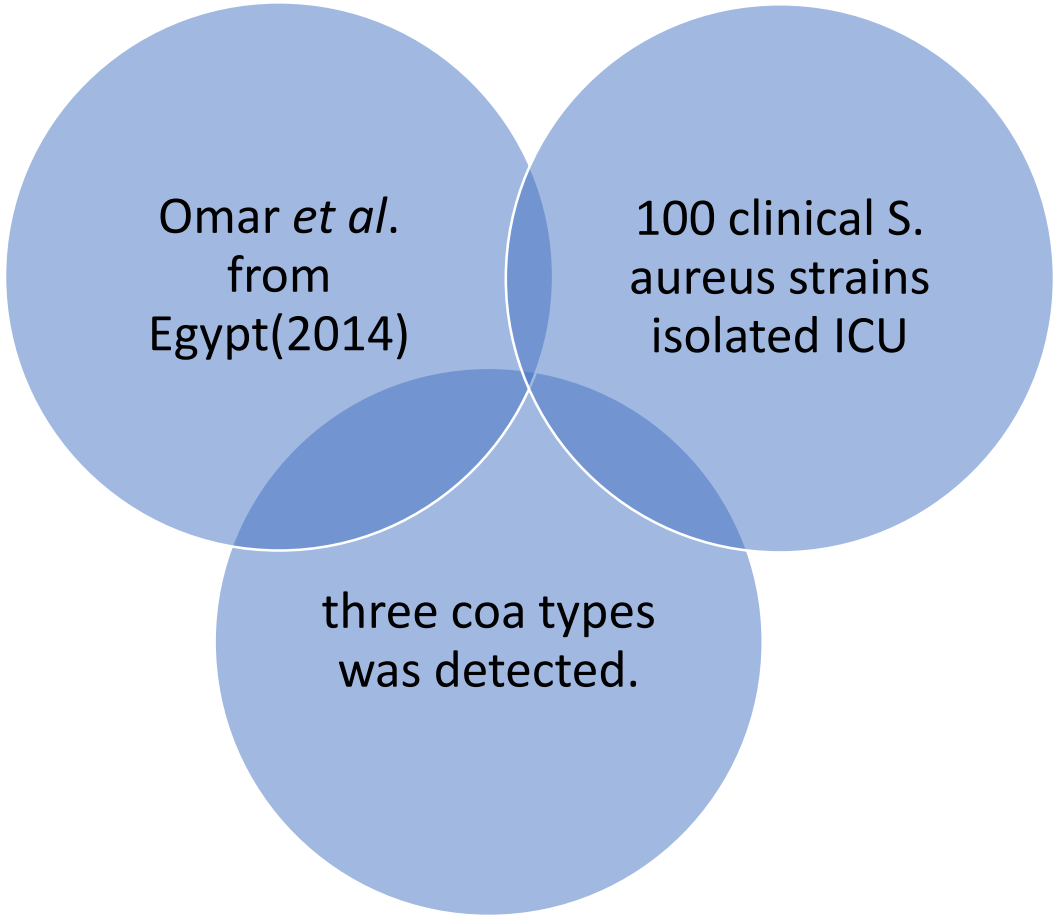
Article type: Research Article; Received: 06 May 2012; Revised: 17 Jul 2012; Accepted: 08 Aug 2012; Epub: 01 Jun 2013; Ppub: Jun 2013

Coa typing



Hindawi Publishing Corporation
International Journal of Microbiology
Volume 2014, Article ID 650328, 11 pages
http://dx.doi.org/10.1155/2014/650328

Hindawi

Research Article

Molecular Typing of Methicillin Resistant *Staphylococcus aureus* Clinical Isolates on the Basis of Protein A and Coagulase Gene Polymorphisms

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Received 12 February 2014; Accepted 24 April 2014; Published 15 May 2014

Academic Editor: Joseph Falkinham

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Increased frequency of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitalized patients requires rapid and reliable characterization of isolates for control of MRSA spread in hospitals. This study evaluated polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as a molecular typing technique for MRSA strains on the basis of protein A (*spa*) and coagulase (*coa*) gene polymorphisms to verify their ability in assessing the relatedness of isolates. Seventy-five MRSA isolates, from different ICUs of Alexandria University Main Hospital, were characterized using antibiotyping and PCR-RFLP analysis of *coa* and *spa* genes. Thirty-two antibiotypes were identified. *coa* gene PCR generated 3 types and 10 subtypes of band patterns. *HaeIII* restriction digestion of amplified *coa* gene products produced 5 major banding patterns and 12 subtypes. *spa* gene PCR products generated 4 major and 11 minor types, and their *HaeII* restriction digestion showed 5 major and 12 minor banding patterns. The combined *coa* and *spa* RFLP patterns generated 22 combined R types. Typing using *coa* PCR and PCR-RFLP had the same discriminatory index (DI) value (0.64), which was comparable to that of both *spa* PCR and PCR-RFLP techniques (0.68). The combined grouping increased the DI value to 0.836. The current study revealed that testing for multiple gene polymorphisms is more useful for local epidemiologic purposes.

Discussion

Abdulghany et a. from Egypt(2014)

among 58 MRSA isolates

15 different coa types

Research Article

The Frequency of Methicillin-Resistant *Staphylococcus aureus* and *Coagulase* Gene Polymorphism in Egypt

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Received 29 June 2014; Revised 22 August 2014; Accepted 1 September 2014; Published 24 September 2014

Academic Editor: Gary Dykes

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The current study aimed to use *Coagulase* gene polymorphism to identify methicillin-resistant *Staphylococcus aureus* (MRSA) subtypes isolated from nasal carriers in Minia governorate, Egypt, evaluate the efficiency of these methods in discriminating variable strains, and compare these subtypes with antibiotypes. A total of 400 specimens were collected from nasal carriers in Minia governorate, Egypt, between March 2012 and April 2013. Fifty-eight strains (14.5%) were isolated and identified by standard microbiological methods as MRSA. The identified isolates were tested by *Coagulase* gene RFLP typing. Out of 58 MRSA isolates 15 *coa* types were classified, and the amplification products showed multiple bands (1, 2, 3, 4, 5, and 8 bands). *Coagulase* gene PCR-RFLPs exhibited 10 patterns that ranged from 1 to 8 fragments with *AluI* digestion. Antimicrobial susceptibility testing with a panel of 8 antimicrobial agents showed 6 different antibiotypes. Antibiotype 1 was the most common phenotype with 82.7%. The results have demonstrated that many new variants of the *coa* gene are present in Minia, Egypt, different from those reported in the previous studies. So surveillance of MRSA should be continued.

1. Introduction

MRSA was identified as a hospital acquired pathogen in the 1960s. Infections with community-acquired MRSA (CA-

rectum may be colonized with *S. aureus*. About 30% of people are transient carriers, and 5 to 7% of them are colonized with MRSA [5].

Discussion

Mohajeri et al in Iran(2016)

- 258 S. aureus isolates
- 5 coa types
- majority of genotype pattern III

Jundishapur J Microbiol. 2016 January; 9(1): e26460. doi: 10.5812/jjm.26460
Published online 2016 January 2. **Research Article**

Genotyping of *coa* and *aroA* Genes of Methicillin-Resistant *Staphylococcus aureus* Strains Isolated From Nasal Samples in Western Iran

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Received 2015 March 31; **Revised** 2015 June 17; **Accepted** 2015 July 8.

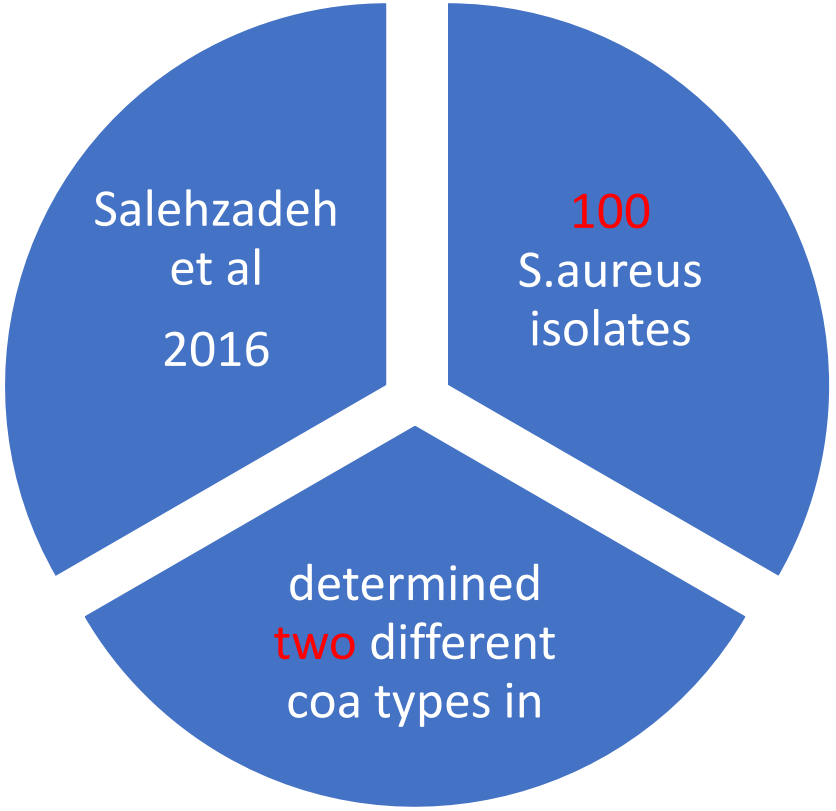
Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterial pathogen frequently isolated in both hospital and community environments. Methicillin-resistant *Staphylococcus aureus* is considered a major nosocomial pathogen that causes severe morbidity and mortality.
Objectives: The main objective of this study was to determine the genotypes of MRSA strains isolated from the nares of hospitalized and community patients in Kermanshah Hospital, western Iran, by PCR-restriction fragment length polymorphism (PCR-RFLP).
Materials and Methods: Of 1387 patients, 1217 patients were screened for more than 48 hours after admission in hospital wards and 170 patients were screened in the hemodialysis unit of Kermanshah Hospital, which is the largest hospital in western Iran. *S. aureus* was identified by standard biochemical tests, including colonial morphology, production of coagulase, and DNase and the API20 Staph test. Methicillin-resistant *Staphylococcus aureus* was identified by the Oxacillin strip test.
Results: In total, 258 *S. aureus* isolates were recovered from 1387 samples, of which 96 isolates were MRSA, 82 were hospital acquired, and 14 were community acquired. Digestion of the *aro A* gene revealed only one distinctive RFLP pattern in the 258 isolates.
Conclusions: Methicillin-resistant *Staphylococcus aureus* is an increasingly common cause of nosocomial infections. Our results are in agreement with those of other studies reporting that a few specialized clones are responsible for most cases of MRSA nasal carriage. In this study, MRSA strains isolated from different wards of hospital were closely related when analyzed by coagulase gene typing. Identifying patients colonized with MRSA during hospitalization and rapidly typing them with these methods may facilitate detection of outbreaks and prevention of the spread of organisms in hospitals.

Keywords: Restriction Fragment Length Polymorphisms, Methicillin-Resistant *Staphylococcus aureus*, *Coa* gene, *aroA* gene, *Staphylococcus aureus*

1. Background

Discussion



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Molecular typing of nosocomial *Staphylococcus aureus* strains associated to biofilm based on the coagulase and protein A gene polymorphisms

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ARTICLE INFO

Article type:
Original article

Article history:
Received: Apr 5, 2016
Accepted: Oct 18, 2016

Keywords:
Adhesion molecules
Biofilm
Coagulase
RFLP
S. aureus
Spa typing

ABSTRACT

Objective(s): *Staphylococcus aureus* is an important bacterial pathogen responsible for a variety numbers of nosocomial and community acquired infections. Biofilm formation is regarded as an important factor in the establishment of *S. aureus* infection. The contribution of the genetic background of *S. aureus* to biofilm formation is poorly understood. The aim of the present work was to genotype *S. aureus* strains associated to biofilm based on the coagulase and protein A genes and to evaluate the association between the genetic background and the biofilm forming ability of clinical *S. aureus* isolates.

Materials and Methods: A total number of 100 *S. aureus* were isolated from nosocomial infections and biofilm formation capability was investigated using phenotypic assay and molecular detection of biofilm associated genes. The strains were genotyped based on coagulase (*coa*) and protein A (*spa*) gene polymorphisms using restriction fragments length polymorphism-polymerase chain reaction (RFLP-PCR).

Results: RFLP-PCR of *coa* gene generated two types and three subtypes. Amplification of *spa* gene resulted in two banding patterns and their restriction digestion generated three subtypes. The combined *coa* and *spa* RFLP patterns generated nine genotypes (G_1-G_9). The genotypes G_4 and G_1 were the most prevalent (32.1% and 24.3%, respectively).

Conclusion: High clonal diversity of *S. aureus* strains able to produce biofilm was observed. Biofilm formation correlates with the *spa* and *coa* clonal lineage in our population and testing for multiple gene polymorphisms could be employed for local epidemiologic purposes.

► *Please cite this article as:*
Salehzadeh A, Zamani H, Keshtkar Langeroudi M, Mirzaie A. Molecular typing of nosocomial *Staphylococcus aureus* strains associated to biofilm based on the *coagulase* and *protein A* gene polymorphisms. Iran J Basic Med Sci 2016; 19:1325-1330; http://dx.doi.org/10.22038/ijbms.2016.7919

Introduction

Staphylococcus aureus is recognized worldwide as an

Association between biofilm formation ability to genetic background of *S. aureus* isolates is not well

Conclusion

- The present study attempted to give a snapshot of the molecular characteristics of *S. aureus* by a cross-sectional descriptive study.
- Evidence plus our results showed that indiscriminate and wide use of antibiotic has been defined as the main driver of MDR and easy access to antibiotic has been considered to account for regional differences in resistance rates.
- We demonstrated that there was low resistance to antibiotics rarely prescribed whilst high resistance rates were observed in the case of antibiotics which are obtainable over-the-counter for the treatment of *S. aureus* related Infections.
- There were 7 different SCs types in the present study suggesting infection in bacteremia patients is caused by *S. aureus* strains harboring different variants of the *coa* genes, which highlights the special attention for systematic surveillance and antimicrobial stewardship programs for *S. aureus* infections within burns patients.

Suggestions

- More extensive studies on the pattern of drug resistance and epidemiology of *Staphylococcus aureus* should be performed
- Carrying out phenotypic and genotypic studies simultaneously in hospitals to identify antibiotic-resistant *Staphylococcus aureus.*
- Less administration of antibiotics such as linezolid, teicoplanin, TMP/SMX to prevent the development of resistance to these antibiotics in isolates of *Staphylococcus aureus*
- Use of appropriate molecular techniques to rapidly identify isolates carrying the toxin genes *eta, etb*, *tst* and *pvl* in *Staphylococcus aureus* strains due to their pathogenicity.
- These findings emphasized that various *coa* types of *S. aureus* are responsible to engage hospitalized patients.

Thank you