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Genetic Comparative Study Of Staphylococcus Aureus Isolated From Infections And Rural Water

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Abstract

One hundred specimens from wounds, burns, and Rural water were collected , wounds and burns isolates were collected from patients laying in hospital from different age and gender . It was found that 50 isolates belong to *Staphylococcus spp*., 38 isolates were identified as *S. aureus* from infections isolates and 12 isolates were identified as *S. aureus* from rural water according to microscopic, cultural and biochemical testing. The study of seven extracellular enzyme as virulence factors including the enzymes: urease, lipase, DNase, haemolysin, coagulase, β-lactamase, and lecithinase. Revealed that 100% of *S.aureus* which isolated from infections had the ability to produce these enzymes, while the isolates of rural water were unable to produce the enzymes DNase, lipase, coagulase , but they were capable to produce haemolysin, urease, lecithinase, and β-lactamase the range for production of these factors were 50 %,84,%, 7,%, and 43% respectively. 14 *Staphyolococcus* isolates from infections isolates and 4 isolates from rural water isolates were selected according of their ability for production most of studies virulence enzymes for detection of genes encoding for the enzymes heamolysine (hly) and (coa) by using of polymerase chain reaction (PCR) technique. The Gene hIy was detected in all isolates from infections and rural water and coa was detected in infections isolates only.

Keywords: *Staphylococcus aureus*, PCR, virulence factor, heamolysine (hly), coagulase (coa), rural water.

1- Introduction

Bacteria contamination of wounds and burns is one of the health problems faced by hospitalized patients due to their infection. It is known as hospital infection (Nosocomial infection(Intensive care worldwide, especially in developing countries. Reports indicate that about (5-10%) of those placed in intensive care units in US hospitals acquire this condition(1). The type of infection, the occurrence and development of the infection depends on several factors, including the virulence, the number of germs that cause the infection, and the extent of the infection(2). The host's sensitivity and the nature of the exposure to infection, the tag that is transmitted by the germs, as well as the type of wound and the degree of burn , treatment with antibiotics, age, gender, and length of stay in the hospital(3,4).

Despite the progress made in the field of medical care for patients with wounds and burns, it has been to antibiotics since their discovery(5,6). The great effect in reducing the rates of this infection, and it has given good results in treatment and control and limit(5) .

However, the misuse of these antibiotics, indiscriminately and without studied and without conducting a test for sensitivity to antibiotics, led to the emergence of Several strains of bacteria that are resistant to antibiotics(7).

S. aureus in drinking water may also serve as a source for colonizing residents exposed to contaminated water. Shine field and his colleagues (8), in their investigation of bacterial interference, found that they could set up a carrier state in the nose of 50% of newborn infants by inoculating 200 to 400 cocci(9). Other investigators found that the inoculum required to induce infection in traumatized skin was very small. Colonization occurred in the majority of sites when inoculated with a few hundred cells, and some infections occurred after an inoculation of approximately 10 cells(10,11)

The great development in the generalization of molecular biology and genetic engineering techniques has led to the use of advanced and rapid techniques in the detection of Genes of virulence or antibiotic resistance and investigation of genetic elements related to pathogenicity without resorting to conventional methods. In isolation, diagnosis, and sensitivity testing to antibiotics (12) some pathogenic bacterial agents are sometimes difficult to diagnose In the laboratory using conventional methods, the use of molecular PCR technology, using specialized primers, was used to detect these factors that were not It is taught protected, either by conventional or partial laboratory methods(13).

2- Materials and working methods:

2-1-Sample collection

A 100 sample were collected from wounds, burns and rural water From October 2020 to March 2021, the swabs were taken from different ages and from both sexes, with 35 samples for a wound, 25 for a burned sample, and 40 for rural water

2-2- Isolation and diagnosis of staphylococcus bacteria

Samples were cultured on both blood agar and MSA (salt mannitol ag). The plate was cultured at 37 c° for 24 hour and isolates were diagnosed accordingly for phenotypic features, microscopy and biochemical tests(14) as in figure 1 & 2

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2-3: water Sample collection.

Water samples were obtained tTechnical paper 5383, Oregon Agricultural Experiment Station. by two procedures. In the first, sterile sample bottles were distributed to residents in a rural area who obtained their water from individual wells. Residents were instructed to take samples after running the water for several minutes. The bottles were collected 3 to 4 h after being filled and stored on ice until cultured within the next 1 to 3 h. The second procedure involved sampling water specimens sent to the Oregon State University Department of Microbiology water laboratory. These samples were usually mailed in and had a maximum 30-h transit time limit before sampling(15,16).

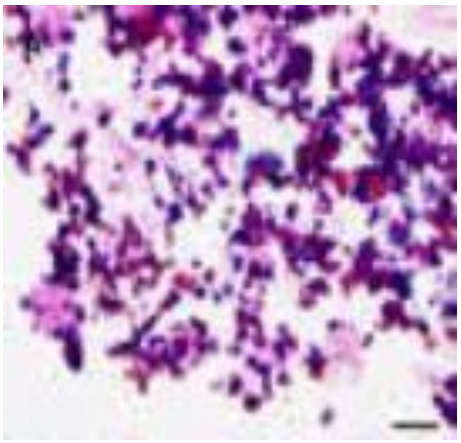

Figure (1) Gram positive staphylococcus bacteria

Figure (2): *S. aureus* cultured on MSA (salt mannitol ag).

2-4- Investigate some virulence factors

The productivity of some virulence factors and seven of the following extracellular enzymes were investigated: urease, lipase, DNase, haemolysin, coagulase, β- lactamase, and lecithinase... using Various laboratory culture media .

2-5- Investigate the prevalence of coa& hIy genes in bacterial isolates

18 isolates depending on their ability to produce the most virulence factors studied in a laboratory from the infection of wounds and burns. 7 Isolates from each of the wounds and burns injuries and 4 isolates from the swabs do not use specialized primers that target Co-specific genes (coa, hIy), as in the Table –937

Table 1 - Sequences of nitrogenous bases for specific primer

No.	Primer	primer sequence	Expected gene size	the manufacture company
1	Coa	5'ATA GAG ATG CTG GTA CAG G3' 5'GCT TCC GAT TGT TCG ATG C3'	440-1400 bp	Alpha DNA
2	hIy	F:GGTTTAGCCTGGCCTT R:CATCACGAACTCGTTC	100-937 bp	Alpha DNA

3- Results and discussion

3-1- Sample collection

A 100 sample were collected from wounds, burns and rural water the swabs were taken from different ages and from both sexes, with 35 samples for a wound, 25 for a burned sample, and 40 for rural water as clear in table 2 which clear Number of samples that contained *Staphylococcus aureus* isolates

Table 2: Number of samples that contained *Staphylococcus aureus* isolates

Type of sample	**The number of Staphylococcus aureus isolates**	**%**	**Total**
Wounds	**20**	**57.14%**	**35**
Burns	**18**	**72%**	**25**
rural water	**12**	**30%**	**40**
Total	**50**	**50%**	**100**

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The presence of staphylococcus in wounds and burns injuries at high rates. This is consistent with most studies.(11,14)

As we noticed during the study, the presence of large numbers of Staphylococcus aureus in rural water as found in (15,16)

3-2- Investigate some virulence factors

The productivity of some virulence factors and seven of the following extracellular enzymes were investigated: urease, lipase, DNase, haemolysin, coagulase, β- lactamase, and lecithinase. using Various laboratory culture media(17) and the results clear in table(3) which clear differ in the ability of productive between the infections isolates and the environmental isolates (rural water)

Table 3: The productivity of some virulence factors

Type of sample	The number of Staphyloccus aureus	Urease%	Lipase%	DNase,%	Haemolysin%	coagulase %	β- lactamase %	Lecithinase %
Wounds	20	100 %	100 %	100%	100%	100%	100%	100 %
Burns	18	100 %	100 %	100%	100%	100%	100%	100 %
Rural Water	12	84%	0	0	50%	0	43%	7%

3-4- Investigate the prevalence of coa& hIy genes in bacterial isolates

18 isolates depending on their ability to produce the most virulence factors studied in a laboratory from the infection of wounds and burns. 7 Isolates from each of the wounds and burns injuries and 4 isolates from the swabs do not use specialized primers that target Co-specific genes (coa, hIy), as in the Table –1

The Gene hIy was detected in all isolates from infections and rural water and coa was detected in infections isolates only

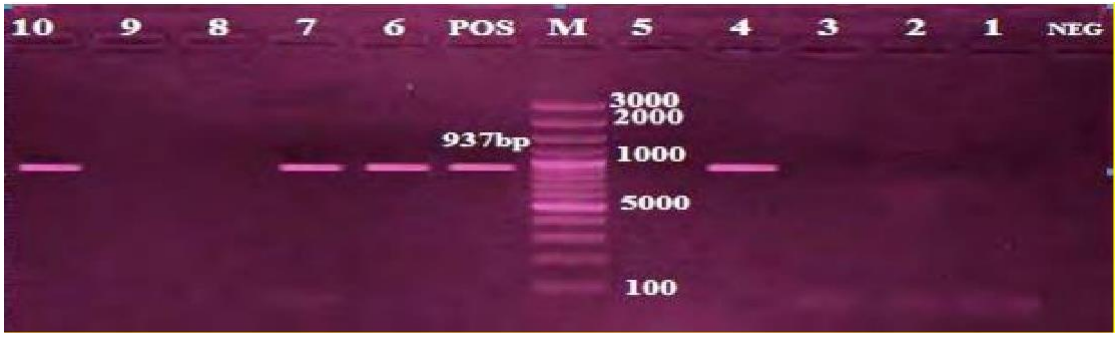

Figure 3: PCR results of DNA isolates of *S, aureus*. bacteria isolated from infections of wounds, burns and sample from rural water by using specialized primers of hIy genes on acarose pain at a concentration (5.1%)and a good difference (60) volts for a period of time.) Two hours)

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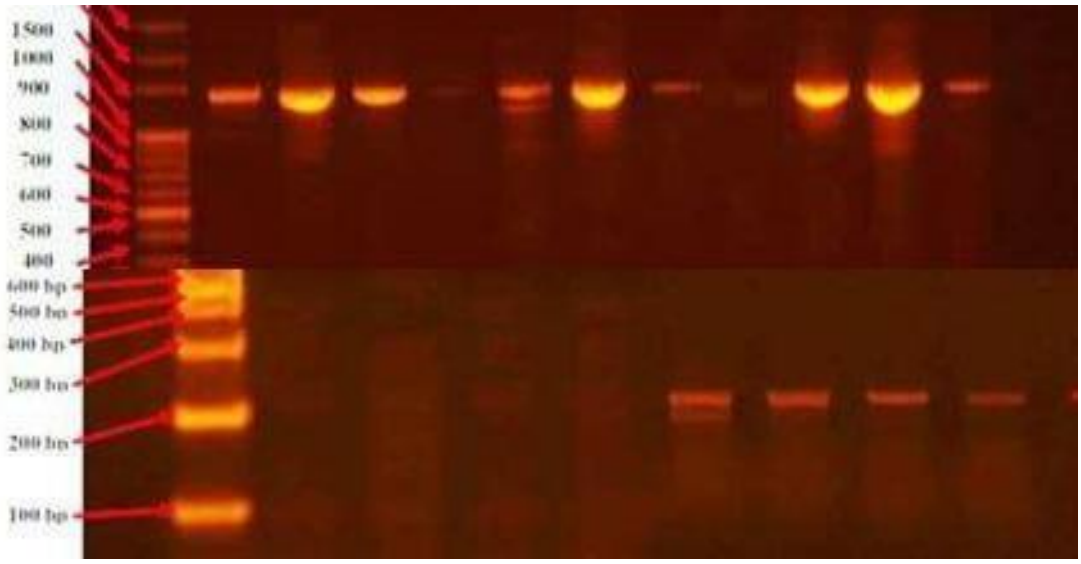

Figure 4: PCR results of DNA isolates of *S, aureus*. bacteria isolated from infections of wounds, burns and sample from rural water by using specialized primers of coa genes on acarose pain at a concentration (5.1%)and a good difference (60) volts for a period of time.) Two hours)

The results of the current study for the presence of the hIy gene which clear in figure (3)came relatively close to what was found by U [4], as it was found that the pathogenicity of these bacteria is due to Productively, the virulence factor of haemolysine enzyme, emulsin, where it was mentioned that the proportion of (18.81)% are genes encoding production gene hla and (18.18(\ Any genes encoding hIb and also the results of the current study coincided with what was found (5), where they stated that haemolysin-α is more A frequency of haemolysin-, at a rate of (4.43)%, and the results of the current study also converged with what was found by (6) .

High resistance to antibiotics has a high potential for pathogenicity through the production of enzyme enzymes.

The results of the current study, depending on the isolation sources, showed the presence of the coa gene at a rate of (100%) in wound infection isolates and burns, and the absence in rural water, as shown in Table (3), and this is what you confirmed the results Electrophoresis, where the results of these isolates showed DNA bundles with partial weights ranging between 440-1400bp. Compared with the 100bp volumetric index and as in Figure -4

The results of the coa gene were consistent with what was found by [8], where they stated that the majority of S aureus isolates are producing this enzyme because it is a working factor virulence in the pathogenicity of this bacterium, and the results of the study also converged with what was mentioned [9], where they used gene coagulase as a virulence factor for methismine-resistant Staphylococcus aureus, and [10] indicated that most strains of *Staphylococcus aureus* are

aureus.S was the producer of the enzyme coagulase as a significant virulence factor in the pathogenicity of this bacterium with a rate of 39%.

The ratio of gene spread to the different source and number of isolate

References

1. Akineden Ö., Annemüller C., Hassman A. A., Lämmler C., Wolter W., Zschöck M. (2001). Toxin genes and other characteristics of *Staphylococcus aureus* isolates from milk of cows with mastitis. *Clin. Diag. Lab. Immunol.* 8, 959–964. 10.1128/cdli.8.5.959-964.2001 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
2. Argudín M. Á., Mendoza M. C., Rodicio M. R. (2010). Food poisoning and *Staphylococcus aureus* enterotoxins. *Toxins* 2, 1751–1773. 10.3390/toxins2071751 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
3. Becker K., Skov R. L., von Eiff C. (2015). Staphylococcus, Micrococcus, and other catalase-positive cocci, in *Manual of Clinical Microbiology, 11th Edn*, eds Jorgensen J. H., Pfaller M. A., Carroll K. C., Funke G., Landry M. L., Richter S. S., et al. (Washington, DC: ASM Press;), 354–382. [Google Scholar]
4. Ben Ayed S., Boutiba-Ben Boubaker I., Ennigrou S., Ben Redjeb S. (2008).

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Accessory gene regulator (agr) typing of *Staphylococcus aureus* isolated from human infections. *Archs. Inst. Pasteur Tunis* 85, 1–4. [PubMed] [Google Scholar]

5. Budd K. E., McCoy F., Monecke S., Cormica P., Mitchell J., Keane O. M. (2015). Extensive genomic diversity among bovine-adapted *Staphylococcus aureus*: evidence for a genomic rearrangement within CC97. *PLoS ONE* 10:e0134592. 10.1371/journal.pone.0134592 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
6. Buzzola F. R., Alvarez L. P., Tuchscherr L. P. N., Barbagelata M. S., Lattar S. M., Calvinho L., et al. . (2007). Differential abilities of capsulated and noncapsulated *Staphylococcus aureus* isolates from diverse *agr* groups to invade mammary epithelial cells. *Infect. Immun.* 75, 886–891. 10.1128/IAI.01215-06 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
7. Capurro A., Aspán A., Ericsson Unnerstad H., Persson Waller K., Artursson K. (2010). Identification of potential sources of *Staphylococcus aureus* in herds with mastitis problems. *J. Dairy Sci.* 93, 180–191. 10.3168/jds.2009- 2471 [PubMed] [CrossRef] [Google Scholar]
8. Clark N. C., Cooksey R. C., Hill B. C., Swenson J. M., Tenover F. C. (1993). Characterization of glycopeptide-resistant enterococci from U.S. hospitals. *Antimicrob. Agents Chemother.* 37, 2311–2317. 10.1128/AAC.37.11.2311 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
9. Cuny C., Wieler L. H., Witte W. (2015). Livestock-associated MRSA: the impact on humans. *Antibiotics* 4, 521–543. 10.3390/antibiotics4040521 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
10. de Freitas Guimarães F., Nóbrega D. B., Bodelão Richini-Pereira V. B., Marson P. M., de Figueiredo J. C. P., Langoni P., et al. . (2013). Enterotoxin genes in coagulase-negative and coagulase-positive staphylococci isolated from bovine milk. *J. Dairy Sci.* 96, 1–7. 10.3168/jds.2012-5864 [PubMed] [CrossRef] [Google Scholar]
11. Enright M. C., Day N. P., Davies C. E., Peacock S. J., Spratt B. G. (2000). Multilocus ~~sequence typing~~ for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J. Clin. Microbiol.* 38, 1008–1015. [PMC free article] [PubMed] [Google Scholar]
12. Faria N. A., Carrico J. A., Oliveira D. C., Ramirez M., de Lencastre H. (2008). Analysis of typing methods for epidemiological surveillance of both methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains. *J. Clin. Microbiol.* 46, 136–144. 10.1128/JCM.01684-07 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
13. Feil E., Cooper J., Grundmann H., Robinson D., Enright M., Berendt T., et al. . (2003). How clonal is *Staphylococcus aureus*? *J. Bacteriol.* 185, 3307–3316. 10.1128/JB.185.11.3307-3316.2003 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
14. Ferens W. A., Davis W. C., Hamilton M. J., Park Y. H., Deobald C. F., Fox L., et al. . (1998). Activation of bovine lymphocyte subpopulations by staphylococcal enterotoxin C. Infect. Immun. 66, 573–580. [PMC free article] [PubMed] [Google Scholar].
15. American Public Health Association. 1976. Standard methods for the examination of water and waste water, 14th ed. American Public Health Association, Inc., Washington, D.C.
16. Casman, E. P., and R. W. Bennett. 1965. Detection of staphylococcal enterotoxin in food. Appl. Microbiol. 13: 181-189.
17. Sakai, H., Procop, G.W.,Kobayashi, N.,Togawa, D. and Bauer, T.W. 2004. Simultaneous detection of Staphylococcus aureus and Coagulase negative Staphylococcus in positive blood culture by real time PCR with two fluorescence resonance energy transfer probe sets. J.Clin.Microbiol., 42(12), pp:5739-5744.