

How to Cite:

Al-Issawi, S. A. kadhim S., & Abdul-Razzaq, M. S. (2022). Detection of some genetic elements and virulence factorse in staphylococcus isolated from wound infections. *International Journal of Health Sciences*, 6(S5), 1138–1147. <https://doi.org/10.53730/ijhs.v6nS5.9098>

Detection of some genetic elements and virulence factorse in staphylococcus isolated from wound infections

Salam Abdul kadhim Shadhan Al-Issawi

Email: salamshadhan77@gmail.com

Mohammad Sabri Abdul-Razzaq

Abstract---Background: Detection of of some genetic elements and virulence factorse from wound contaminated in bacteria : Aims this study: is to show the genetic elements that are present in staphylococci which confer the ability of staphylococci to colonize and invade the skin Methods: This study included 12 Staphylococci isolates out of a total of 300 clinical wound samples. Patients were admitted to three main hospitals in Hilla city, Iraq, during the period from first October, 2021 to the end of December 2021.. Results: It was observed that there staphylococci when classified according to coagulase synthesis , only 25 isolates of staphylococci (40.3%) are coagulase positive and the others 37 isolates (59.7%) are coagulase negative staphylococci. Among coagulase negative staphylococci , the most common species that are isolated in this study is S.epidermidis , followed by S.haemolyticus and S.hominis where as all coagulase positive bacteria are related to S.aureus. The diagnosis depends mainly on traditional techniquis and VITEC2 techniquis. these 62 isolates are undergone to show the ability of bacteria to resist methicillin antibiotics. the results have indicated that only 7 isolates of S.aureus ,3 isolates of S.epidermidis , and one isolate for each S hominis and S.haemolyticus are resistant methicillin , where as only two isolates are found to be resistant to the antibiotics vancomycin which are related to S.aureus only. So , only 12 isolates (19%) of staphylococci are resistant to methecillin and only these isolates are subducted for molecular studies. Arginine catabolic mobile elements Aand B are investigated by using PCR techniquis , the results showed that only 4 isolates of S.aureus gave positive for ACM-A , and one isolates for each S.epidermidis , S.hominis and S.haemolyticus gave positive for this element , where as 5 isolates of S.aureus , 2 isolates S.epidermidis , one isolate for each isolates of S.hominis and S.haemolyticus gave positive resultes for ACM-B .

Keywords---virulence factorse, genetic elements, staphylococci.

Introduction

Staphylococci are gram positive bacteria that are seen as cocci under the microscope. according to its ability to produce coagulase enzyme , these bacteria are clasified into coagulase -positive bacteria and coagulase -negative bacteria . (Marsilio, et al.,2018) The more bacteria related to this genus that is positive for coagulase is staphylococcus aureus which is considered one of the most important pathogen in some chlinical cases in the human. However coagulase negative staphylococci should also be considered where there are two important species should be taken into account in some clinical cases such as staphylococcus epidermidis and saprophyticus (Balachander,et al.,2021)

Although most species of staphylococci are considered as normal flora of the skin , but under certain circumstances , they will transform into opportunistic pathogens and their ability to produce different virulence factors (Tong,et al.,2015)

Basides , according to the ability of staphylococci particularly staphylococcus aureus ,the bacteria is classified into methicilline resistance staphylococcus aureus (MRSA) and methicilline sensitive staphylococcus aureus (MSSA)(Jayakumar,et al.,2021)

This genus appears to be able to resistance different types of antibiotics , particularly B-lactam antibiotic ,However , vancomycin may be used for treatment some disease caused by this germ particularly staphylococcus aureus that causes respiratory infection. (Stogios,et al.,2020) Staphylococcus aureus on the other hand possesses many virulence gene that share in its ability to cause a diseases. These virulence genes may be present as distractive loci or as genetic elements (Cheung, et al.,2021) The most important genetic elements , is arginine catabolic mobile elements which are present in staphylococcus aureus and staphylococcus epidermidis which confer the bacteria the ability to resist some heavy metals particularly copper ions, and also facilitate the bacteria to colonize the skin and mucus membranes (Al-Jabri,et al.,2021). The aim of this study is to detect ACME among staphylococcus isolates.

Materials and Methods

Patients and Samples collection

this cross-sectional study was designed to evaluate some virulence factors of staphylococcus bacteria isolated from patients with wound infections in Babel Governorate, Iraq. the study included 300 clinical samples from patients with wound infections of all ages and both sexes, in three main hospitals in Hilla City, Hilla Teaching Hospital, Imam Al-Sadiq Hospital, and Al-Kifl General Hospital, and outpatients. During the period extending from October 2021 until the end of December 2021, 300 clinical samples were collected in general from the site of the wound infection, using sterile transfer media and send directly to the laboratory for the purpose for culturing on blood agar and MacConkey agar Petri dishes, the collected specimen swabs were inoculated. For 24 hours, the plates were incubated aerobically at 37°C. The expanding colonies were then morphologically recognized and stained with Gram stain. Various biochemical assays were also carried out in order to distinguish and identify bacterium species. The catalase

test was used to separate Gram-positive cocci such as *Streptococcus* and *staphylococcus* species, followed by the coagulase test to differentiate *Staphylococcus* coagulase-negative and coagulase-positive strains. and used manitol salt agar medium ,novobiocin disc and VITEK2 system (Munyeshyaka,et al.,2021).

Methicillin and vancomycin Screening test:

It was performed by using a pure culture of previously identified bacterial isolate. The most effective antibiotic for each bacterial isolate was determined as recommended by CLSI, (2021).

Place the appropriate antimicrobial-impregnated disc on the surface of the agar , To add disc one at a time to the agar plate using forceps Sterilize the forceps by cleaning them with a sterile alcohol pad and allowing them to air dry or immersing the forceps in alcohol then igniting, Once all disks are in place, replace the lid, invert the plates, and place them in a 35°C air incubator for 18 to 18 hours. Inhibition zones were measured using a ruler and compared with the zones of inhibition determined by the antibiotic inhibits microbial growth, a clear ring, or zone of inhibition, is seen around the disc. The bacteria are classified as sensitive, intermediate, or resistant to an antibiotic by comparing the diameter of the zone of inhibition to defined thresholds which correlate with MICs. by disc diffusion method according to the (CLSI ;2021).

Detection of arginine catabolic mobile elements:

We detected ACME methicillin-resistance to *Staphylococci* isolates from patients , using PCR primers ACME-A (F- 5' - GGATTCGCCCAAGTGATGACC-3'), and the R- 5' -GACTGCTGGGTATGACGT-3') 510 bp (McManus, et al 2017) The ACME-B (F- 5' - CGATATCATCTATACCTAGTAC G-3') and the (R- 5' - GAAAATCCTCAAGTAAGAAG TG-3') , 510 bp (Ellington, et al., 2008) the conditions of both two genes 95 C° for 2 minut of Initial denaturation , 95 C° for 30 Sec of denaturation , 55.6 C° for 30 Sec of annealing , 72 C° for 50 Sec of extension , 72 C° for 5 min of final extension and 35 cycle.

Results and Discussion:

clinical wound samples were collected from patients with infection in skin wounds (ages between 41 days and 72 years) of both sexes, who were admitted to three hospitals in Hilla city from first October 1, 2021 to the end of December 2021. Of the 300 clinical wound samples, 286 (95%) were positive wound samples and 14 (5%) were negative wound samples. 268 (95%) positive wound cultures ,were divided into 221 (73%) Gram-negative bacteria, and 65 (22%) Gram-positive bacteria based on Gram stain and culture medium.

Among gram positive bacteria 62 isolates of *staphylococcus* were isolated , while three isolates of other Gram-positive bacteria were excluded from this study as shown in Table (3-1)

Table (3-1) screening for the growth of bacterial isolates from wound infection :

Isolates	No.	%
Gram negative bacteria	221	73%
Gram positive bacteri	65	22%
No growth	14	5%
total	300	100%

It was noticed that the predominant bacteria isolated of superficial in Diabetic foot and skin ulcer were Staphylococcus. The same results were found by (Kothari, et al.,2022) who found that staphylococci were predominant among Gram positive bacteria.

In other study done by (Abass et al.,2022) who observed that Gram positive bacteria recorded (43.6%), which include *S. aureus* as predominant isolated bacteria (34.14 %) isolates.

However, *Staphylococcus aureus* is the pathogen most frequently isolated in diabetic foot infection (DFI), with high drug resistance (Xie, et al.,2017). The results suggest that *Staphylococcus aureus* strains obtained from chronic wounds seem to be more often resistant to antibiotics and harbor more virulence genes compared to strains isolated from blood. (Budzyńska, et al 2021).

Among of the 65 isolates that are positive growth related to the gram positive bacteria, 62 isolates (95.4%) are belonging to the genus staphylococci according to the Gram stain analysis, cultural features, biochemical characteristics, it was found that 25 isolates are positive for Coagulase which are mostly related to the *S. aureus* and the others 37 isolates are Coagulase negative shown in the table (3-2).

Table (3-2) number of staphylococcus according to the type of wounds and some effect of antibiotics:

Bacteria	Source of wounds	Coagulase positive staphylococci	Resistance to methicillin	Resistance to vancomycin
Staphylococci (62) isolates	Wound (41) isolates (66%)	(25) isolates 40.3%	(12) isolates 19.3%	(2) isolates 3.2%
	Diabetic foot (14) isolates (22.8%)			
	Post operate (4) isolates (6.4%)			
	abscess (3) isolates (4.8%)			

Besides , methicilline resistant staphylococci are also detected among these isolates . It wase showed that only 12 isolates (19.3%) are resistant to this antibiotic and are classified with methicilline resistant staphylococcus.

Also, among 62 isolates , only two isolate (3.2%) showed resistant to the antibiotic vancomycin, there isolates are classified with in vancomycin resist staphylococcus group. (the results are showed in table (3-2)

However ,the 12 isolates that are ciassified with in MERSA , are also re-identified by using VITEK2 system to know the species of staphylococci and also these isolates are suspected for molecular studies.

high drug resistance (Xie et al .,2017) , the results suggest that Saphylococcus aureus strains obtained from chronic wounds seem to be more often resistant to antibiotics and harbor more virulence genes compared to strains isolated from blood.(Budzyńska,et al 2021).

VITEK2 system analysis showed that methicillin resistant staphylococci (12 staphylococci) are diagnosed as the followimg : 7 isolates belong to S.aureus , and 3 isolates belong to S.epidermidis and one isolate for each S.haemolyticus and S.hominis. Wherease both two isolates that are resistant to vancomycin is belonging to S.aureus . as shown in table (3-3).

Table (3-3) prevalence of methicillin and vancomycin resistance staphylococcus spp.

Antibiotics type	S.aureus 25 (40.3%)	S.epidermidis 35 (56.5%)	S.haemolyticu s 1(1. 6%)	S.hominis 1(1.6%)	No.
Methicillin	7(11.3%)	3(4.8%)	1(1.6%)	1(1.6%)	12 (19.3%)
Vancomycin	2(3.2%)	0	0	0	2(3.2%)

According to the data obtained above it was shown that staphylococcus spp . can be obtained from different types of wounds where they are shaped the high levels of bacteria found in the skin and soft tissue . these results are identical to thate obtained by (Zatout,et al., 2020) who found that staphylococcus spp . many be isolated from wound at high levels. The same results are noticed by (Bessa,et al 2015). Who said that staphylococci are the predominant in wound and skin infections.

Since coagulase is positive in 25 isolated bacteria , it is strictly related to S. aureus which is positive for this enzyme particularly the pathogen strains .

But only 12 isolates are resistance to methicillin and these isolates and shaped with 7 isolates for S.aureus , 3 isolates for S. epidermidis and one for each S.haemolyticus and S.hominis . these results are giving good signals for that methicillin resistance staphylococcus is not restricted for S. aureus but for other species of staphylococci .

3.2.Molecular detection of virulence factors:

3.2.1-Detection of arginine catabolic mobile element (ACME) gene A&B in staphylococci isolated from wound infections.

Only twelve isolates (19%) staphylococci which are resistant to methicillin subjected for molecular studies where DNA samples were previously obtained from these isolates by using Favor Prep Genomic DNA Mini kit DNA extraction kit.

A total of 12 methicillin resistant staphylococcus spp isolates ACM-A elements is detected in 4 isolates of *S.aureus* , and one isolate in each *S.epidermidis* , *S. haemolyticus* , and *S. hominis* . so , 7 isolates only (58.3%) are positive for this gene and 5 isolates (41.7%) give negative result for it. (as shown in fig : 3-1) .

In contrast to ACM-B element, the results of this study showed only 9 isolates (75%) of staphylococci are positive for this gene and 3 (25%) negative isolates, are distributed as the following , 5 isolates of *S.aureus* , 2 isolates of *S. epidermidis* , and one of *S. hominis* and *S.haemolyticus* . as shown in fig (3-2). The results are summarized in table (3-4).

Table (3- 4) detection of ACM-A &ACM-B element gene .

Staphylococcus spp	ACM-A	ACM-B
<i>S.aureus</i> (7)	4	5
<i>S.epidermidis</i> (3)	1	2
<i>S.haemolyticus</i> (1)	1	1
<i>S.hominis</i> (1)	1	1
total	7	9

Two mobile genetic elements are seen separately or together in staphylococci species isolated from patients with wound infections.

These genetic elements when be present , they are transferred from other bacteria by gene transfer mechanisms , so , they may be present or absent depends mainly on such mechanisms .

However , (Al- Jabri et al.,2021) have indicated that the genetic composition of ACM-elements and their distribution among staphylococci differ widely from one strain to another and from one species to another , these observations come correctly to the results of this study where the presence of these elements differ from one isolate to another.

(Wu et al.,2020) was found that these elements particularly ACM-A and ACM-B elements may be present in some staphylococcus spp and they come mainly due to insertion of these elements by phage invasion or by transmissible vectors such as plasmids .the same observations are found by (Diep, et al .,2006) Who had indicated that elements were not found in staphylococci chromosome .

Indeed some studies found that these elements do not increase the virulence of staphylococci but they contribute in of staphylococci but they contribute in of supporting the other virulence factors to increase transmission (Diep,et al.,2006)(Highlander,et al.,2007) .

But (O'Connor ,et al., 2018) showed that these elements may contribute in the virulence of staphylococci particularly in some cases of periodontitis to resist some environmental factors at oral cavity.

Almebairik,et al.,(2020) have observed that these elements when be present it may be located with chromosomal cassette elements (scc) and the later may have different elements that carry different virulence genes .

In astudy done by Shamansouri,et al.,(2016) it way indicated that these elements are also present in other species of staphylococci such as *S. epidermidis* and these results confirm the results of this study when ACME-A are present in *S. epidermidis* and other staphylococci.

The methicillin-resistant *S. epidermidis* (MRSE) lineage was largely divided into three groups: two staphylococcus-2 clusters and one staphylococcus-23 cluster (Lee, et al.,2018) , *S. epidermidis* is a dominant reservoir of multidrug resistance genes, as demonstrated by horizontal gene transfer(HGT), according to multiple smaller scale and local investigations (Lee, et al.,2018; Dhawan,et al.,2015) , a common occurrence of SCCmec type IV and ACME element predominantly type I arranged in a composite island was discovered through genomic analysis. The genetic composition of ACME differed widely between isolates of the same or different staphylococcus spp (Al-Jabri,et al.,2021) , the results of this study are similar to those of our current study.

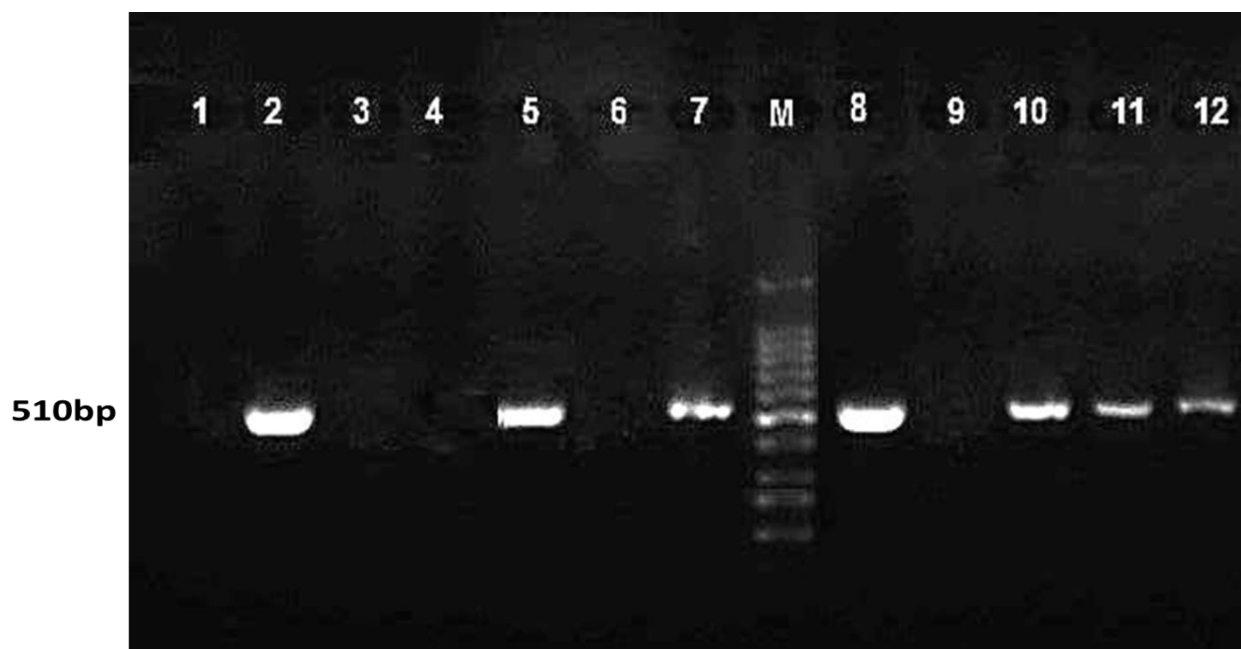


Figure (3- 1) Gel electrophoresis of PCR product of ACME-A gene (510bp) the (No.5, 8, 11 ,12,*S.aureus* . No,10,*S.epidermidis*.No ,7,*S.haemolyticus*. No,2,*S.hominis*) isolates with positive result for ACME-A from isolates wound infection L= ladder(100-1500). The electric current was allowed at 80 volt for 1hr.

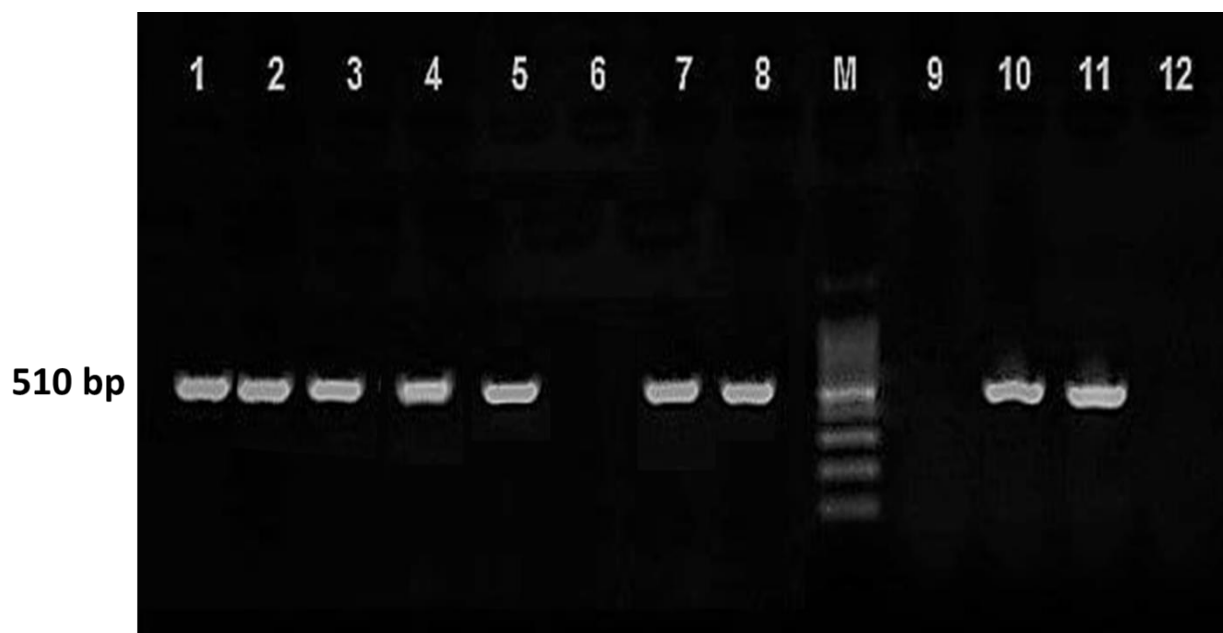


Figure (3- 2) Gel electrophoresis of PCR product of ACM-B elements gene(510bp) the (No.1, 3, 5, 8, 11 ,12 *S.aureus*.No,4,10 *S.epidermidis*. No 7 *S.haemolyticus*, and No,2 *S.hominis*) isolates with positive result for ACME-B from isolates wound infection L= ladder(100-1500). The electric current was allowed at 80 volt for 1 hr.

References

- Almebairik, N.; Zamudio, R.; Ironside, C.; Joshi, C.; Ralph, J.D.; Roberts, A.P.; Gould, I.M.; Morrissey, J.A.; Hijazi, K.; Oggioni, M.R. Genomic Stability of Composite SCCmec ACME and COMER-Like Genetic Elements in *Staphylococcus Epidermidis* Correlates With Rate of Excision. *Front. Microbiol.* 2020, 11, 166.
- Abass, Abeer A., Mayyada F. Darweesh, and Israa Abdul Ameer Al-Kraety. "Bacterial profile in skin ulcer infection with special resistance to antibiotics in AL-Sader hospital in Al-Najaf city." *AIP Conference Proceedings*. Vol. 2386. No. 1. AIP Publishing LLC, 2022.
- Al-Jabri, Zaaima, et al. "Whole Genome Sequencing of Methicillin-Resistant *Staphylococcus epidermidis* Clinical Isolates Reveals Variable Composite SCCmec ACME among Different STs in a Tertiary Care Hospital in Oman." *Microorganisms* 9.9 (2021): 1824.
- Balachander, Kannan, and Deborah Gnana Selvam Alexander. "Antibiotic Resistance, Virulence Factors and Phylogenetic Analysis of Efflux Proteins of Coagulase Negative *Staphylococcus* Isolates from Sewage Samples." *Pharmaceutical Science and Technology* 5.1 (2021): 14.
- Bessa, Lucinda J., et al. "Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection." *International wound journal* 12.1 (2015): 47-52.

- Budzyńska, Anna, et al. "Virulence Factor Genes and Antimicrobial Susceptibility of *Staphylococcus aureus* Strains Isolated from Blood and Chronic Wounds." *Toxins* 13.7 (2021): 491.
- Cheung, Gordon YC, Justin S. Bae, and Michael Otto. "Pathogenicity and virulence of *Staphylococcus aureus*." *Virulence* 12.1 (2021): 547-569.
- Dhawan, B.; Rao, C.; Udo, E.E.; Gadepalli, R.; Vishnubhatla, S.; Kapil, A. Dissemination of Methicillin-Resistant *Staphylococcus Aureus* SCCmec Type IV and SCCmec Type v Epidemic Clones in a Tertiary Hospital: Challenge to Infection Control. *Epidemiol. Infect.* 2015, 143, 343–353.
- Diep, B. A., Gill, S. R., Chang, R. F., Phan, T. H., Chen, J. H., Davidson, M. G., Lin, F., Lin, J., Carleton, H. A. & other authors (2006). Complete genome sequence of USA300, an epidemic clone of community-acquired methicillin-resistant *Staphylococcus aureus*. *Lancet* 367, 731– 739.
- Ellington, Matthew J., et al. "Distribution of the ACME-arcA gene among methicillin-resistant *Staphylococcus aureus* from England and Wales." *Journal of antimicrobial chemotherapy* 61.1 (2008): 73-77.
- Highlander, S.K.; Hulten, K.G.; Qin, X.; Jiang, H.; Yerrapragada, S.; Mason, E.O., Jr.; Shang, Y.; Williams, T.M.; Fortunov, R.M.; Liu, Y.; et al. Subtle genetic changes enhance virulence of methicillin resistant and sensitive *Staphylococcus aureus*. *BMC Microbiol.* 2007, 7, 99.
- Jayakumar, J., Kumar, V. A., Biswas, L., & Biswas, R. (2021). Therapeutic applications of lysostaphin against *Staphylococcus aureus*. *Journal of Applied Microbiology*, 131(3), 1072-1082.
- Kothari, Bhavin. "Clinical study and surgical management of diabetic foot: An observational study." *International Journal of Surgery* 6.1 (2022): 130-133.
- Lee, J. Y. H., Monk, I. R., Gonçalves da Silva, A., Seemann, T., Chua, K. Y. L., Kearns, A., et al. (2018). Global spread of three multidrug-resistant lineages of *Staphylococcus epidermidis*. *Nat. Microbiol.* 3, 1175–1185. doi: 10.1038/s41564-018-0230-7.
- Marsilio, Fulvio, Cristina E. Di Francesco, and Barbara Di Martino. "Coagulase-positive and coagulase-negative *Staphylococci* animal diseases." *Pet-To-Man Travelling Staphylococci*. Academic Press, 2018. 43-50.
- McManus, Brenda A., et al. "First detailed genetic characterization of the structural organization of type III arginine catabolic mobile elements harbored by *Staphylococcus epidermidis* by using whole-genome sequencing." *Antimicrobial agents and chemotherapy* 61.10 (2017): e01216-17.
- Munyeshyaka, Emmanuel, et al. "Contribution of Medical Wards Contamination to Wound Infection among Patients Attending Ruhengeri Referral Hospital." *International Journal of Microbiology* 2021 (2021).
- O'Connor, Aoife M., et al. "Significant enrichment and diversity of the staphylococcal arginine catabolic mobile element ACME in *Staphylococcus epidermidis* isolates from subgingival peri-implantitis sites and periodontal pockets." *Frontiers in microbiology* 9 (2018): 1558.
- Reference
- Shamansouri, S., V. K. Zadeh, and M. Khazaei. "Study on the percent of frequency of ACME-Arca in clinical isolates resistant to methicillin-staphylococcus epidermidis in Isfahan, Iran." *Journal of Fundamental and Applied Sciences* 8.2 (2016): 1046-1057.

- Stogios, Peter J., and Alexei Savchenko. "Molecular mechanisms of vancomycin resistance." *Protein Science* 29.3 (2020): 654-669.
- Tong SY, Davis JS, Eichenberger E, et al. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev.* 2015;28(3):603–661.
- Wu, Kaiyu, et al. "Arginine catabolic mobile element in evolution and pathogenicity of the community-associated methicillin-resistant *Staphylococcus aureus* strain USA300." *Microorganisms* 8.2 (2020): 275.
- Xie, Xiaoying, et al. "Bacterial profile and antibiotic resistance in patients with diabetic foot ulcer in Guangzhou, Southern China: focus on the differences among different Wagner's grades, IDSA/IWGDF grades, and ulcer types." *International journal of endocrinology* 2017 (2017).
- Zatout, A., et al. "Coagulase negative staphylococci in Anti-Cancer Center, Batna, Algeria: antibiotic resistance pattern, biofilm formation, and detection of *mecA* and *icaAD* genes." *African Journal of Clinical and Experimental Microbiology* 21.1 (2020): 21-29.