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THE PREVALENCE OF VANCOMYCIN-RESISTANT STAPHYLOCOCCUS AUREUS AMONG PENITENTS IN DIYALA PROVINCE

Haider Aziz Salman
Degree of Bachelors in Biology, College of Sciences, University of Diyala, Iraq
Corresponding author: haederazez97@gmail.com

Abstract

Staphylococcus aureus is an opportunist that causes systemic infections and eye infections in the human being body. This organism increases its resistance to many categories of antibiotics all day and turn out to be more resistant. Given this fact, A total of one hundred fifty-four samples from different clinical cases had been collected from patients who had: Food diabetes 51 (33.1%), wound 39(25.3%), urinary tract infection 27(17.5%), Gingivitis19 (12.3%) and otitis 18(11.6%). And attended at to Diyala Hospital and private clinics in Diyala province during the period from December 2022 to May 2023. Our results show 63/154 (40.9%) was S. aureus. These isolates were identified according to culture, microscopic examination, biochemical tests, and APIstaph system identification kits. The current study show the highest percentage of S. aureus infections was observed in Food diabetes 23(36%), followed by wound and the frequency of S. aureus was 15(24%), then urinary tract infection 10 (16%), Gingivitis 8(13%) While the lower incidence was 7 (8%) otitis. The results showed 37/63(58.73%) of isolated S. aureus were VRSA strain. %), regarding of virulence factor the results showed Eta gene is present in all cases in percent 100%, while other gens appear in different percentages; SeA,94.5 Seb 39.68% ,Sec 34.92% Sed 28.57% ,Tst 34.92%, and ,Etb 17.46%.

Keywords: Saureus, virulence factors, wound, vancomycin-resistant.

Introduction

Staph aureus is one of the most prevalent pathogenic bacteria because its biology is based on many virulence factors that cause illness. The disease's cause and the disease's host. (1).It's also an "ESKAPE" species, which may lead to a range of severe illnesses (3) As a result, it is regarded as a significant and rising worldwide threat that affects a wide range of individuals and causes serious nosocomial infections. (2).Drug resistant S. aureus strains are seen in a few S. aureus strains (4) developed. The strains of Staph. aureus that was resistant to antibiotics containing beta-lactams, such as Penicillins, Amoxicillin, Methicillin, Ampicillin, Cephalosporins, Oxacillin, and others (5). (6). S'. the inclination is to the right. Antibiotic resistance was acquired by aureus,

resulting in a worldwide clonedistribution of antimicrobial resistance expressions. Instead of MRSA strains, there are numerous bacterial diseases that cause mortality in the public and clinics (7). S. aureus infection, like VRSA strains, has been around for a long time (7). Since the indiscriminate use of antibiotics is not a standard process, hospital facilities are not sanitary, and patients and health personnel are overburdened Infectious bacteria, such as S.auroreus, are spread more easily. (8) As a result, it makes sense to assess the state of microbial resistance to the most widely used antibiotics for treating S. aureus-caused eye infections (9).In recent decades, encapsulation of antimicrobial medicines innanoparticle systems have emerged as a promising carrier approach for increasing therapeutic efficacy while decreasing unwanted side effects. (Troncar) Antibiotic treatment through NPS offers numerous benefits, including controlled and uniform dispersion in the target area, higher solubility, longer release, improved patient compliance, fewer side effects, and improved cellular internalization (10).

Materials and Methods

The cross-sectional study was conducted at Diyala Hospital and private clinics in Diyala province in Iraq during the period from December 2022 to May 2023. approval has been obtained from Diyala health Director to conduct the study

Using simple random sampling technique, patients of either gender were enrolled for obtaining specimens from different clinical cases . The sample size was calculated using the formula: (11)

$$\frac{Z_{1-\alpha/2}{}^2 p(1-p)}{d^2}$$

Standard normal variate Z1-α/2 was 1.96 at 5% type 1 error (p=0.05) and 2.58 at 1% type 1 error (p=0.01). As p<0.05 are typically regarded as significant in research, 1.96 was utilised in the formula in which p was the population expected proportion based on previous studies (12) , and d was the absolute inaccuracy or precision determined by the researchers.

Briefly, the patient was requested to look up while lowering the eyelid down and the sample was collected from one or both eyes based on the nature of the infection. Sterile cotton swab that had been premoistened with sterile physiological saline was used gently to collect eye discharge. Swab was rubbed softly over the lower conjunctival sac from medial to lateral side and back again (13). Then simple were cultured on several different media, such as blood agar, MacConkey agar and

mannitol salt agar the latter being a selective medium for S. aureus. The samples were incubated at 37°C for 24 hours after which cultured bacteria were isolated and identified according to colony morphology, shape, size, colour and pigment production.

Deoxyribonucleic acid (DNA) extractions were carried out using a commercial kit (Presto™ Mini gDNA Bacteria Kit, Geneaid, Thailand) to obtain DNA templates for use in PCR assays. The DNA of P. aeruginosa isolates was extracted as per the manufacturer's instructions.

For cell harvesting pre-lyses, the bacterial strains were cultured on mannitol salt agar for 18 hours at 37°C. Then, they were harvested by centrifugation for 1 minute at a speed of 14,000rpm, with the supernatant being discarded.

Further, 20µL of proteinase potassium (K) (solution and 180µL of buffer Guanidinium thiocyanate(GT) were added to the pellet and mixed, with the sample tubes inverted every 3 minutes for the duration of the incubation period.

After mixing for 10 seconds with 200µL of buffer Guanidine Brochloride (GB), the cell lysate was incubated for 10 minutes at 70°C, with sample tubes mixed by inversion every 3 minutes to induce lysis. The elution buffer was pre-heated 200 l/sample at 70°C for DNA elution.

For DNA binding, the lysate samples were treated with 200µL of 100% ethanol and thoroughly mixed by shaking. The mixture was transferred to a spin column in a 2ml collection tube, and placed in a new 2ml collection tube for the genome DNA (GD) column.

For DNA elution, the spin column was placed in a 1.5 microcentrifuge tube, and 100µL of pre-heated elution buffer was added to the middle of the column matrix. After letting the mixture stand for 3 minutes to ensure that all of the elution buffer had been absorbed, the spin column was centrifuged for 30 seconds at 14,000rpm to elute the purified DNA. The extracted DNA was stored in the freezer at -20°C until use.

The concentration and purity of the DNA was measured by using an instrument (Nano Drop) and agarose gel electrophoresis.

During the process, 1µl of the extracted DNA was added to the instrument in order to detect DNA concentration and purity by analysing the optimal degree)OD((260/280 ratio to verify the protein and DNA concentration.

For agarose gel electrophoresis, 1x Tris-borate-EDT(TBE) buffer was placed in the electrophoresis tank, after which the agarose tray was immersed in the electrophoresis tank. It was ensured that the buffer was roughly several millilitres above the agarose surface. Each well was filled with 5µl of the sample and 2µl of dye, and the tank was then filled and closed. Electrophoresis was performed using 70

volt/cm of gel run swat electrophoresis. With the use of gel paper, the agarose was extracted from the tank and visualised.

For the optimization of the primers used, 2.5µl of the master mix was mixed with 5-6µl of DNA, along with 1µl of the forward and revers primers. Optimisation was programmed for SeA, Seb,Sec,Sed,Tst, eta, mecA ,and Etb gens

, and primer of gene grades were chosen, and the annealing temperature of PCR were set at 55°C, 58°C and 52°C.

Detection of SeA, Seb,Sec,Sed,Tst, eta, mecA and Etb genes was carried out by mixing 12.5ml master mix, 5-6ml DNA, 1ml each of forward and reverse primers, and nuclease-free deionised water to a final volume of 20ml, as per the manufacturers' instructions.

PCR cycling programme parameters used in the reaction for the detection of the genes of interest were noted (Tables. 1).

Data was analysed using SPSS 20. Chi-square test was used to analyse the data. P<0.001 was considered statistically significant. (13).

Table (1): The sequence and source of the gene primers used in the study

Prime r	Oligonucleotide sequence (5'-3')	Location within gene	Size of amplific d product (bp)	Mult iplex PCR set	
sea	GSEAR-1	GGTTATCAATGTGOG GGTGG	349-368	102	A
	GSEAR-2	CGGCACTTTTTTCTC TTCGG	431-450		
seb	GSEBR-1	GTATGGTGGTGTAAC TGAGC	666-685	164	A
	GSEBR-2	CAAATAGTGACGAG TTAGG	810-829		
sec	GSECR-1	AGATGAAGTAGTTG ATGTGTATGG	432-455	451	A
	GSECR-2	CACACTTTTAGAATC	863-882		
		AACCG			
sed	GSEDR-1	CGAATAATAGGAGA AAATAAAAG	492-514	278	A
	GSEDR-2	ATTGGTATTTTTTTTC GTTC	750-769		
mecA	GMECAR-1	ACTGCTATCOCACCCT CAAACC	1182-1201	163	B
	GMECAR-2	CTGGTGAAGTTGTAAT CTGG	1325-1344		
eta	GETAR-1	GCAGGTGTTGATTTAG CATT	775-794	93	B
	GETAR-2	AGATGTCCCTATTTTT GCTG	848-867		
etb	GETBR-1	ACAAGCAAAAGAATA CAGCG	509-528	226	B
	GETBR-2	GTTTTTGGCTGCTTCTC TTG	715-734		
tst	GTSSTR-1	ACCCCTGTTCCCTTAT CATC	88-107	326	B
	GTSSTR-2	TTTTCAGTATTTGTAA CGCC	394-113		

Antimicrobial susceptibility test:

Antibiotic resistance phenotypes (Methicillin/Oxacillin sensitivity test): All isolates of S. aureus were checked for the sensitivity to 1 µg Oxacillin disc and 5 µg Methicillin disc (Difco) by the disk diffusion method that instructed by NCCLS. The resistance breakpoints were ≥ 12 mm to ≤ 10 mm for 1 µg Oxacillin and ≥14 mm to ≤ 10 mm for 5 µg Methicillin. The capacity of extra antibiotic discs to inhibit MRSA or MSSA was estimated according to the instructions provided by NCCLS using commercially available discs that include: Augmenitin (AC 30 µg), tetracycline (T,30 µg), erythromycin (E,15 µg), ceftizoxime (CEF 20 µg), ciprofloxacin(Ci 5 µg), clindamycin(CC, 2 µg), clarithromycin (Cl 15 µg) and vancomycin(V, 30 µg). The zone of inhibition produced by S. aureus against each antibiotic was measured and interpreted as resistant and susceptible according to standards of Clinical Laboratory and Standards Institute(14) .

Results and discussion

Prevalence of S. aureus among various clinical cases

Out of (154) different clinical cases samples, we were able to extract 63 (40.9%) pure S. aureus. Culture, microscopic inspection, biochemical testing and API staph system identification kits were used to identify these isolates. Table (1) shows the prevalence of S. aureus among . various clinical cases . The current study show the highest percentage of S. aureus infections was observed in Food diabetes 23(36%), followed by Wound and the frequency of S. aureus was 15(24%), then urinary tract infection 10 (16%), Gingivitis 8(13%) While otitis was 7 (8%) (Table.2)

Table (2): Prevalence of S. aureus among clinical cases .

clinical cases	Number	%
Food diabetes	23	36
Wound	15	24
urinary tract infection	10	16
Gingivitis	8	13
otitis	7	8
Total	63	100
X2		47.7*
P value	0	

* Highly significant difference (P<0.01)

The present study's findings revealed that S. aureus isolated from clinical cases infections, which may be caused by Food diabetes, can operate as a reservoir for opportunistic microorganisms. If antibiotics are used to treat periodontal disease or other infections, they can lead to an increase in Staphylococcus spp. in the eye .s.aureus strains can cause antibiotic resistance is widespread and can Periodontitis develops as a result of antibiotic therapy. The fact that S. aureus is more prevalent in the skin might result in a more severe illness. The current percentages of isolated S. aureus are consistent with those reported by (15), who found that Food diabetes was 36 (33.8%), followed by wound and otitis at 19(26.8%) and 12(16.9%), respectively. Also, accord with the findings of (16) who found a prevalence of S.aureus in the food wound of 21% and skin swabs of 11% in 110 patients attending a eye hospital with a variety of oral illnesses. 13 Salivary carriages of S. aureus was detected in 41% of patients with decreased salivary flow rates attending an eye medicine clinic, with concentrations ranging from 3.7x101 to 5.2x103 cfu ml. Because of the variety of the normal eye flora and the healthy carriage ofS. aureus in specific patient groups, the case for S. aureus in the etiology of eye dysaesthesia and mucositis is difficult. However, given the high rates of S. aureus recovery in patients with oral mucosal symptoms such as pain, burning, erythema, and swelling, physicians should consider the potential of this pathogen playing a role in eye mucosal illness.

Prevalence of Methicillin Resistant S. aureus (VRSA)

In the current study all 63 coagulase positive isolates of S. aureus were subjected to disc diffusion method to 5 µg Methicillin disc and 1 µg Oxacillin disc to determine MRSA; the test results discovered that 37(58.73%) of isolated S. aureus were MRSA strain figure (1).

Figure(1):Detection of (VRSA)

The Susceptibility of VRSA and MSSA isolates to antimicrobial agents

The Susceptibility of VRSA and VSSA isolates to antimicrobial agents where shown in the Figure (2,3). The results of current study showed the rate of MRSA was 37/63 (58.73%) from various Clinical cases is lower than the rate reported from Iraq in previous reports in which VRSA was isolated from 85% of health workers in Basrah city (17), also it is very lower than that reported by Hussein (18), among health care workers in Kurdistan region of Iraq, in 2015 where the VRSA prevalence was 53% On the other hand, study in Iran was 69% (19) while in a study conducted in India, the percentage was much lower 16.6% (20) MRSA prevalence 51.4% at the Korean hospital from the Staph aureus collected from blood and nasal colonizers (21) In general VRSA was highly prevalent in Asian countries (Hussain et al., 2019) In the German study there was a decrease in MRSA rate (22) In Turkey 2017, high rates of Staph aureus highly resiste to penicillin and ampicillin (23) A study in Isfahan Iran, in 2018 showed that MRSA was 51.9% among oral infection patients and 16% among health workers (24) . HA-VRSA occurred at a higher rate than CA-VRSA in the world, but in iraq the rates were similar for the HA-MRSA and CA-VRSA (19.4% and 17%, respectively), as mentioned by (16) . This result can be explained by long hospitalization, random use of antibiotics, lack of awareness, and receiving antibiotics before coming to hospital, which are some of the potential predisposing factors for the appearance of MRSA in the hospital and community. Results of current study differs from that reported in the United States of America where a high incidence of MRSA occurred in a hospital-acquired S. aureus infection (HA-MRSA) (59%), compared to a community-acquired infection of S. aureus (17%)19 . This difference can be explained by the CA-MRSA biology appearing to be different from the HA-MRSA and the MSSA, which may allow CA-MRSA to cause diseases other than those expected from VSSA) 24).regarding Susceptibility of MRSA and MSSA isolates to antimicrobial agents the results showed that VRSA appeared more resistant to antibiotic than MSSA, Wang also found higher antibiotic resistance rat in MRSA compared to MSSA except with Trimethoprim/Sulfamethoxazole (25) Multi-Drug Resistance (MDR) was more evident among the VRSA than VSSA (26), VRSA in this study were Multi Drug Resistant (MDR), this result was similar to previous research. (26) As this study they reported high resistance to Cifoxitin (100%) amoung MRSA isolates. highest susceptibility for Vancomycin & Genatmycin (27) MRSA is resistant to all types of antibiotics containing β -lactam (28) the resistance is conducted with low affinity for β-lactam antibiotics resulting in resistance to all β-lactams antibiotics or due enzymes that hydrolytically destroy β-lactams, MRSA may contain one or both of these mechanisms (29).

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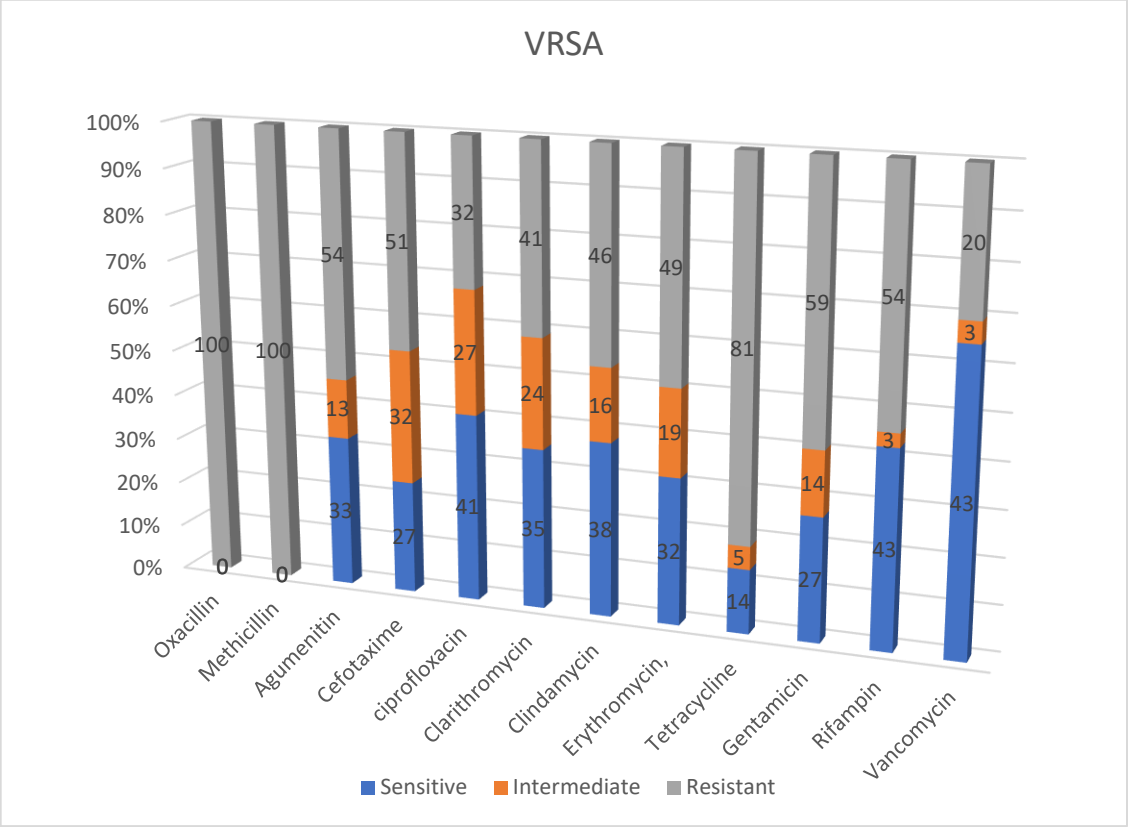

Figure (2). The Susceptibility of VRSA isolates to antimicrobial agents

Virulence gens of VSSA

Detection of the VRSA virulence genes by PCR technique All MRSA isolates were tested for seven genes(TssT, Sea, Seb,Sec,Sed, Eta and Etb gene) as well as mecA gen .(figure 3,4). All isolates and 100% contained Eta and mecA genes , Followed by SeA(94.5%), Seb (39.68%) ,Sec (34.92%),Sed (28.57%),Tsst (34.92%), and Etb (17.46%) that most of MRSA samples harbored at least two virulence gene, multiple toxin gene combinations were also observed. MRSA isolates recovered from eye infections samples contained all genes, MRSA isolate from conjunctivitis samples harbored seven gene, while there blepharoconjunctivitis samples contained mecA, Sea, Seb,Sec,TSST and Eta gene. blepharitis continued mecA, Sea, Seb, TSST and Eta gene; while there other external eye infections contained MecA, Sea, and Tst, hospital wards VRSA contained mecA, Sea, Seb, and Eta gene, VRSA isolated from Tooth impaction harboring MecA, Sea, Eta gene genes. This result agreed with many studies that showed all MRSA isolate harboring the MecA gene (30) other studies considered that Methicillin resistance can happen in MecA absence, MRSA could have another mechanism (s) for resistance; e.g., altered target site or maybe reduced drug accumulation. MecA gene absence may also be due to a technical error upon detection. (31), Staph aureus could be containing several enterotoxins (SEs) that could cause

poisoning symptoms when taken. (32) Staph aureus enterotoxin also may be implicated as virulence factors in some cases of toxic shock- like syndromes. In a local study, Sea, Seb rate was (86.78%, 52.2% respectively) (33) In general, the Sea gene was the most common compared to the Seb, Sec, and Sed genes, and this corresponds to what was stated in this study results, The TSST coded by Tst gene (34) The Tsst gene was detected in (35.13%) MRSA isolates in this study, The percentage was close to these studies by (35) (33%,32.6%) respectively, Concerning the Sec gene, Delta hemolysins is 26 peptide amino acids encoded by the gene, the mechanism of secreting delta toxin was not yet been understood (36) high prevalent of Eta gene reported in this study (100%), The result was similar to (Motallebi, et al., 2019) and (100%) (Hoseini Alfatemi et al., 2014), the current study showed the percentage Etb were (18.91%) the result agree with Ezeamagu (37)) who reported the Eta was the lower percentage 10 % among the virulence gens in VRSA. VRSA genes variation could be observed in different countries, in the same country, in different cities, or within the same city, in different hospitals, even within a single hospital in different parts. papers discussed above, that possible carriage of VRSA and contaminated hospital environment led to the development of infection.

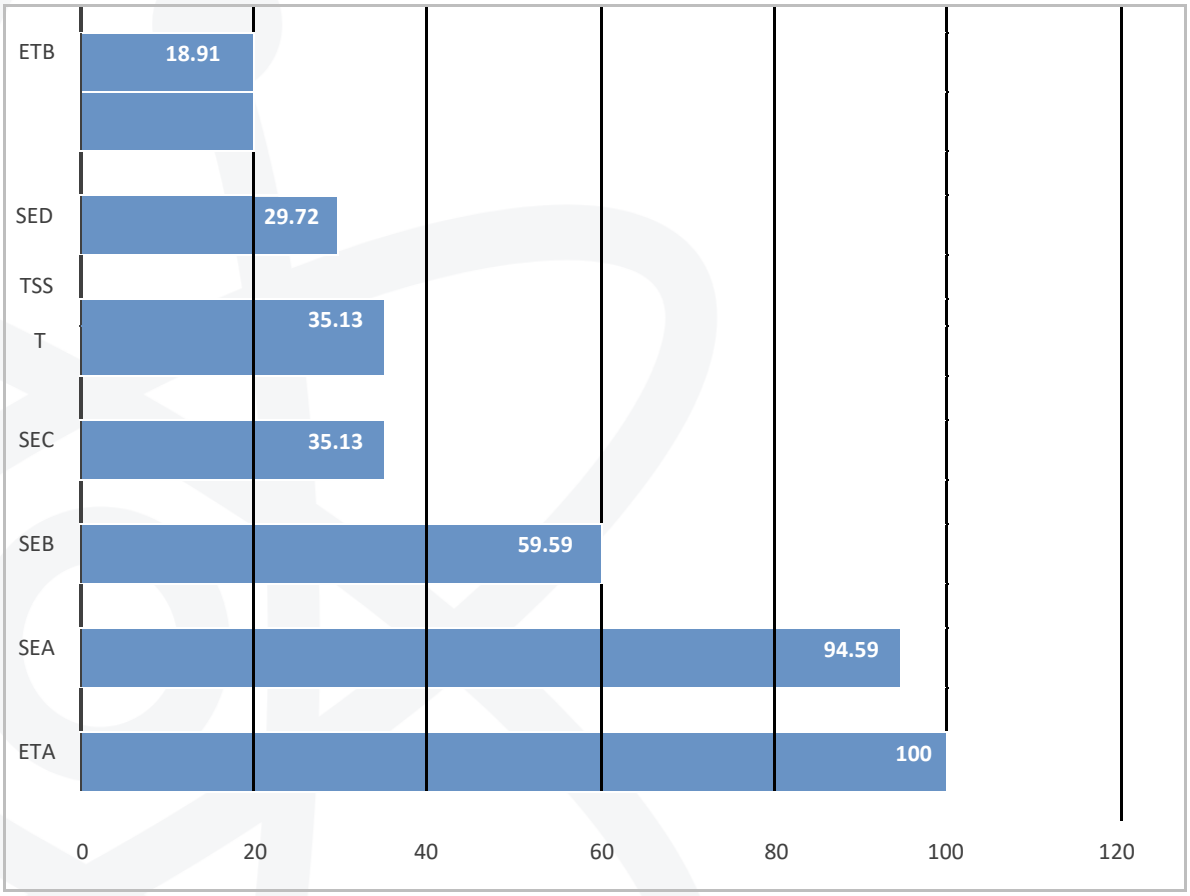

Figure (3). virulence genes distribution of VRSA isolates

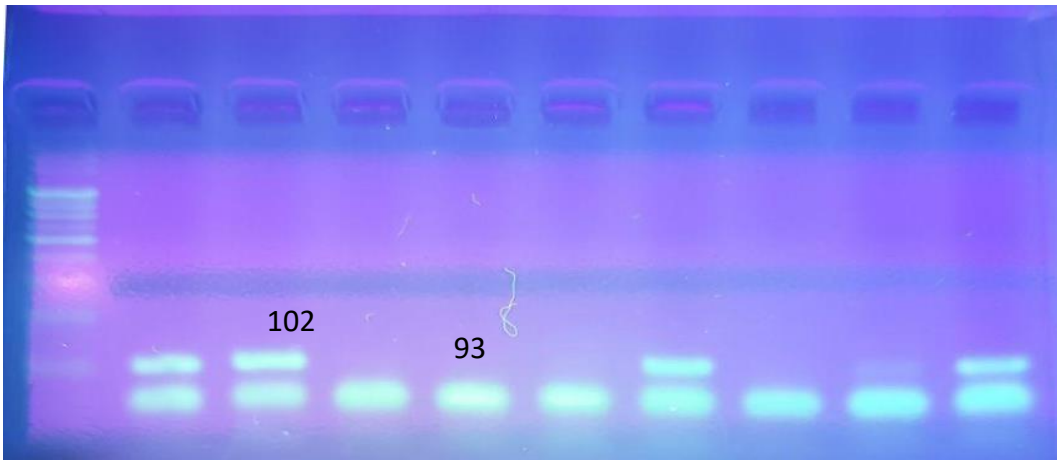

Figure (4): Agarose gel electrophoresis image shows PCR product of(Eta &Sea)genes analysis of S. aureus. Lane M Marker ladder (100-500bp), lanes (1- 10):gene of S. aureus isolate with 163bp.

Conclusion

The prevalence rate of vancomycin-resistant Staphlococcus aureus isolates among external Cinicl cases was high. Increased resistance rate to ampicillin, penicillin, erythromycin, trimethoprim-sulphamethoxazole, tobramycin, and tetracycline was observed. Ciprofloxacin and gentamicin were found to have better activity against vancomycin-resistant Staphlococcus aureus isolated from external ocular infections.

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