Isolating and Identification germs of Staphylococcus aureus from of butchers' Shops Instrument in the Shekhan District

Abdullah. M. Al-Saiegh[1]*, Raghdah Mahdi[2], Sabah Meskeen[3], Shreen ftah[4], Wesam Zedan[5]

ABSTRACT

The aim of the study was conducted to isolation microbial contamination in butchers instruments and meat in shekhan district, and identify the most important bacterial species associated with these instruments. 65 samples were taking from different localities of Shekhan district distributed among (9 samples from kalakchi area, 15 samples from chera area, 21 samples from qasrok area and 20 samples from shekhan) After taken sample by a swab from tools of butcher's shops (Knife ,hooks , cutting board and meat grinder) after that the samples were taken to the laboratory of shekhan technical colleague of health for completion process of culturing, isolated and examined of the bacterial species and studying the morphological biochemical characteristics of bacteria by using culture media. The results of the current study showed growth six different species of bacteria were Staphylococcus aureus 24 samples (36%) Staphylococcus epidermidis 9 samples (14%), Staphylococcus saprophytes 5 samples (8%), Streptococcus 9 samples (14%), Bacilli species 2 sample (3%) , Gram negative species 16 samples (25%) . At the conclusion of this study, two types of bacteria were isolated the first of which was gram negative and its proportion was (25%) the second was gram positive and its proportion was (75%), Identified six species of bacteria was staphylococcus aureus, staphylococcus epidermidis, staphylococcus saprophytous, streptococcus and bacilli species.

Keywords: Instruments Butchers, Bacteria, Staphylococcus aureus, Sheikhan distract.

I. INTRODUCTION

Staphylococcus aureus is both a commensal bacterium and a human pathogen. Approximately 30% of the human population is colonized with Staphylococcus aureus .Simultaneously, it is a leading cause of bacteremia and infective endocarditis (IE) as well as osteoarticular, skin and soft tissue, pleuropulmonary, and devicerelated infections (Tong et al, 2015) . Staphylococcus aureus is a major agent of health-care-associated infections that causes a wide range of diseases from mild to life-threatening conditions. It is one of the most prevalent causes of nosocomial bacteremia, hospital-acquired pneumonia, and surgical site infections (Oniciuc et al, 2017). Additionally, this bacterium has a high potential to acquire antimicrobial resistance (Oniciuc et al, 2017).

[1] Department of Community Health, Technical College of Health Shekhan, Duhok polytechnic University, Duhok, Kurdistan Regional Government- Iraq

Instruments define as action or process to achieve a specific purpose like, skinning, cutting, by using a tools like knife, skinner knife, Meat grinder, hooks and cutting boards . Meat is animal flesh that is eaten as food, humans have been hunting animals for their meat and other products, allowing animals to be domesticated, such as sheep, cattle, camel, fish and chickens (Boler and Woerner, 2017). It's recognized as an important source of essential amino acids (proteins), iron, B vitamins as well as other nutrients minerals and fat (Zhang et al, 2017). Fresh meat is a highly nutritious substrate with water activity of about 0.99, meaning that it is suitable for the growth of most microorganisms. Raw meat is contaminated with pathogenic bacteria that produce spoil by handling. As warm-blooded animals naturally carry bacteria such as Salmonella spp. in their intestines, raw meat may be contaminated with bacteria during the slaughter process such as evisceration and dressing procedures. In addition, the equipment and tools used in the processes, the hands and clothing of personnel as well as the environment may also contaminate the meat with bacteria (Fiona FONG, 2017) Meat has potential to carry foodborne pathogens that can cause illness and lead to food safety problems. These pathogenic bacteria are able to invade our bodies or produce toxins to cause illness. They cannot be seen or smelled on the meat, but can generally be destroyed by cooking conditions (i.e. cooking to a core temperature of at least 75°C instantaneously or other effective time/ temperature combinations) Pathogenic bacteria may need to compete with other bacterial flora (e.g. spoilage bacteria) for growth on the meat. Certain pathogenic bacteria such as Staphylococcus aureus are relatively poor competitors and may be outgrown by other flora. Spoilage bacteria will cause food to deteriorate or lose quality by developing a bad odor or feeling sticky on the outside of the meat, signs that consumers would normally notice. These spoilage bacteria are normally not harmful, however, when consumed in very large numbers, they can cause gastrointestinal disturbance. Consumers should throw away the meat that shows any signs of food spoilage (Fiona FONG, 2017). Meat normally eaten after it been cooked and seasoned or processed in a variety of ways. Un processed meat will spoil or rot within hours or day as a result of infection with decomposition by bacteria and fungi. (Lawrie and Ledward, 2006) the consumption of contamination is an important risk factor for diarrheal illness and is commonly identified as a source of outbreak transmission this is occur by the bacteria the bacteria have a number of shapes, ranging from spheres to rods and spirals. Bacteria were among the first life forms to appear on earth, and are present in most of its habitats. (Fredrickson et al, 2004) also the bacteria live in symbiotic and parasitic relationships with plants and animals. Most bacteria have not been characterized, and only about half of the bacterial have species that can be grown in the laboratory. (Rappé and Giovannoni, 2003). A slaughterhouse is a facility where animals are slaughtered for consumption as food (Canencia et al, 2016) , and a butcher is a person who slaughter animals . The aim of studying was conducted to determine the type of microbial that contamination the surface of butchers instruments and meat in shekhan district, isolate and identify the most important gram positives bacterial species associated with these instruments and identify the species of Gram Positives bacteria which that growing on butcher's instrument.

II. MATERIALS AND METHODS

In this study taken 65 sample from Shekhan district and other areas which belong to it (Kalakchi, Chera, Qasrok,Baadra) .

in each area she had own Butcher's shops, In Shekhan there was(5) butcher's shops , Qasrok there was (7) bucther's shops , Kalakchi (3) butcher's shops , Chera (5) butcher's shops and Baadra there was no butcher's shops found in it.

We visited Shekhan, Kalakchi, Chera, Qasrok and Baadre to take sample from butcher's instrument (Knife, hooks, cutting board and meat grinder), the samples distributed as 20 samples from shekhan, 9 samples from kalakchi area, 15 samples from chera area, 21 samples from qasrok area and we visiting baadre but there was no any butcher's shops. The samples taken were given numbers and letters for the purpose of distinguishing between the tools and the areas from which they were taken, for example

(SH1_k1_C1_B1_H1) (SH2_k2_C2_B2_H2) and so on. After that the samples were taken to the Laboratory of Shekhan technical health of college for completion process of culturing and making the tests to differentiate between bacteria and these tests are (Catalase Test, Coagulase test, Mannitol fermentation). The date of samples collection start from 15/11/2017 and finish at 20/4/2018. the Data were analyzed by using the Statistical Package for Social Sciences (SPSS, version 23) and excel and word software 2013.

III. RESULT

The isolated results from the butchers instrument in Shekhan district showed an growth six different species of bacteria were Staphylococcus aureus (36%) Staphylococcus epidermidis (14%), Staphylococcus saprophytes(8%), Streptococcus (14%), Bacilli species(3%) , Gram negative species (25%) as shown in (figure 1.5, 1.6, 1.7, 1.8, 1.9 and1.10) and table 1.

Table 1: Number and percentage of bacterial species isolated from the butchers instruments in all shekhan district

Bacteria species	Shekhan	Kalakchi	Chrra	Qasrok	Total	Percentage
Staphylococcus aureus	8	3	6	7	24	36%
Staphylococcus epidermidis	3	2	2	2	9	14%
Staphylococcus saprophytes	0	2	0	3	5	8%
Bacilli species	1	1	0	0	2	3%
Streptococcus	2	1	2	4	9	14%

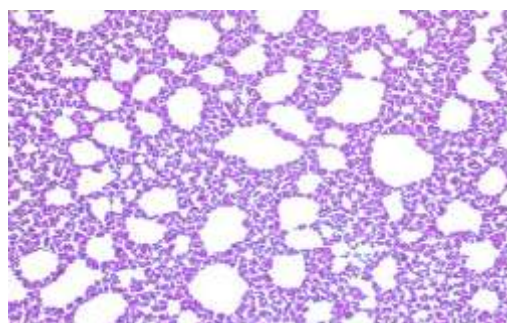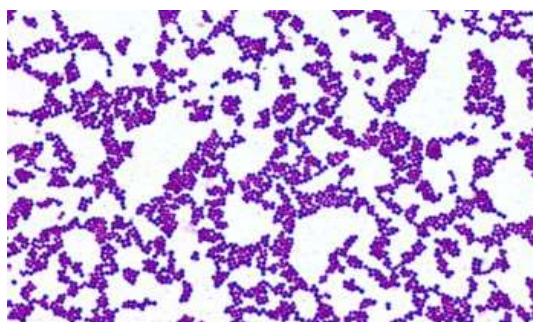|						
Gram negative species	6	0	5	5	16	25%
Total	20	9	15	21	65	100%

Figure 1.5:Staphylococcus aureus (gram stain, strength 100x)   Figure 1.6 Staphylococcus epidermidis

Figure 1.7 Gram negative species   Figure 1.8 Staphylococcus saprophytes
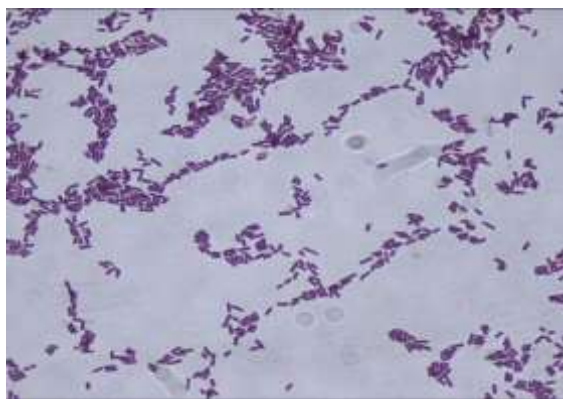
Figure 1.9 Bacilli species   Figure 1.10 *Streptococcus*

Gram stain analysis

Gram stain is a method of staining used to distinguish and classify bacterial species into two large groups (gram-positive and gram-negative), in this study from 65 samples 16 (25%) samples of them are gram negative and 49 (75%) are gram positive as show in (figures 1.11) .
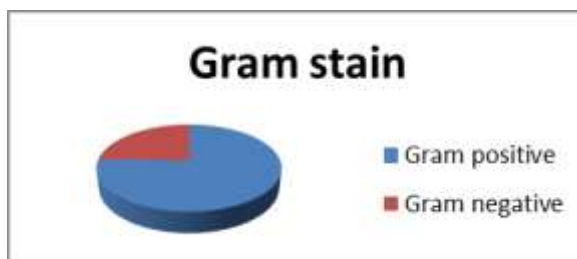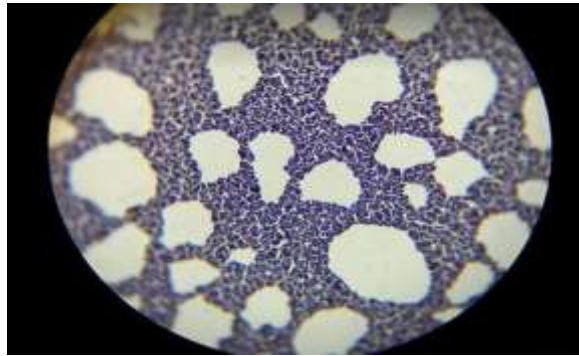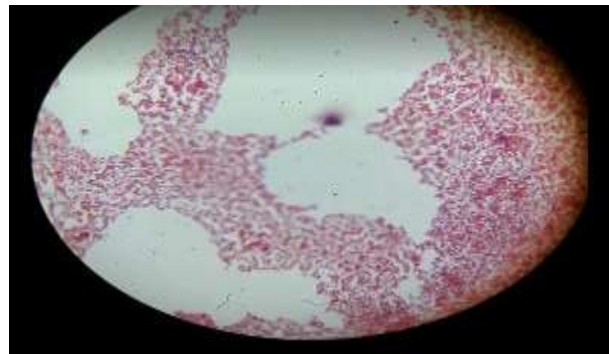

**Figure 1.11**: Show the percentage of gram positive and gram negative

Figure 1.12: Gram positive   **Figure 1.13**: Gram negative

Catalase, coagulase and mannitol tests analysis

Catalase this test is used for deffrensition between Staphylococcus and Streptococcus as show in (figures 1.15) Where the results showed an increase percentage of Staphylococcus 58% while the Streptococcus 14% as show in (figures 1.14) It has also been used coagulase test for determination the types of Staphylococcus Where the results showed 36 % (Staphylococcus aureus) and 8 % (Staphylococcus saprophytous and 14% (Staphylococcus epidermidis) as show in (figures 1.16) also have been used Mannitol fermentation test for determination the percentage of Staphylococcus saprophytous and Staphylococcus epidermidis the result was 14 % Staphylococcus epidermidis and 8 % Staphylococcus saprophytous asshow in (figures 1.17) .
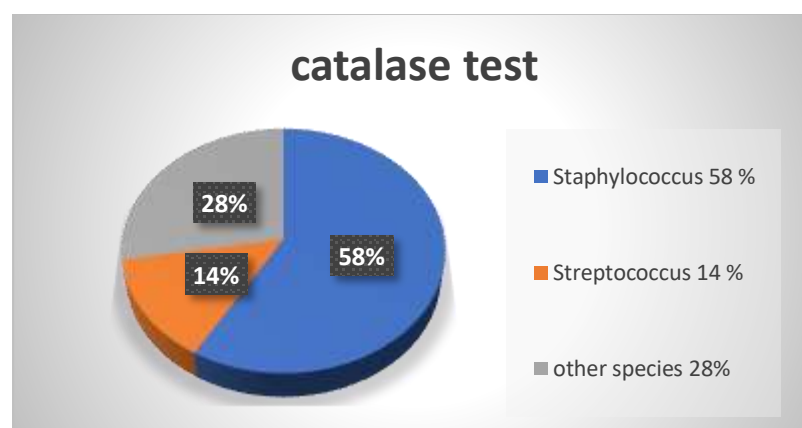
Figure 1.14: The different percentage between *Staphylococcus* and *Streptococcus*

Figure 1.15: Catalase test

Figure 1.16: Coagulase test

Figure 1.17: Mannitol fermentation

Instrument analysis

The samples was collected from butchers instruments for the purpose of determining the percentage of bacteria on each instrument, knife recorded the different percentage of microorganism the result was Staphylococcus aureus 9%, Streptococcus 6 % , Staphylococcus epidermidis 5% , and there wasn't growth of Staphylococcus saprophytous on knife in any place, gram negative species 8 % and least of them were Bacilli species 2%, cutting board in all shekhan district had been identified different microorganism , and the range of Staphylococcus aureus 9 % and other species were, Staphylococcus epidermidis 5 % , Staphylococcus saprophytes 3%, Streptococcus 3 % gram negative species 9 % and the range of Bacilli species were (1%). meat grander only two species of bacteria isolated from it because there wasn't meat grander in every slaughter house shop and the percentage of microorganism had been identified from the shekhan area was Staphylococcus aureus 8% and

Streptococcus 2%. the isolated microorganism from hook were Staphylococcus aurous 11% , S. epidermidis 4%, Staphylococcus saprophytes 5%, Streptococcus 3% , gram negative species 8 % , and there wasn't Bacilli species growth on hook in any place and the percentage of each microorganism as shown in table 2 .

Table 2 : The percentage of bacterial growth on butchers instrument

Instrument Name	Staphylococcus aureus	Staphylococcus epidermidis	Staphylococcus saprophytes	*Streptococcus*	G-	Bacilli species
Knife	9%	5%	0%	6%	8%	2%
Cutting board	9%	5%	3%	3%	9%	1%
Meat grander	8%	0%	0%	2%	0%	0%
Hook	11%	4%	5%	3%	8%	0%
Total	37%	14%	8%	14%	25%	3%

IV. DISCUSSION

The aim of this study is to determine the type of bacteria that grow on the tools of the butchers to preventing its grow on the meat and tolls most should be cooked thoroughly before consumption, where the results showed a high percentage of bacteria Staphylococcus aureus and the rate of 37% due to the fact that these bacteria are present naturally in the nose, respiratory system, skin, In addition, it is also considered an aerobic bacteria (Masala et al, 2001). Where studies confirmed that Staphylococcus aureus can causes of bacteremia, infective endocarditis. also cause various skin and soft tissue infections, (Tong et al, 2015) S. aureus also considers as predisposing factor for developing surgical site infection (Bouvet et al, 2017) the lowest range of bacteria was bacilli species 3%, this is a gram-positive, rod-shaped bacteria They will test positive for the enzyme catalase when there has been oxygen used or present. (Baron, 1996) . its can causes systemic infections like meningitis, endocarditis, osteomyelitis, and bacteremia. also self-limited food poisoning and localized infections related to trauma (e.g. ocular infections), soft tissue infections, (Tuazon et al, 1979) . It was also recorded Staphylococcus epidermidis the ratio was 14%, this is a Gram-positive bacterium, and one of over 40 species belonging to the genus Staphylococcus. It is part of the normal human flora, typically the skin flora, and less

commonly the mucosal flora (Schleifer and Kloos,1975). The effect of S. epidermidis is occur in infection of catheter the percentage of infection is about 50%-70% the high rate of intravascular catheterization among hospital patient (O'GARA and Humphreys, 2001) The other bacteria had been isolated is Staphylococcus saprophytes 8%, these bacteria is a Gram-positive belonging to the coagulase-negative Staphylococcus genus. These bacteria is a causes urinary tract infections (Kuroda et al, 2005) the another isolated bacteria is Streptococcus (14%) this is a genus of coccus spherical shape Gram-positive bacteria , they play an important role in human diseases. Infections rarely occur, but they are usually pathogenic, although they are present as part of the skin flora (Ferretti et al, 2001) Infection of Group A Streptococcal (GAS) may spread through direct contact with mucus or sores on the skin. (Group A Streptococcal (GAS) Disease, 2012) GAS infections can cause >500,000 deaths per year (Cohen-Poradosu and Dennis, 2007). The conclusions from this study identified a two types of gram stain bacteria positive and negative, identified a six species of gram positive bacteria, the highly percentage of bacteria was staphylococcus aureus (37%) and the low percentage of bacteria was bacilli species(3%), By catalase test the range of staphylococcus are more than streptococcus .

REFERENCES

1. Baron, S., 1996. Alphaviruses (Togaviridae) and Flaviviruses (Flaviviridae)--Medical Microbiology. University of Texas Medical Branch at Galveston.

2. Boler, D.D. and Woerner, D.R., 2017. What is meat? A perspective from the American Meat Science Association. *Animal Frontiers*, *7*(4), pp.8-11.

3. Bouvet, C., Gjoni, S., Zenelaj, B., Lipsky, B.A., Hakko, E. and Uçkay, I., 2017. Staphylococcus aureus soft tissue infection may increase the risk of subsequent staphylococcal soft tissue infections. International Journal of Infectious Diseases, 60, pp.44-48.

4. Canencia, O.P., Dalugdug, M.D., Emano, A.M.B., Mendoza, R.C. and Walag, A.M.P., 2016. Slaughter waste effluents and river catchment watershed contamination in Cagayan de Oro City, Philippines. J Biodivers Conserv Environ Sci, 9(2), pp.142-8.

5. Cohen-Poradosu, R. Dennis, L. 2007. Group A Streptococcus Epidemiology and Vaccine Implications. *Clinical Infectious Diseases*. 45 (7): 863-865. doi:10.1086/521263. PMID 17806050.

6. Ferretti, J.J., McShan, W.M., Ajdic, D., Savic, D.J., Savic, G., Lyon, K., Primeaux, C., Sezate, S., Suvorov, A.N., Kenton, S. and Lai, H.S., 2001. Complete genome sequence of an M1 strain of Streptococcus pyogenes. Proceedings of the National Academy of Sciences, 98(8), pp.4658-4663. Doi:10.1073/pnas.071559398.

7. Fiona, FONG. 2017. Food Safety Platform Bacteria in Raw Meat vs Cooked Meat. Food Safety Focus (130th Issue, May 2017) .

8. Fredrickson, J.K., Zachara, J.M., Balkwill, D.L., Kennedy, D., Shu-mei, W.L., Kostandarithes, H.M., Daly, M.J., Romine, M.F. and Brockman, F.J., 2004. Geomicrobiology of high-level nuclear waste-contaminated vadose sediments at the Hanford Site, Washington State. Appl. Environ. Microbiol., 70(7), pp.4230-4241. doi:10.1128/AEM.70.7.4230-4241.

9. Group A Streptococcal (GAS) Disease. 2007.Centers for Disease Control and Prevention. Archived from the original on December 19, Retrieved November 21,2012.

10. Kuroda, M., Yamashita, A., Hirakawa, H., Kumano, M., Morikawa, K., Higashide, M., Maruyama, A., Inose, Y., Matoba, K., Toh, H. and Kuhara, S., 2005. Whole genome sequence of Staphylococcus saprophyticus reveals the pathogenesis of uncomplicated urinary tract infection. Proceedings of the National Academy of Sciences, 102(37), pp.13272-13277. Doi: 10.1073/pnas.0502950102.PMC 1201578. PMID 16135568.

11. Lawrie, R.A. and Ledward, D.A., 2006. Lawrie's Meat Science. 7th English, ed. Cambridge: Woodhead Publishing Limited. ISBN 978-1-84569-159-2.

12. Masalha, M., Borovok, I., Schreiber, R., Aharonowitz, Y. and Cohen, G., 2001. Analysis of Transcription of theStaphylococcus aureus Aerobic Class Ib and Anaerobic Class III Ribonucleotide Reductase Genes in Response to Oxygen. Journal of Bacteriology, 183(24), pp.7260-7272. doi:10.1128/JB.183.24.7260-7272.2001. PMC 95576 . PMID 11717286.

13. O'GARA, J.P. and Humphreys, H., 2001. Staphylococcus epidermidis biofilms: importance and implications. Journal of medical microbiology, 50(7), pp.582-587.

14. Oniciuc, E.A., Nicolau, A.I., Hernández, M. and Rodríguez-Lázaro, D., 2017. Presence of methicillin-resistant Staphylococcus aureus in the food chain. Trends in Food Science & Technology, 61, pp.49-59.

15. Rappé, M.S. and Giovannoni, S.J., 2003. The uncultured microbial majority. Annual Reviews in Microbiology, 57(1), pp.369-394. doi:10.1146/annurev.micro.57.030502.090759.

16. Schleifer, K.H. and Kloos, W.E., 1975. Isolation and characterization of Staphylococci from human skin I. Amended descriptions of Staphylococcus epidermidis and Staphylococcus saprophyticus and descriptions of three new species: Staphylococcus cohnii, Staphylococcus haemolyticus, and Staphylococcus xylosus. International Journal of Systematic and Evolutionary Microbiology, 25(1), pp.50-61. doi:10.1099/00207713-25-1-50. ISSN 0020 .

17. Tong, S.Y., Davis, J.S., Eichenberger, E., Holland, T.L. and Fowler, V.G., 2015. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical microbiology reviews, 28(3), pp.603-661.doi:10.1128/CMR.00134 14. PMC 4451395 . PMID 26016486.

18. Tuazon, C.U., Murray, H.W., Levy, C., Solny, M.N., Curtin, J.A. and Sheagren, J.N., 1979. Serious infections from Bacillus sp. Jama, 241(11), pp.1137-1140.

19. Zhang, X., Owens, C.M. and Schilling, M.W., 2017. Meat: the edible flesh from mammals only or does it include poultry, fish, and seafood?. Animal Frontiers, 7(4), pp. 12-18.