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THYROIDITIS IN THYROID DYSFUNCTION WOMEN

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ABSTRACT : In this study (33) infected portion was obtained from a thyroidectomy process were collected from women suffering with thyroiditis with the age range between (18-65) years. All of these samples were obtained from general educational hospital in Ramadi city. The collection period extended from September 2020 to January 2021. The results of this study revealed that 25 (75.75%) of samples was positive for bacterial culture, while 8 (24.25%) of samples have no growth. Different types of bacteria were isolated which caused thyroiditis. The bacterial strains isolated in our study, which proved to be Gram-positive, were as follows : 6 (23.07%) isolates of *Staphylococcus lentus* were identified followed by 4 (15.38%) isolates of *Staphylococcus hominis*, while 1 (3.84%) isolates of *Staphylococcus warneri*, *Staphylococcus haemolyticus*, *Staphylococcus aureus*, *Kocuria Kristinae* were found. While, the isolated bacterial strains in our study that were negative for gram stain were as follows : 9 (34.61%) isolates of *Burkholderia cepacia* were identified followed by 1 (3.84%) isolates of *E.coli* , *Enterobacter cloacae*, *Leclercia adecarboxylata* were found. The predominant bacteria was *Staphylococcus lentus* for gram positive and for gram negative was *Burkholderia cepacia.* All isolates (100%) from thyroid gland were biofilm producers, among (26) isolates, detected 3 (11.53%) as strong, 19 (73.07%) as moderate and 4 (15.38%) as weak. The results refer revealed the presence of many bacterial isolates that cause thyroiditis of both Gram-positive and Gram-negative types.

Key words : Thyroiditis, thyroid dysfunction, virulence factors, bacterial isolate.

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INTRODUCTION

The word thyroiditis refers to inflammation of the thyroid gland, which has several causes. Medical diagnosis is used to classify thyroiditis into chronic, acute and subacute types, acute forms of thyroiditis occur either due to acute infectious thyroiditis caused by a bacterial or fungal infection (usually arising from a piriform sinus fistula or blood spread) or radiation (Rothacker and Walsh, 2018). Infectious diseases of the thyroid gland are rare but potentially life-threat (Rahim *et al*, 2019). An altered microbiota composition increases the prevalence of Graves' disease (GD) and Hashimoto's thyroiditis (HT) (Fröhlich and Wahl, 2017). Microbes influence thyroid hormone levels by regulating iodine uptake, degradation (Fröhlich and Wahl, 2019). Microbiota, which consists of numerous different microorganisms, including bacteria, viruses, archea, fungi colonizing our bodies, is dominated by bacteria and plays an important role in the individual life of each person, according to scientific sources, the average number of all bacteria in a person is estimated at 39 trillion and exceeds the number of cells building the body of an adult (Opazo *et al*, 2018). Acute thyroiditis (pyogenic thyroiditis, acute suppurative thyroiditis and bacterial thyroiditis), it is a rare disease, not considered common, usually bacterial in origin, but may be caused by fungi, parasitic organisms, or even *Pneumocystis carinii* (Chusid, 2017). The most common etiologic agents are *Staphylococcus aureus, Streptococcus pyogenes* and *Pneumococcus pneumoniae*, although other bacteria, including *Hemophilus injluenzae*, *Escherichia coli* and meningococcal organisms, as well as anaerobes, have been reported as causing infection (Larem *et al*, 2021). Acute thyroiditis is the most prevalent in the pre-antibiotic era, previous studies reported its presence in more than 50% of cases and previous studies by Berger et al documented only 224 cases of acute thyroiditis. Immunocompromised individuals are more susceptible to infection. This disease is more common in women, usually between the ages of 20 and 40, as well as in children and

the elderly. The low incidence of this disorder is due to multiple factors, the most important of which are: generous lymphatic drainage and the thyroid-rich blood supply making it relatively resistant to the bacterial "housekeeping" setting (Osborn and Deschler, 2018). The researchers also suggested other causes for this rare disorder to be the encapsulation of the thyroid gland, which may to some extent protect against inflammation from nearby structures. Also, a high amount of iodine in the thyroid gland may lead to an unsuitable environment for bacteria growth. Infection may reach the thyroid gland in one of several ways, including the spread of blood from distant places, such as the urinary tract, or through the lymphatic vessels as a result of a local infection such as mastoiditis or pharyngitis, internal fistulas near the thyroid gland, or persistent thyroglossal ducts, Symptoms of acute thyroiditis include sudden pain in the anterior neck on one side. Neck pain may spread to the ear or lower jaw along with infection, sweating, fever and other symptoms of bacterial poisoning. Other symptoms, such as pharyngitis and dysphagia, may appear (Shrestha and Hennessey, 2015). Sometimes, patients with acute infectious thyroiditis may be in a euthyroid state, as well as hyperthyroidism due to the destruction of thyroid tissue, which leads to the secretion of thyroxine and triiodothyronine. Chronic and devastating infection can also lead to hypothyroidism (Sweeney *et al*, 2014). Despite improvements in diagnostic testing and the availability of modern antibiotic treatments, acute suppurative thyroiditis (AST) leading to thyroid abscess is a rare disease. Therefore, this infection continues to cause disease cases, and a large death rate, especially when the necessary treatment is not taken (Karanikolic *et al*, 2018). A statistical study confirmed the number of AST patients, 92% are from the younger generations, the most common causes of infection are *Staphylococcus, Streptococcus*, *Pneumocystis carinii*, and *Mycobacterium* species. Unusual pathogens often occur in immunocompromised patients. *Candida*, *Klebsiella pneumoniae* and *Brucella melitensis* occurs exclusively with patients suffering from leukemia, diabetes mellitus and AIDS (Wanger *et al*, 2017).

MATERIALS AND METHODS

The current study was done at the General Educational Hospital in Ramadi city from September 2020 to January 2021. This study include 33 infected portion of the thyroid gland was obtained from a thyroidectomy process in the range of age (18-65) years. A piece was taken from the infected part of the resected thyroid gland and then placed in the brain heart infusion broth directly and incubated aerobically at 37°C for 24 hours and then samples had been inoculated on the culture media (MacConkey Agar, Blood Agar base) and incubated aerobically at 37°C for 24 hours. All isolates were diagnosed by conventional microbiological methods (colonial morphology, Gram staining and cultural characteristics) according to Cheesebrough (1998) and automated diagnosis using Vitek2 compact system.

Antibiotic sensitivity tests (disk diffusion)

Antibiotics (Gentamicin (CN), Ciprofloxacin (CIP), Imipenem(IPM), Azithromycin (AZM), Ceftriaxone (CRO), Aztreaname (ATM), Cefixime (CFM), Cefotaxime (CTX), Amoxicillin (AMC), Ceftazidime (CAZ), Amikacin (AK), Cefepime (CPM), Rifampicin (RIF) were used for detection of the susceptibility of the bacteria isolates to these antibiotics. The results of this experiment were recorded according to the standard guidelines recommended by National Committee for Clinical Laboratory Standards (CLSI, 2020).

Detected of virulence factors in bacterial isolates

Hemolysis activity : This test was carried out for detection of hemolytic zone around the colony, it was done according to Beecher and Wong (1994).

DNase production : For detection of the thermostable nuclease, it was done according to Brakstad and MæLand (1995).

Urease production : This test was used to test the ability of bacteria to produce the enzyme urease, it was done according to Achal and Pan (2011).

Gelatinase production : It was done according to Mehta *et al* (2020).

Protease production : This test was used to screen proteases producing bacteria, it was done according to Maurer (2004).

Coagulase test : To detect conjugated (bound) coagulase, it was done according to Trusty and Brown (2005).

Catalase test : It was done according to Evans *et al* (1994).

Detection of biofilm production

Quantitative method were used in this study to detect biofilm production according to Kýrmusaoðlu (2019).

RESULTS

Among the 33 women, who underwent thyroidectomy and had the infected portion obtained, the our results of our study showed that 25 (75.75%) were positive culture had thyroiditis, and 8 (24.25%) women had a negative culture for absence the bacterial growth, as shown in Fig. 1 by growing the infected part in the culture medium.

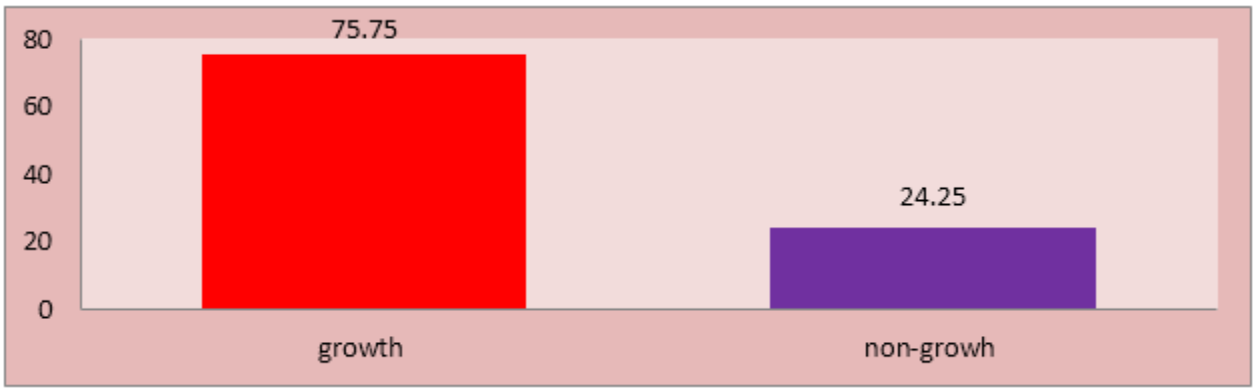

Fig. 1 : Distribution of samples with bacterial infection.

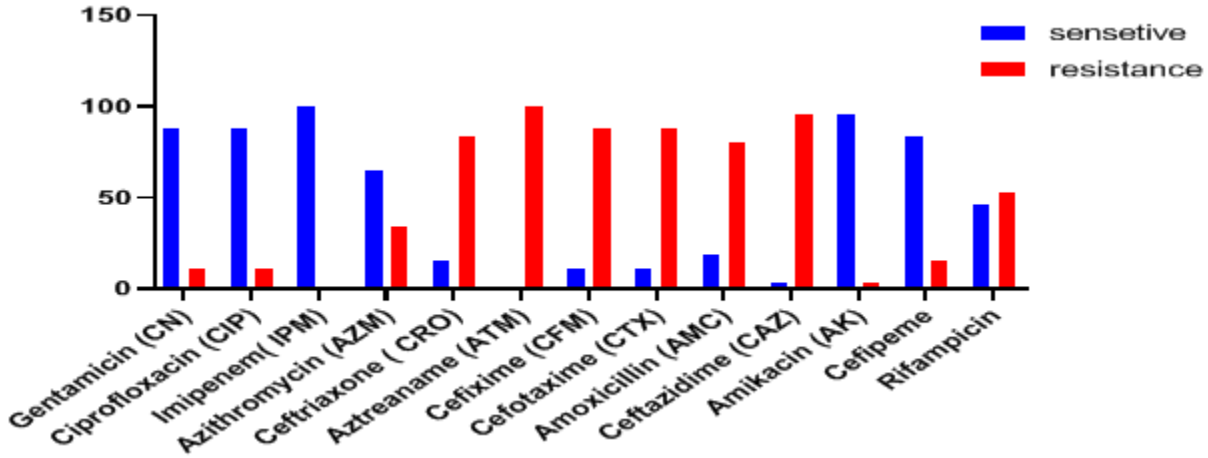

Fig. 2 : Percentage of effect of antibiotics on bacteria which isolated from thyroid gland.

As shown in Table 1, different types of bacteria were isolated from the infected portion of the resected thyroid gland. The total number of bacterial isolates obtained was 26 bacterial isolates from 25 women with thyroiditis were as follows: 14 (53.84%) bacterial strains were Gram-positive cocci (GPC) and 12 (46.15%) were Gram negative bacilli (GNB). The bacterial strains isolated in our study, which proved to be Gram-positive, were as follows : 6 (23.07%) isolates of *Staphylococcus lentus* were identified followed by 4 (15.38%) isolates of *Staphylococcus hominis*, while 1 (3.84%) isolates of *Staphylococcus warneri*, *Staphylococcus haemolyticus*, *Staphylococcus aureus*, *Kocuria Kristinae* were found. While the isolated bacterial strains in our study that were negative for gram stain were as follows : 9 (34.61%) isolates of *Burkholderia cepacia* were identified followed by 1 (3.84%) isolates of *E. coli*, *Enterobacter cloacae*, *Leclercia adecarboxylata* were found.

Antimicrobial susceptibility testing was performed using the disc diffusion method for 13 different antimicrobial agents, as shown in Fig. 2. As a result, varying responses to different antimicrobial discs were detected that all isolates were resistant to Aztreaname group (100%), also it has been found all these isolates were resistant to Ceftriaxone, Cefotaxime and Ceftazidime groups (100%) except the *Staphylococcus* was showed that resistant in lesser degrees (76.92%) to Ceftriaxone, Cefotaxime groups, while (92.30%) to Ceftazidime group. And most of these isolates showed resistance to Cefixime and Amoxicillin groups (100%). While, *Staphylococcus* and *Kocuria Kristinae* were showed their resistance in lower degree to Cefixime (84.61%), (0%) and to Amoxicillin (76.92%), (0%) consecutively. All isolates isolated in our study were sensitive to Imipenem, Ciprofloxacin groups (100%) except the *Staphylococcus* was showed that sensitive in lesser degrees (76.92%) to Ciprofloxacin group, as well as all isolates also were sensitive to Gentamicin group (100%) except the *Staphylococcus* and *Enterobacter cloacae* were showed that sensitive in lesser degrees (84.61%), (0%) consecutively. All isolates isolated sensitive to Amikacin group (100%), while *Staphylococcus* was showed that sensitive in lesser degrees (92.30%). And all isolates also were sensitive to Azithromycin (100%) except the *Staphylococcus* was showed that sensitive in lesser degrees (46.15%) and all isolates also sensitive to Cefepime group (100%) except the *Staphylococcus* and *Burkholderia* were showed

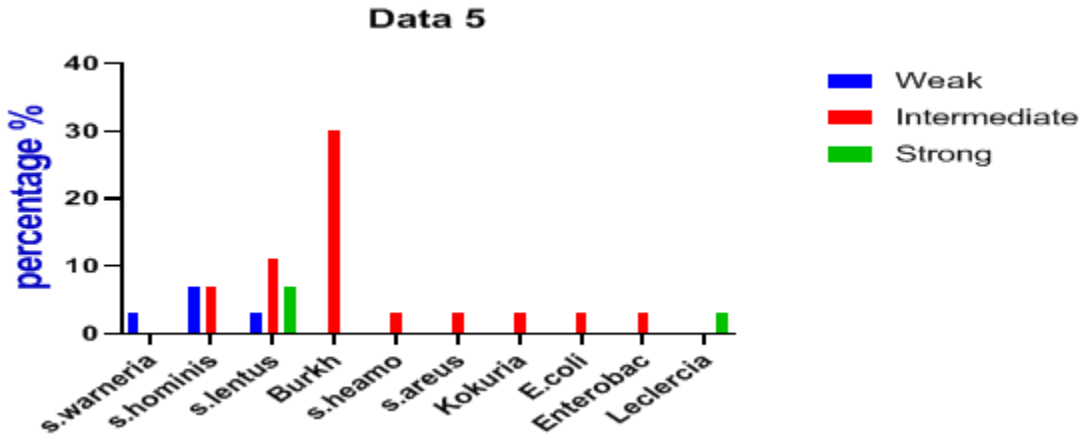

Fig. 3 : Percentage for detection of biofilm formation of bacteria which isolated from thyroid gland infection.

Table 1 : Percentage of bacterial isolation.

Isolated bacteria	Percentage%
Gram-positive cocci	**14(53.84%)**
Staphylococcus lentus	**6 (23.07%)**
Staphylococcus hominis	**4 (15.38%)**
Staphylococcus warneri	**1 (3.84%)**
Staphylococcus haemolyticus	**1 (3.84%)**
Staphylococcus aureus	**1 (3.84%)**
Kocuria kristinae	**1 (3.84%)**
Gram negative bacilli	**12 (46.15%)**
Burkholderia cepacia	**9 (34.61%)**
E.coli	**1 (3.84%)**
Enterobacter cloacae	**1 (3.84%)**
Leclercia adecarboxylata	**1 (3.84%)**

Table 2 : Percentage of some virulence factors from bacteria which isolated from thyroid gland.

Virulence factors	Percentage%	
	Positive result	Negative result
Hemolysis activity	**21(80.76%)**	**5 (19.23%)**
DNase production	**1(3.84%)**	**25 (96.15%)**
Urease production	**22(84.61%)**	**4 (15.38%)**
Gelatinase production	**12(46.15%)**	**14(53.84)**
Protease production	**13(50%)**	**13(50%)**
Coagulase test	**1(3.84%)**	**25 (96.15%)**
Catalase test	**(100%)**	**0(0%)**

that sensitive in lesser degrees (92.30%), (66%) consecutively. Most of the isolates resistance to Rifampicin (100%) while *Staphylococcus* and *Kocuria kristinae* showed that resistant in lesser degrees (15.38%), (0%) consecutively.

All isolates isolated were tested to produce some virulence factors (production of hemolysis, DNase, urease, gelatinase, Protease, Coagulase and Catalase enzyme), as shown in Table 2. As a result, positive hemolysis was detected in (80.76%) of isolates, only *S. aureus* produced DNase and Coagulase (3.84%). On the other, (84.61%) of isolates produced urease, while (46.15%) produced gelatinase, (50%) of isolates produced Protease, while all isolates (100%) produced Catalase.

All isolates (100%) from thyroid gland were biofilm producers, among (26) isolates, detected 3 (11.53%) as strong, 19 (73.07%) as moderate and 4 (15.38%) as weak as shown in Fig. 3.

DISCUSSION

The rare occurrence of suppurative thyroiditis is due to several causes, the most important of which are due to the rich vascular/lymphatic supply, well-developed fascial encapsulation and the high iodine content of the thyroid gland. Acute suppurative thyroiditis (AST) leading to thyroid abscess is a rare clinical disease, and thyroid abscess and AST account for only 0.1 to 0.7% (Karanikolic *et al*, 2018). Common symptoms of thyroiditis are: hyperthermia, swelling of the anterior midline in the neck, sore throat, redness of the skin, dysphagia, hoarseness and restricted head movements. The left lobe of the thyroid gland is affected by the inflammatory process, more often than the right lobe (Bandara and Samaranayake, 2019). The our results of this study showed that 25 (75.75%) were positive culture had thyroiditis and 8 (24.25%) women had a negative culture. These results which included the high percentage of positive culture. The reasons could be that most of these patients belong to a poor socioeconomic group, with a large number of elderly patients, those with poor conditions such as malnutrition and most of them with co-morbidities such as diabetes, chronic liver disease and some take immunosuppressive therapy (Schaaf *et al*, 2010). Also, The results of our study showed, 14 (53.84%) bacterial strains were, Gram-positive cocci (GPC) and 12 (46.15%) were, Gram negative bacilli (GNB). It was consistent with previous research from the same study environment and elsewhere within the

body that confirmed *S. aureus* to be the most common bacterial pathogen (Manyahi, 2012). While these results contradicted those of previous studies, in Asia and other African places (Atef *et al*, 2019 and Capson-Tojo *et al*, 2020), this might be due to, the high antibiotic resistance of Gram-negative bacteria and therefore their persistence in infected wounds. The difference of these studies can in fact be attributed to the differences in the populations examined; Diversity of surgical procedures, which are performed on the participants in the study. The results of our study showed that *Staphylococcus lentus* was the predominant Gram positive bacteria in thyroiditis infected patients. These findings are in line with Mazal and Sieger (2010), *Staphylococcus lentus* has been associated, with infection in animals, but few and rare studies have reported *S. lentus* as a pathogenic agent in humans. Here the researcher reported 72 cases of *S. lentus* in their institutions over a 9-year period. Previous literature revealed only one case of infection in humans, and to the best of their knowledge, this is the largest series of *S. lentus* infection in humans reported to date. While, that *Burkholderia cepacia* was the commonest gram negative bacteria isolated. This results agreed with Parajuli *et al* (2017). The researcher attributed the reason to high level of drug resistance (95.8%) along with the production of β-lactamases. Different types of bacteria were isolated from thyroid gland. The results of our study agreed with Karanikolic *et al* (2018). Where their study indicated that *Staphylococcus* species was isolated from the abscess of the right lobe of the thyroid gland after finding it and sending the excised thyroid gland for microbiological examination. Also, the results of our study agreed with Singh *et al* (2019), where he referred in his study to isolation *E. coli* from a thyroid abscess of a patient, who was suffering from diabetes with poor glycemic control and urinary tract infection. He suggested that the reason for this may be a urinary tract infection. In the literature, there have been some reports of cases of thyroid abscess caused by *Escherichia coli.* The results of our study also agreed with Ghaemi *et al* (2014) report a 9 year old girl suffering from an abscess in the thyroid gland. The diagnosis of acute suppurative thyroiditis was made and parenteral antibiotic was started on the second day of admission. The patient underwent surgical operation. Culture of pus yielded *Staphylococcus aureus*. The spread of the infection from the thyroid gland to the lungs is very rare and has been described only in a few patients. Cannizzaro *et al* (2008) report a very unusual case of thyroid abscess associated with a lung infection both caused by *K. pneumoniae*. The spreading of infection from the thyroid gland to the lung can be hematogenous or down the trachea.

All of the isolated bacteria were resistant to Aztreaname and most of these isolates showed resistance to Cefixime, Amoxicillin and Rifampicin. This may be due to the antibiotics have been in use for a much longer time and their oral route of administration that affects their rate of absorption into the bloodstream (Al-Marzoog and Hameed, 2018). The high resistance to second and third-generation cephalosporins (Ceftriaxone ,Cefotaxime and Ceftazidime) may suggest high expression or production of extended spectrum beta-lactamases among Gram-negative bacteria as previously reported in Ghana (Agyepong *et al*, 2018). While these results differ from (Fusté i Domínguez, 2012), those who reported that Gram-negative isolates are sensitive to ceftazidime, cefotaxime and ciprofloxacin, and this may be due to hospital infection associated with these microorganisms posing a serious threat to immunocompromised patients. Hence, infectious disease care centers and hospitals routinely use antimicrobial agents, resulting in increased cases of antibiotic resistance by bacteria. The low sensitivity of *Staphylococcus* to the combination of beta-lactam/beta-lactamase inhibitor treatment with antibiotics and fluoroquinolones (ciprofloxacin), is comparable to other studies in Ghana (Gyasi-Sarpong *et al*, 2014), this poses a strong challenge in treating the infection because these agents are the most readily available treatment options (Khan *et al*, 2020). Also, the results of our study showed that most of the isolates were sensitive to Gentamicin group, this is in line with Chaves and Tadi (2020) reported that Gentamicin is an aminoglycoside antibiotic. It has strong bactericidal activity against gram-negative aerobic bacteria which leads to gentamicin as a good choice for the treatment of many common infections. The reason is that gentamicin has minimal gastrointestinal absorption, so it is administered by injection. A large percentage of bacterial isolates showed sensitivity towards Azithromycin. Azithromycin is a derivative of erythromycin with greatly enhanced activity against gram-positive bacteria by inhibition of bacterial protein synthesis (Sandman and Iqbal, 2020). Also, the vast majority of bacterial isolates appeared sensitive to Cefepime. Cefepime has a broader spectrum of antibacterial activity than third-generation cephalosporins, a fourth-generation cephalosporin, that is more active in vitro against Gram-positive aerobic bacteria (Cheng *et al*, 2020). All isolates isolated in our study were sensitive to Imipenem and Amikacin with the exception of *Staphylococcus*, they were less sensitive to Amikacin, these results we agree with Agyepong *et al* (2018), who mentioned that the carbapenems such as (imipenem,

meropenem), colistin and amikacin were sensitive, these antibiotics are used as a last step in the treatment of serious infections. These results were shown in Fig. 2. Furthermore, since carbapenems have been introduced to the Ghanaian market for a limited time, they are relatively more expensive than basic antibiotics and therefore not commonly used. This resulted in relatively low natural selection and thus low antibiotic resistance among bacterial isolates. In addition, the sensitivity of most Gram-negative bacteria to amikacin leads to a lack of regrowth of bacteria. This is likely due to the strong, irreversible attachment to the ribosome, remaining intracellular long after plasma levels have decreased. This leads to a higher dose depending on its concentration, they act as bacteriostatic or bacteriostatic agents (Wilschanski *et al*, 2003).

In this study, as shown in Table 2, 21(80.76%) hemolysin production, by Gram- positive, Gram-negative bacteria isolated from thyroid gland was studied and it was found that, 12 (57.14%) of isolates was able to produce β -hemolysin, 9 (42.85%) of isolates was able, to produce α- hemolysin on, blood agar plate and 5 (19.23%) of isolates did not produce hemolysin, this agree with result mentioned by Mishra and Padhy (2018). The production of hemolysin, which determines as a virulence factor, enhances the ability of the organism to invade the tissues of the host and increases the pathogenic capacity of the organism (AL-Hamawandi, 2014). The erythrocytes are not only affected by hemolysin, but also leucocytes (white blood cells). So, the clinical importance of hemolysin in wound infection may be due to its ability to lyse platelet and WBCs. On the other hand the bacteria which have no ability to produce this factor extracellularly may have further means to evade the action of WBCs or to prevent wound healing (Al-Taai, 2018). The results of our study showed, 1 (3.84%) of isolated bacteria were positive for DNase test, while 25 (96.15%) were negative result, this agree with result mentioned by Türkyilmaz and Kaya (2006). DNases are endonucleases whose function is to break down DNA, resulting in a high concentration of low nucleotides. Hydrolysis of DNA is indicated by observing a clear region around bacterial growth on DNase agar after addition of HCl (Lau *et al*, 2020). Also, the result show 22 (84.61%) of isolated bacteria were positive for the urease test, while 4 (15.38%) were negative result . Urease is a nickel-dependent metalloenzyme that catalyzes the hydrolysis of urea into ammonia (NH_3) and carbon dioxide (CO_2) (ROUGHTON, 2021). Our results show that 12 (46.15%) of isolated bacteria have gelatin hydrolysis activity. The bacteria that showed a positive result were isolated because they have the ability to secrete the enzyme gelatinase and thus hydrolyze gelatin into soluble carbohydrates (Chakraborty *et al*, 2011). 13 (50%) of isolated bacteria showed proteolytic activity against skim milk containing media (Table 2). Clear proteolytic zones were observed around the bacterial growth after the incubation period. Using skim milk agar as a non-specific protease substrate (Suaifan *et al*, 2019). The results of our study showed 1 (3.84%) of isolated bacteria were positive for coagulase test, while 25 (96.15%) were negative result, this agree with Türkyilmaz and Kaya (2006), because were able to produce an enzyme coagulase that converts soluble fibrinogen in plasma to insoluble fibrin. Also the results of our study showed all of these isolates 26 (100%) were positive for catalase testing, because they were able to produce an enzyme Catalase, which splits hydrogen peroxide H_2O_2 which is a powerful oxidizer to oxygen and water H_2O.

Numerous studies have shown that many chronic infectious diseases are linked to the formation of bacterial biofilms. In addition, biofilm-associated infections often fail to respond to antibiotic treatment. The ability of bacteria to form biofilms, is an important advantage in causing infection associated, with medical devices and provides a major therapeutic challenge (Omar *et al*, 2017 and Huemer *et al*, 2020). Detection of biofilm formation, which is one of the most important virulence factors detected in this study. All isolates (100%), from thyroid gland were, biofilm producers, among (26) isolates, detected 3 (11.53%) as strong , 19 (73.07%) as moderate and 4 (15.38%) as weak, as shown in Fig. 3. These results are similar to Iyamba *et al* (2020), where he showed that *E. coli* produced (48%) strong biofilm and Citrobacter produced a moderate biofilm. Biofilms are communities of microbes in which cells of microbes adhere to each other on a nonliving or living surface within a matrix produced with self of extracellular polymeric substance (Girmaye *et al*, 2018). It appears that the microbes that produce biofilms produce special mechanisms that cause many microbial species to come into close contact with surface bonding, thus promoting cell-to-cell contact for growth and biofilm formation (Maier, 2021). Infection occurs after microbial cells adhere to surfaces and multicellular communities develop into biofilms. In addition, bacterial biofilms can play an important role in thyroiditis (Murray *et al*, 2021). The results of our study showed a great diversity in the production of fluffy biomass among isolates from thyroid gland (Fig. 3). Biofilms are dependent on many factors such as sugar content and concentration, environment, sample types, geographic origin, surface adhesion properties, proteolytic enzymes and biofilm-

associated genes for their formation (Iyamba *et al*, 2020).

CONCLUSION

The results refer revealed the presence of many bacterial isolates that cause thyroiditis of both Gram-positive and Gram-negative types. These isolates possess a number of virulence factors that enabled them to cause this type of infection. Some of the bacterial isolates possess the ability to form biofilms and to varying degrees enhance their ability to cause infection.

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