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EFFECT OF AEROBIC BACTERIA ON HIDES AND SKINS AND LEATHER QUALITY

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DEDICATION

To My Father

To the Soul of my Mother

To brothers, sisters and friends

To all whom I love

PREFACE

This work was carried out at the

Department of Microbiology, Faculty of

Veterinary Medicine, University of Khartoum,

Under supervision of Dr. Suliman Mohamed El Hassan

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ABSTRACT

The present work was carried out to isolate and identify aerobic bacteria associated with raw cattle hides and sheep skins and to examine their effect on tissue of hides and skins, in Gezira region.

A total of 160 samples were collected. They were collected from slaughterhouse, warehouse and tannery. Samples collected were hides and skins treated with salt, washed and air dried. Samples taken 2 hours after slaughter and samples delivered without treatment.

The bacterial damage was clear in raw hides and skins delivered without treatment. They showed signs of putrefaction, offensive odour and hair slipping. A number of bacteria were isolated.

A number of bacteria were isolated also from traditional salted hides and skins and this was probably due to delay in curing and absence of bacteriocides.

A number of bacteria were isolated from samples taken from washed and air dried hides and skins and samples 2 hours taken after slaughter, this probably due to poor hygiene, large number of labors and bad condition of collection room of raw hides and skins.

A total of 414 organisms were isolated and consist of 379 Gram-positive bacteria and 35 were Gram- negative bacteria.

One hundred and thirty four bacterial strains were isolated from fresh and washed cattle hides and sheep skins in slaughterhouse these include: *Staphylococcus spp, Micrococcus spp, Corynebacterium spp*, *Aerococcus homorri*, *Enterococcus casselifarus, Aerococcus viridans, Enterococcus faecalis*, *Gamella haemolysan*, *Stomococcus spp, Pseudomonas spp* and *Eschericha coli.*

Also one hundred and sixty three strains were isolated from salted and dried cattle hides and sheep skins in warehouse these include:

Staphylococcus spp, Micrococcus spp, Corynebacterium spp, *Enterococcus spp*, *Streptococcus faecalis*, *Stomatococcus mucilaginosus*, *Bacillus spp*, *Morexell bovis*, *Proteus vulgaris bigroup II*, *Pseudomonas spp* and *Eschericha coli.*

One hundred and seventeen bacterial strains were isolated from raw hides and skins delivered without treatment to tannery these include:

Staphylococcus spp, Micrococcus spp, *Corynebacterium spp*, *Lactobacillus jensenii*, *Streptococcus spp*, *Enterococcus spp, Stomatococcus mucilaginous*, *Bacillus spp*, *Aerococcus viridans* , *Proteus vulgaris biogroupII, Escherichia coli* and *Pseudomonas spp*.

Staphylococcus spp, Micrococcus spp, Corynebacterium spp, Bacillus spp, Escherichia coli and *Pseudomonas spp* were the predominant microorganisms isolated in this study.

Staphylococcus sacchrolyticus, Staphylococcus capitis, Staphylococcus hyicus, Micrococcus lylate, *Corynebacterium bovis, Corynebacterium xerosis*, *Lactobacillus jensenii*, *Bacillus cereus*, *Staphylococcus intermedius, Bacillus amylogliguesta, Staphylococcus saprophyticus, Staphylococcus auricularis, Staphylococcus hominis, Staphylococcus epidermidis, Staphylococcus xylosus, Micrococcus varinas, Micrococcus lentus, Corynebacterium bovis* , *Proteus vulgaris bigroup II* and *Morexella bovis* were isolated from putrefied hides and skins.

In this study the histological examination of putrefied area showed the most affected structures of skin layer were epidermis and dermis. The epidermis became thin with no cellular structure and appearing ribbon like and detached from dermis. The dermis became loose structures. This indicated the most affected tissue is epidermis and dermis which are valuable tissue in leather industry.

بسم الله الرحمن الرحيم

أجريت هذه الدراسة لعزل و تعريف البكتريا الهوائية التي توجد في الجلود الخام للأبقار و الضان وكذلك معرفة تأثيرها على أنسجة الجلد، في منطقة الجزيرة.

تم جمع 160 عينة وقد أخذت العينات من السلخانة و وكالات الجلود و المدبغة. وقد أخذت هذه العينات من جلود معالجه بالملح و بالغسيل و بالتجفيف الهوائي وأخرى بعد ساعتين من السلخ وعينات ماخوذه من جلود الأبقار و الضان بدون معالجه.

اتضح إن تاثيرالبكتريا واضح في الجلود الخام التي لم يتم معالجتها، و قد عزلت منها إعداد كبيره من البكتيريا وقد ظهرت عليها علامات التعفن، وهى سهولة نزع الصوف والرائحة الكريهة.

و كذالك تم عزل إعداد كبيره من البكتريا من الجلود المملوحه بالطريقة التقليدية، ربما يعزى ذلك إلى عدم المعالجة السريعة و عدم استعمال مضادات البكتريا مع الملح.

تم عزل إعداد من البكتيريا من عينات جلود ماخوذه بعد ساعتين من السلخ و يعزى ذلك لعدم الاهتمام بالنظافة وكثرة ألعماله وردائه غرفة تجميع الجلود.

تم عزل 414 بكتريا شملت 379 من البكتريا الموجبة جرام و 35 من البكتيريا السالبه لصبغة جرام.

تم عزل 134 بكتريا من جلود الابقار والضان مغسولة وطازجة من السلخانة وتشمل :

Staphylococcus spp و *Micrococcus spp* و *Corynebacterium spp* و *Enterococcus casselifarus* و *Aerococcus viridans* و *Aerococcus homorri* و *Stomatococcus spp* و *Gamella haemolysa* و *Enterococcus faecalis* و *Pseudomonas spp* و *Escherichia coli* .

تم عزل 163 بكتريا من جلود الابقار والضان المملحة والجافه من وكالات الجلود و تشمل :

Staphylococcus spp و *Micrococcus spp* و *Corynebacterium spp* و *Enterococcus spp* و *Streptococcus faecalis* و *Stomatococcus mucilaginosus* و *Bacillus spp* و *Morexella bovis* و *Lactobacillus jensennii* و *Pseudomonas spp* و *Escherichia coli* و *Proteus vulgaris biogroup* II

تم عزل 117 بكتريا من جلود الابقار و الضان الغير معالجه من المدبغة و تشمل :

Staphylococcus spp و *Micrococcus spp* و *Corynebacterium spp* و *Enterococcus spp* و *Streptococcus spp* و *Lactobacillus jensennii* و *Aerococcus viridans* و *Stomatococcus mucilaginosus* و *Bacillus spp* و *Pseudomonas spp* و *Escherichia coli* و *Proteus vulgaris biogroup* II

باكتريا *Staphylococcus spp* و *Micrococcus spp* و *Corynebacterium spp* و *Bacillus spp* و *Pseudomonas spp* و *Escherichia coli* هي أكثر أنواع البكتريا التى تم عزلها.

وجد ان باكتريا *Staphylococcus sacchrolyticus* و *Staphylococcus capitis* و *Staphylococcus hyicus* و *Micrococcus lylate* و *Corynebacterium bovis* و *Corynebacterium xerosis* و *Lactobacillus jensenii* و *Bacillus cereus* و *Bacillus amylogliguesta* و *Staphylococcus intermedius* و *Staphylococcus saprophyticus* و *Staphylococcus auricularis* و *Staphylococcus hominis* و *Staphylococcus capitis,* و *Staphylococcus xylosus* و *Staphylococcus epidermidis* و *Micrococcus varinas* و *Micrococcus lentus* و *Corynebacterium bovis* *Proteus vulgaris biogroup II* و *Morexella bovis* ترتبط مع التعفن البكتيرى لجلود الابقار و الضان.

في هذة الدراسة أوضح الفحص النسيجي للجلود المتعفنة أن أكثر تراكيب الجلد تأثراً هما ألبشره والأدمة. ألبشره أصبحت رقيقه وبدون خلايا وتظهر فى شكل شريط ومنفصلة عن الأدمة. الأدمة أصبحت رقيقة التراكيب. ألبشره والأدمه هما أقيم الأنسجة فى صناعة الجلود.

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INTRODUCTION

Livestock products meat, milk, egg, wool, hides and skins on average account for 28% of agricultural GDP of sub-Saharan Africa countries. Hides and skins account for a significant portion of value of livestock out put and for some countries it is an important source of foreign exchange earning. However, it is generally observed that full potential of hides and skins as a product is not realized in most countries because of several reasons, the most important being low quality of the product produced with consequent poor demand in both manufacturing industries and export market (ILRI, 2000).

Livestock rearing in Sudan is done under very diverse conditions varying from open Savannah grasslands, organized commercial farms, zero and semi-zero grazing. The quality of products obtained from livestock reared in these varying environments is directly influenced by these conditions. In the case of hides and skins the quality and yield of leather obtained from such animals is dependent on these factors. The hides and skins produced in Sudan generally carry a poor image in the global markets because of various constraints found through out the production chain starting with animal husbandry conditions, lack slaughter facilities, inappropriate flaying, and poor handling and preservation of these raw hides and skins (Jabbar *et al*., 2002).

The Sudan has the second largest livestock population in Africa, next to Ethiopia, The average livestock population during 1996-2002 was 34.4 million cattle, 36.8 million goats and 42.06 million sheep (Annual Statistical Bulletin, 1996-2002).

In the year 2003, Sudan produced 280000, 215000 and 148000 metric tons of fresh cattle hides, sheep skins and goat skins (Annual Statistical Bulletin, 2003).

The skins and hides sector and the leather industry are important in the economic of the Sudan. In the year 2002, Sudan exported 2300313, 2919745 and 1415038 pieces of fresh cattle hides, sheep skins and goat skins (Annual Statistical Bulletin, 2002).

Production of high quality hides and skins must be taken into consideration as this is the first step in producing high quality leather. However, hides and skins taken off slaughtered animals are some times badly cured and this leads to the serious problems of bacterial putrefaction. About 10% of hides and skins face undesired conditions such as incomplete bleeding, dirt, faeces, high moisture, direct sun light, soiled hair or wool and late curing. These factors favour bacterial growths that cause deterioration of hides and skins.

Bacterial damage is some times not evident straight away and indications or signs of putrefaction are loss of hair in some areas and unpleasant smell (Jabbar *et al*., 2002).

Putrefaction is indicated by offensive odour, change of colour and slipping of the hair. Putrefaction causes the general structure of the skin to become loose and flabby (Knew, 1952; Devassy and Argaw, 1989).

The major problem which faces the progress of this industry is damage to hides and skins caused by bacterial putrefaction. In most cases

bacterial damage of raw hides and skins is serious established problem and previous attempts were made to study this problem in the Sudan.

The present study was carried out to isolate and identify aerobic bacteria associated with damage of hides and skins and to examine histological the extent of tissue damage on hides and skins.

Table (1): Hides and Skin Production in the Sudan during the period 1997 – 2003; obtained from Statistical Bulletin of the Ministry of Animal Resources

Years	Cattle		sheep		goat	
	Quantity per 1000 pieces	Weight per 1000 tons	Quantity per 1000 pieces	Weight per 1000 tons	Quantity per 1000 pieces	Weight per 1000 tons
1997	1592	12.7	9978	16.0	11186	12.3
1998	1612	12.9	10099	16.2	11322	12.5
1999	1685	13.5	10939	17.5	12343	13.6
2000	3002	24.0	11077	17.7	12305	13.5
2001	3336	26.4	12255	19.6	12287	13.5
2002	3350	36.8	12389	19.8	12367	13.6
2003	3510	28.0	13497	21.5	13488	14.8

Table 2: Hides and skins exports from the Sudan during the period 1996–2002, obtained from Statistical Bulletin of the Ministry of Animal Resources

Years	cattle	Sheep	goat	Total
1996	989488	1453451	1532118	4728956
1997	508828	145351	1225289	3187568
1998	499519	486663	1190706	2176888
1999	1311019	1566031	620277	3497327
2000	2241469	2053755	977161	5272385
2001	4400517	2597005	1589738	8587260
2002	2300313	2919745	1415038	6635096

CHAPTER ONE

1. LITERATURE REVIEW

1.1 Definition

Mammalian skin is an organ full filling many physiological function such as regulation of body temperature, storage of body requirement, protection, elimination of waste products, sensory detection and communication, stress and state of health (Delmann and Eurell, 2000 ; Varnali, 2002).

The pelt is the untanned hide or skin with hair on it (Bloom and Fawcett, 1968).

1.1.1 Hides

Hides are defined as the whole pelt from large animals such as cattle, buffalo, camel and horse.

1.1.2 Skins

Skins are defined as pelt from small animals such as a sheep, goat and calf.

1.2 Anatomy

Fresh hides and skins consist of water, protein, fatty material and some mineral salts. Hides and skins are made of three defined layers. The epidermis, the corium or dermis and the hypodermis (FAO, 1960).

1.2.1 The Hair.

Each was a hair follicle with a hair root at its end, fed by a tiny blood vessel. Hair consists of protein keratin. Hair is entirely a product of epidermis (Dellmann and Eurell, 2000).

1.2.2 Epidermis.

The interface between the delicate tissues within a body and hostile universe, a protective layer of keratinous cells (Dellmann and Eurell, 2000; Varnali, 2002). The epidermis is a cellular structure, which has no blood vessels however; nourishment for the cell is obtained from the blood and lymph of the dermis (Devassy and Argaw, 1989). The epidermis is insoluble and serves to water proof the body surface. It is readily attacked by bacteria (Varnali, 2002).

1.2.3 Sweat Glands.

These are lined with epidermis and not form a coil but enlarged at the deep end and are variably flexuous (Sisson and Grossman's, 1975).Discharge sweat through the pores of the grain (Dellmann and Eurell, 2000). These glands control the rise temperature of the body.

1.2.4 Sebaceous Glands

Sebaceous glands located at side of hair follicles, discharge a waxy oily substance to protect hair, these glands maintain the body temperature (Dellmann and Eurell, 2000).

1.2.5 Corium

Corium is network of collagen fibers and is strongest part of skins. Towards the center, fibers are coarser and stronger, predominant angle, at which they are woven, can indicate properties of leather. The corium or

dermis is true skin, which is converted into leather. It contains three different types of connective tissue, collagen, elastin (elastic tissue) and reticulin (Dellmann and Eurell, 200).

1.2.6 Hypodermis

Hypodermis is flesh-next to meat, its fibers are more horizontal, fatty or (adipose) tissue may also be present. This is the loose connective, which joins the corium to the underlying part of body. It is consisting of the collagen and elastin fibers (Dellmann and Eurell, 2000). At the time of flaying, apart of this tissue remains attached to the hides or skins, although this layer exists in all the flayed hides and skins (Devassy and Argaw, 1989).

1.3 A Brief History of Leather

The primitive men whom lived during the Ice Age of 500,000 years ago wore animal skins to protect their bodies against the cold. Archeologists have found bone and flint scrapers, dating back to that time, which were used to scrape the flesh off animal skins.

Unfortunately, the raw skins quickly decayed and rotted away, because the early men did not know how to preserve them. Tanning was probably discovered by accident, vegetable matter contains tannin which will convert raw skins into leather, and it is easy to imagine a skin left lying in rain puddle containing leaves, bark and so on and so the skin was turned into a crude from of leather. As time went by, men perfected the art of leather making and extended the use of leather beyond that of clothing and

footwear. For example, it was discovered that water supply with them. Leather was used for tents, beds, carpets, amour and harnesses.

Leather became an important item of trade among the ancient Egyptians who taught their arts to the Babylonians and Israelites. Our earliest recorded evidence of the use of leather for shoes was discovered in the form of a painting on the wall of tomb at Thebes in Upper Egypt. This tomb belongs to the reign of Thotmes111. This painting shows craftsmen making sandal type shoes, and one noticeable feature is that the tools used are very similar to the hand-tools of today.

The Babylonians were acknowledged masters of tanning and finishing kid skins, which became known as "Babylonian leather" the art of making this leather crossed the Arab world and came to rest at Kano in northern Nigeria, the Nigerian tanner sent their kid leather across the trans-Saharan trade routes to Mediterranean ports when it was sold as "Moroccan" leather and exported to the other Mediterranean countries. The Moors took this leather with them to Spain, where a tanning industry flourished at Cordoba. British tanners later copied the leather and called "Cordovan" leather, which became corrupted to "Cordwain" thus British Cordwainers Technical College owes its name to a leather made in Babylon many centuries ago. (Intra programmers, Training Department, 1967).

1.4 Types and Uses of Skins and Hides

Traditionally, hides and skins have always been important in Africa's rural setting as the leather made was used for several basic needs including clothing (Jabbar *et al*,2002). Leather, and hence hides and skins, find their way into a wide variety of the leather goods manufactures need different

specification of leather and types of hides and skins. Although some kinds of leather can be made from several types of hides and skins, the special properties of any particular class usually makes it more suitable for manufacture of certain types of leather, and less for others. Leathers produced from cattle hides are of two basic types which also determine their end-use: heavy leather and light leathers. Heavy leather is usually rigid, thick and strong, and is generally transformed into footwear soles, industrial belting, harness and saddlery products. The great variety of lighter leathers from cattle hides are mostly mineral (chrome) tanned. These types, including their splits and used to make a wide range of end products (UNCTAD-GATT, 1968; FOA,1991).

Leather from sheep, goat and kid skins are also mainly mineral tanned, and utilized chiefly as shoe upper material. In addition, extensive used is made of these leathers in manufacture of fancy goods and clothing. Women's dress gloves are to large extent made from the skins of very young kids. The principal end-uses of leathers from sheep skins are garments, shoe-lining and gloves. Such leathers are also used for fancy goods, handbags and sports goods, but they are rarely transformed into shoe uppers (UNCTAD-GATT, 1968; FOA, 1991)

1.5 Hides and Skin Production

Africa account for about 16, 2, 30 and 22 % of world cattle, buffalo, goats and sheep population (FAO, 2001).

The Sudan has the second largest livestock population in Africa, next Ethiopia, accounting for average of 15.7%, 17.7 % of Africa's cattle, goats and cheep population, respectively. The average of livestock population

during 1995-2001 was 34.4 million cattle, 36.8 million goats and 42.06 million sheep, with corresponding growth rate of 4.09, 4.65 and 1.88 % respectively. Sudan's share of cattle, goat and sheep population in African is the lower than it is share of hides and skins production (FAO, 2001). Suggesting that Sudan may have a lower off-take rate of cattle, goats and sheep or lower collection rate of hides and skins than some other African countries (FAO, 2001).

In the year 2003 Sudan produce 30.495.000 and 64.400 metric tons of fresh cattle hides, goat skins and sheep skins respectively (Statistical Bulletin 2003) as shown in table 1. The champers of commerce estimate that the annual value of raw cattle hides, sheep skins and goat skins produced is U.S $ 12 million, 17 million and 5 million, respectively (FAO, 2001).

1.6 Slaughter, Flaying and Preparation of Hides and Skins

1.6.1 Slaughter

Animal should have free access to drinking water for at least 24 hours before slaughter to ease hides removed (Knew 1952; Devassy and Argaw, 1989).

Bleeding, whatever procedure the animal is killed the bleeding operation is best performed with carcass hoisted by the hind legs (Devassy and Argaw, 1989).

1.6.2 Flaying

Flaying is removal of animal pelt from carcass it is done either mechanically by using a machine or by hand using knife. It must be done

with care in order to obtain a finished leather of desired quality (Devassy and Argaw, 1989).

In most cases poor flying, lack of skills and absence of for instance hide putters in modern abattoirs lead to production of low quality hides and skins (Jabbar *et al*; 2002). It is at this stage that value of hide or skin is made (UNCTAO-GATT, 1968).

1.6.3 Preparation of Hides and Skins

1.6.3.1 Cattle Hides

After flaying hides are rolled to a collection rooms, where they are washed with water to clean them from debris and blood and to decrease the hides temperature. The hides are then removed to cemented yard where is no direct sun light. After turning leg, neck and tail inside with hair outside the hides are stacked (Devassy and Argaw, 1989).

1.6.3.2 Sheep Skins

After flaying, skins are taken to the collection room and after three hours they are transported to different warehouse (Knew, 1952; Devassy and Argaw, 1989). It is of great important to make flesh side of any flayed skins as clean as possible (Devassy and Argaw, 1989).

1.7 Preservation of Hides and Skins

Curing or preservation is keeping the hides and skins in a good condition without putrefaction until they are processed in tanneries (UNCTAD- GATT, 1968; Devassy and Argaw, 1989).

1.7.1 Air Dry

In these methods the moisture content is reduce 10-14 % (Knew, 1952). The techniques are drying on the ground, drying by suspension (Frame drying), by suspension over cords or wires, tent and parasol drying (Devassy and Argaw, 1989).

In Sudan drying methods are done in the states especially southern states where the salt is expensive.

1.7.2 Salting

1.7.2.1 Wet Salting

Hide or skin is prinked with salt amounting to 25-30 % of the skins weight. Then put in piles for 15-20 days (Knew, 1952), the bacteria is not destroyed but a condition is created where they become ineffective (Devassy and Argaw, 1989).

1.7.2.2 Dry Salting

Hides and skins are giving the advantage of both dry and salting, this for export purpose (Knew, 1952; Devassy and Argaw, 1989).

1.7.2.3 Brining

This technique popular in South America uses saturated 33% brine solution for initial treatment, green flesh and washed hides are soaked in brine for 24 hours (Knew, 1952; Devassy and Argaw, 1989). Now a day this method is not done in the warehouse. It is often done the side of the leg or not on the back of the animal and thus a very good pieces of leather can spoil (UNCTAO-GATT 1968). The objectives of brine cure are to affect a

cure that will enable hides to reach the hide processors in a condition that will produce quality leather (FAO, 1991).

1.7.3 Chilling or freezing

Chilling or freezing lower rate of growth of bacteria (e.g. icing) of hides and skin or refrigeration (Buckman, 2002).

1.8 Factors Affecting Quality of Hides and Skins.

The quality of hides and skins is influenced by factors throughout the production chain including animal husbandry and disease management, slaughter facilities and practices, handling and preservation methods (Jabbar *et al*; 2002). Table (3):

Table below shows grading and classification values of raw leather in Sudan

Leather kind	First class	Second class	Third class	Fourth class
Cattle	5	20	25	50
Sheep	5	15	50	30
Goat	5	15	40	40

Source: National Center for Leathers Progressing, Ministry of Animal Resource

These results are lower comparing with the international standards especially European and American standards where they miss the lower grades (third and fourth)

Leather stage	Leather spoliation %
A live animal	50
Slaughtering	25
Collection and preservation	25

1.8.1 Animal Husbandry Practices.

The damage caused to the hides and skins under pastoral and small holder husbandry conditions during the animal live is mostly attributed to various types of mechanical actions. Loss of value attributed to these types of damage is estimated to be 40% of total value of hides and skins. The defects are identified according to the damage caused or by the causative agent (Jabbar *et al*; 2002, FOA, 1991).

1-8-1-1 Scratches and Horn Rakes.

Scratches are amongst the most common mechanical damage found on both hides and skins. Scratches give leather an unsightly appearance and if it is deep (Knew, 1952). Cause considerable loss of tear strength especially on skin, the quality also degraded as the tanners try to obscure the faults on the grains by embossing or printing (Jabbar *et al*, 2002).

Horn rakes are a general problem as animal husbandry practices in Sudan, discourage dehorning there for cattle injure, the hide mostly in cruches in fight or during transportation. In some case the damage is a quite serious as the wound is generally deep (Devassy and Argaw, 1989, Jabbar *et al*; 2002).

1.8.1.2 Branding.

Branding is often done on the most valuable part of hides, that on the back and rumps (Knew, 1952). Branding cattle with hot irons causes high losses in hides and leather industry. Any thing from 10-40 % of value of he hide is lost by the unsightly and irreparable damage (FOA, 1991, Jabbar *et al;* 2002).

1.8.2 Effect of Diseases on Quality of Hides and Skins.

1.8.2.1 Parasite

1.8.2.1.1 Ticks

One of the major causes of down grading of hides and skins is attributed to tick-mark (Jabbar *et al*; 2002). A certain amount of the local damage occurs where the tick attaches itself by inserting the jaws and hypostome, the area become leather scarred and loose in texture at the site of damage (Kew, 1952).Ixodidae causes damage in skins (Damms,1994).

1.8.2.1.2 Mites.

The damage caused by the mites was restricted to the epidermis and upper dermis causing scabs (Venkatesan *et al*; 1989). Demodex, Sarcotes and Corioptes mange cause a high rate of damage (Buchner *et al*; 1994).

Bovine dermodecosis and bacteria associated with it were reported in Sudan (Ibrahim, 1989).

1.8.2.1.3 Lice.

Lesions made by lice, scar the grain surface of hide by the inflammation set up where the parasite has attached itself (Knew, 1952). Adult and nymphal stages make inflamed area on skin, they were found on hide of slaughtered steers (Waston *et al*; 1997). A sheep pelt defect was caused by *Bovicola ovis*, a sheep biting louse (Health *et al*; 1996).

1.8.2.1.4 Grub.

Grub damage is usually seen along either side of backbone of the animal. It makes series of holes through the hide (Marzo, 1995).

1.8.2.1.5 Arthropods, Helminthes, and Protozoa.

Dermatoses of cattle hides caused by arthropods, helminthes and protozoa have a severe effect on quality of leather and hide produced (Matthes and Hrep, 1987, Soulsby, 1977).The authors review the dermestids that are know to damage in warehouse or in ships is particular importance since holes make them useless and nullity the commercial value of this important export item. The injurious species was identified as dermestes maculates (Grillo *et al*; 1980).

1.8.2.2 Mycotic**.**

Mycotic dermatitis of cattle, or bovine sterptothricosis it responsible for severe damage to the hides and considerable economics loss to the skins (Buxton and Frster, 1977). There is a skin defect know as rash associated with lice and mycotic dermatitis (Marzo, 1995). Habb *et al*;

1995), reported that the visual examination of veal calves at Zurich abattoir revealed the presence ringworm defects to the leather. To prevent fungal growth on stored wet blue pelt fungicidal Busan72 was used (Galloway and Cooper, 1973).

1.8.2.3 Viral

Cow and hog pox causing further inflammation and damage to the grain surface of the skin (Knew, 1952). Sheep pox is characterized by generalized pocks (Rebertoson, 1976). The virus causes inflamination of epidermis and dermis. Lumpy skin disease is characterized by cutaneous nodules, Pseudolumphy skin disease is characterized by development of exudative cutaneous plaques (Robertoson, 1976)

1.8.2.4 Bacterial Damage**.**

The most important bacteria which cause damage to the during the animal life is *Dermatophillus congolensis*. Dermatophilus infection or cutaneous streptothricosis is a chronic, exudative dermatitis characterised by scab formation (Robertoson, 1976).

Unsworth (1946) and Esuruoso (1977) reported that, bacteria act as secondary infection, in bovine demodicosis lesions in different animals. *Staphylococcus aureus*, *Staphylococcus albus*, *Streptococcus pyogenes*, are associated with the lesions of demodectic mange (Gmeiner, 1908).

In Sudan, Ibrahim (1989) isolated *Staphylococcus aureus*, *Corynebacterium pyogenes*, *Psedomonas aeruginosa*, *Bacillus subtilis*, and *Morexella bovis* as secondary invader from bovine demodicosis cases.

1.8.3 Slaughter Facilities and Practices Affecting on Quality

The type of facilities use in country also determine quality of hides and skins produce such as Homestead slaughter and rural slaughter slabs.

1.8.3.1 Rubbed Grain

The rubbed grain damage is produced by dragging the unflayed carcass over rough and even ground and can even be caused by rough concrete (Knew 1952; Jabbar *et al*; 2002).

The grain is generally rubbed off or (Frizzed) and is definite cause of loss in value to the tanner (Knew 1952, Jabbar *et al*; 2002).

1.8.3.2 Bad Battern

The bad battern is caused by indiscriminate ripping. Ripping being initial opening cuts down the center of the belly and four legs (Knew 1952, Jabbar *et al*; 2002).

1-8-3-3 Flay Cuts, Scores or Gouges

This damage is caused by careless use of the knife or by use of unsuitable knives (Knew 1952, Jabbar *et al*; 2002). Deep cut on the flesh side of the skins caused by carelessness when removing the skins after slaughter (Intra programmers, training department, 1967)

1.8.4 Causes and Impact of Post Slaughter Defect on Quality

Investigation carried out by UNIDO, African leather programmed found that at least 60% of hide and skins defect are caused during slaughter due handling and preservation procedures (FAO, 1991; Jabbar *et al*; 2002).

Loss of quality hides and skins due to post slaughter activities is therefore very significant for leather industry (Knew 1952; Jabbar *et al*; 2002).

1.8.4.1 Grain Crack

Grain crack caused by dry in crumpled condition and by multifloding causes grain crack or any pressure and stain, combined with the low moisture content will in this trouble some damage (Furlong, 1950; Jabbar *et al*; 2002) folding and unfolding when the hides are dry will always be a danger and cracks are very frequently caused in this manner (Knew 1952; Jabbar *et al*; 2002).

1.8.4.2 Mechanical Damage

Mechanical damage are caused when the hides are being prepared for air-drying by framing method, it is customary to remove excess flesh left on the hide by defleshing. This is normally done using a concave knife on a table where the hide spread with the flesh side facing up. Most time the operators taking off the flesh do not take care as they try to make the hide too clean by getting maximum amount of flesh out. During this process hides suffer flay cuts and gouges are made. (Knew 1952; Jabbar *et al*; 2002).

1-8-4-3 Defects during the Wet Salting

In wet and dry salted hides the salt acts as bactericidal agent as well as a preservative against insect damage, but certain halophillic bacteria (salt loving) can thrive in salt hide and can give rise to common defect known as "red heat" (Knew 1952; Jabbar *et al*; 2002).

1-8-4-4 Bacterial damage

Bacterial damage indication or signs of putrefaction are loss of hair in some areas and unpleasant smell (Jabbar *et al*; 2002). In Sudan loss of cattle hides and sheep skins is about 10% especially in summer and the wet season and this is due to delayed curing, temperature and moisture. In addition hides and skin are a good media for the growth of bacteria. Incomplete bleeding, moisture, dirt and warmth are factors which favour the multiplication of organism that leads to putrefaction on hides and skins (Knew, 1952). The flesh side contains bacteria which were held and prevented from invading the body defensive mechanism of animal, as soon as the animal is slaughtered; the process of decay begins (Marzo, 1995). In case of hide and skins from imperfectly bled carcasses, blood will remain in vessel or capillaries if cleaning and drying is delayed, bacteria will multiply rapidly along the blood vessel then through the substance of hide or skin.

As a result hides and skins fibers in the area are destroyed (FAO, 1955). If drying is too slow the bacterial activity will start before the moisture content has been reduced sufficiently. On the other hand, if dry occur, too quickly, the middle of hide or skin will begin to gelatinize by bacterial activity, (Marzo, 1995). In the case of wet salting methods the salt must be clean and dirt-free to avoid bacterial contamination which will lead to putrefaction (Cooper and Gallaway, 1965; Marzo, 1995). Isolation and typing of bacteria responsible for damaging raw cured hides have been reported by several investigations (Taneous, 1961). The generation time of bacterial is less than four hours which means that under exponential growth

rate conditions these bacteria double their number in less than four hours (Cooper *et al;* 1973).

1.9 Bacteria isolated from treated hides and skins

Halophilic bacteria grow in salt while extremely halophilic bacteria grow in 20 % concentration and higher. Staphylococcus and Micrococcus grow in 5-15 % salt and tolerance of Bacillus range from 2-25 % NaCl (Bergey's Mnual, 1974). *Enterococcus faecalis*, *Enterococcus faesium, Enterococcus mundtii*, *Enterococcus casseiflavus* and *Aerococcus spp* grow in 6.5 % NaCl. *Corynebacterium bovis* grow in 9 % NaCl. *Morexella bovis*, *Morexella urethralis, Morexella locunata, Morexella nonlquefaciens, Morexella phenylpurica* and *Acitrobacter lwoffii* grow in 4 % NaCl (Bergey's, 1974; Barrow and Feltham, 1993).

The growth of extremely halophilic bacteria is very slow at room temperature (Kallenberger *et al*; 1988). To preserve bovine hides until they are processed in to leather; they are often cured in large raceways of saturated brine. This process prevented the growth of most microorganisms, but under some conditions these hides are still susceptible to decomposition by halophilic archaebacteria (Gihering *et al*; 2003).

The microorganism isolated from salted hides and skins were *Staphylococcus, Micrococcus, Bacillus, and Corynebacterium* were the predominant bacteria isolated (Kheiri, 2001).

1.10 Bacteria isolated from untreated hides and skins

The flesh side of the hides contains bacteria which were held and prevented from invading the body by defensive mechanisms of the animal,

as soon as the animal is slaughtered; the processes of the decay begins (Marzo, 1995).

In Switzerland, Gobat and Jemmi (1990) studied in slaughterhouse, samples and examined for *Listeria monocytogenes* and other *Listeria spp*. Skin and hides were the main source of contamination.

Rei, Small, Avery and Buncic (2002) studied food borne pathogenic bacteria in cattle hides. The hide of cattle is known to be source of microbial contamination. The bacteria isolated on the hide's areas were *Eschericha. Coli Salmonella spp*, and *Campylobacter spp*. Surface wetness of the hides was slightly associated with *coliform* and *E. Coli* (Donkersgoed *et al*; 1997).

Ruhrmann, 1987 identified that the most important organism involved in hides and skin in slaughterhouse, the main microflora of the hides was primarily Gram- positive, non spore- forming rods and Gram-positive cocci and predominated off 233 Gram positive, catalase producing cocci isolated, 132 were Staphylococci and 101 Micrococcus. The commonest Staphylococci were *Staphylococcus xylosus* (25 strains), *Staphylococcus sciuri* (23) and *Staphylococcus cohnii* (21). *Staphylococcus simulans* (8), *Staphylococcus hyicus* (5), *Staphylococcus epidermidis* (5), *Staphylococcus saprophyticus* (5), *Staphylococcus hominis* (3), *Staphylococcus warneri* (3), *Staphylococcus aureus* and *Staphylococcus haemolyticus* (1 each) were also identified. The commonest *Micrococcus* was *Micrococcus varians* (13 strains).

In the Sudan, Ibrahim (1989) succeeded to isolate *Staphylococcus aureus, Staphylococcus albus, Streptococcus pyogenes* Group (D). *Protus*

vulgaris, Bacillus subtilis, Corynebacterium pyogenes, pseudomonas aeruginosa and Morexella bovi from cattle hides infected by Demodectic manage.

Keiri (2001) studied aerobic bacteria associated with spoilage of hides and skins. Organisms isolated were Staphylococcus, Micrococcus, Bacillus, and Corynebacterium was the predominant bacteria isolated from sample after 11 hours among the latter isolated was *Morexella bovis* and *Erwinia herbicola* which are gelatinolytic bacteria.

1.11 Effect of bacteria in structure of hides and skins (Histopathology)

Marzo, (1995) reported that if drying is too slow the bacterial activity will start before the moisture content has been reduced sufficiently. On the other hand, if drying occurs too quickly, the middle of hide or skin will begin to gelatinize into glue- like material by bacterial activity. A simple qualitative method based on gelatinollytic activity has been proposed for technical application in hide stores and tanneries by unskilled operator (Schmitt and Deasy, 1963).

Wood, *et al*; (1970), succeeded to use ring plate test for estimation of bacterial contamination of salt curing hide and wet salted hides. Not all bacteria which occur on hides are necessarily responsible for decomposing the collagen. Collagen is known to be resistant to degradation by most enzymes released by bacteria for decomposing protein (Veis, 1964). In raw hides, bacteria showed a higher rate of collagenolysis than with cured hides. The collagenolysis was highest at salt concentration below 7.0 % (Woods, *et al*; 1971).

Cooper *et al*; (1973) reported that the growth rate of hide bacteria and pure strains of collagenolytic and non collagenolytic bacteria is high in delayed curing and uncured hides. The generation time of bacteria is less than four hours which means that under exponential growth rate condition these bacteria double their number in less than four hours. The putrefaction of hides is due to collagenolytic activity of bacteria (Waldo *et al*; 1986).

FAO (1995) reported that bacteria as result hide and skin fibers in the area are destroyed. The period of delayed curing can extend for as much as 6-12 hours after salting hide for stack-salting. This due to fact that salt has to penetrate into the grain layer of hide. Halophilic organisms isolated, damage the grain layer of brine cured hide and so lower the value of the leather (David *et al*; 1996).

CHAPTER TWO

2. MATERIAL AND METHODS

2.1 Sterilization

a- Flaming.

It was used to sterilize glass slides, cover slips, needles, scalpels points of scissors and mouth of culture tubes by passing them through Bunsen burner flame without allowing becoming red hot.

b- Red heat.

It was used to sterilize loop wires, points of forceps and searing spatulas by holding them over Bunsen burner flame until became red-hot.

c- Hotair oven

It was used to sterilize glassware such as bottles, flasks, test tubes, Petri dishes, Pasteur pipettes, graduated pipettes and forceps. They were sterilized in hot air oven at 180 °C for one hour.

d- Steaming at 100 °C.

Repeated steaming (Tyndallization) was used for sterilization of sugars and media that could not be autoclaved without determent effect to their constituents. It was carried out as described (Cruckshank *et a.,* 1975).

e- Moist Heat (Autoclave).

Autoclaving at 121°C (151b/inch²) for 15 minutes was used for sterilization of media and plastic wares.Autoclaving at 115°C (101b/ inch²) for 10 minutes was used for sterilization of some media.

2.2 REAGENTS

2.2.1 Hydrogen peroxide (H_2O_2).

This reagent was obtained from Agropharm limited, Buckingham. It was prepared as 3% aqueous solution and stored in dark and cool place. It was used for catalase test.

2.2.2 Kovac´s reagent.

This reagent composed of 5 grams para-dimethylaminobenzaldehyde, 75 ml amyl alcohol and 25ml concentrated hydrochloric acid. It was prepared as described by Barrow and Feltham (1993) by dissolving the aldehyde in the alcohol by heating in water bath. It was then cooled and acid was added. The reagent was stored at 4°C for later use in indole test.

2.2.3 Tetramethyl-p-phenylene dihydrochlroide.

This was prepared in a concentration of 3% aqueous solution and was used for oxidase test.

2.2.4 Potassium hydroxide.

This reagent was obtained from (BDH) and prepared as 4% aqueous solution for Voges-Proskaur test.

2.2.5 Methyl red solution.

This was prepared according to Barrow and Feltham (1993) by dissolving 0.04 gram of methyl red in 40 ml ethanol and the volume was made to 100 ml with distilled water. It was used for Methyl Red test.

2.2.6 Nitrate reagent.

Nitrate test reagent was consisting of two solutions (A) and (B). They were prepared according to Barrow and Feltham (1993). Solution (A) was composed of (0.33%) sulphanilic acid dissolved by gentle heating in 5/N acetic acid. Solution (B) was composed of dimethylamine-alfa-naphthylamine dissolved by gentle heating in 5/N acetic acid.

2.2.7 Alpha-naphthol solution.

It was manufactured by British Drug House; London (BDH) This reagent was prepared as 5% aqueous solution and was used for Voges-Proskaur (VP) test.

2.3 INDICATORS.

2.3.1 Andrade s indicator.

It was composed of acid fuchsin 5 grams, distilled water 1 liter and N-NaOH 150 ml. The acid fuchin was dissolved in distilled water, then the alkali solution was added, mixed and was allowed to stand at room temperature for 24 hours with frequent shaking until the color changed from red to brown. This was used for sugar fermentation.

2.3.2 Bromothymol blue.

It was obtained form BDH. It was prepared according to Barrow and Feltham (1993) by dissolving 0.2 grams of powder in 100 ml distilled water. It was used for Oxidation Fermentation test.

2.3.3 Phenol red.

It was supplied by Hokins and William Itd, London. It was prepared as 0.2% aqueous solution.

2.3.4 Plasma.

The plasma used for coagulase test was rabbit plasma, prepared by centrifugation citrated rabbit blood.

2.4 Collection of blood for enriched media.

Blood for enriched media was collected aseptically into sterile flask containing glass bead by venipuncture of jugular vein of healthy sheep kept for this purpose. The blood was defibrinated by shaking the sterile flask that containing glass bead while and after collection.

2-5 PREPARATION OF MEDIA

2-5-1 Blood agar.

This medium was composed of dehydrated blood agar base obtained from (Oxoid) and defibrinated sheep blood. The blood agar base contained heart infusion, tryptose, sodium chloride and agar. It was prepared according to manufacturer′ s instruction by dissolving 40 grams in one liter of distilled water by boiling, mixed and sterilized by autoclaving at 121°C

for 15 minutes. Then cooled to about 50°C, Defibrinated sheep blood was added aseptically to give final concentration 10%, mixed gently and 15 ml of complete medium was poured into each sterile Petri dish. The poured plates were allowed to solidify at room temperature on flat surface.

2.5.2 Nutrient agar.

The medium was obtained from (Oxoid) it was composed of beef extract, peptone, sodium chloride and agar. The medium was prepared according to manufacturer's by dissolving 28 g. of the powder in one liter of distilled water by boiling and sterilized by autoclaving at 121°C for 15 minutes. Then cooled to about 50°C and distributed in 15 ml amount per plate. The poured plates were left to solidify at room temperature on leveled surface.

2.5.3 MacConkey agar.

The dehydrated form consist of peptone, lactose, bile salts, sodium chloride, agar and 1% natural red .The medium was prepared according to manufacturer's (Oxoid) instructions by dissolving 52 g of MacConkey agar in one liter of distilled water, brought to boil to dissolved the ingredients completely, then sterilized by autoclaving at 121°C for 15 minutes and poured into sterile Petri dishes in 15 ml amount. The poured plates were left to solidify at room temperature on the flat surface.

2.5.4 Urea Agar.

The base medium contained peptone, sodium, dextrose, potassium dihydrogen phosphate, phenol red and agar. The medium was prepared according to manufacturer's (Oxid) instructions by dissolving 2.4 g of

powder in 95 ml distilled water by boiling and sterilized by autoclaving at 121°C for 15 minutes and cooled to 50 -55 °C. Five ml of sterile urea solution was added aseptically. The medium was distributed in 10 ml amounts in sterile test tubes and allowed to set in sloping position.

2.5.5 Hugh and Leifson´s (O/F) medium.

The medium was composed of dipotassium hydrogen phosphate, peptone, sodium chloride, agar and 0.2 % aqueous solution of bromocresol purple. The medium was prepared as described by Barrow and Feltham (1993). Two grams of peptone powder, 5 grams of sodium chloride, 0.3 g. of potassium hypophosphate and three grams of agar were added to 1 liter of distilled water then heated in water path at 55°C to dissolve the solids. The pH was adjusted to 7.1 and filtered. Then the indicator bromothymol blue (0.2 % aqueous solutions) was added and the mixture was sterilized by autoclaving at 115 °C for 15 minutes. Filtered sterile glucose solution was added aseptically to give final concentration of 1% .Then the medium was mixed and distributed aseptically in 10 ml amount into sterile test tubes of no more than 16 mm diameter.

2.5.6 Mannitol salt sugar.

One hundred and eleven grams of (Oxiod) CM 85 dehydrated medium were suspended in a liter of distilled water, mixed, steamed to dissolve and then the pH was adjusted to 7.5. It was then autoclaved at 121°C for 15 minutes, cooled and poured into Petri dish. The poured plates were allowed to solidify at room temperature on the flat surface.

2.5.7 Motility medium.

Motility medium was prepared as described by Barrow and Feltham (1993). It was consisted of peptone 10 grams; meat extracts 3 grams, sodium chloride 5 grams, agar 4 grams, gelatin 80 grams and distilled water 1 liter. First gelatin was soaked in water for 30 minutes then the other ingredients were added. The pH was adjusted 7.4. This medium was dispended in volume of 5 ml into 20 ml test tubes containing the appropriate Gragie tubes, and then the medium in test tubes were sterilized by autoclaving at 121°C for 15 minutes.

2.5.8 Simmon´s citrate agar.

Twenty three grams of Simmon´s citrate agar (Oxoid) were suspended in 1 liter of distilled water, dissolved by boiling, sterilized by autoclaving at 121°C for 15 minutes, then poured aseptically into sterile McCartney bottles and allowed to solidify in slope position.

2.5.9 Urea agar medium.

An amount of 2.4 grams of Urea agar base (Oxiod) were suspended in 95 distilled water, dissolved by boiling, sterilized by autoclaving at 115°C for 20 minutes and cooled to 50°C. Then 5 ml of sterile urea solution was aseptically added, mixed well, poured in 10 ml amount into sterile McCartney bottles and allowed to set in slope position.

2.5.10 Ammonium salt sugar.

Ammonium salt sugar (ASS) was prepared as described by Barrow and Feltham (1993). One gram of (NH_4) H_2PO_4. KCl (0.2g), $MgSO_4.7H_2O$

(0.2g), yeast extracts (0.2g) were added to 1 liter distilled water. The solids were dissolved by steaming, then the indicator bromothymol blue (0.04ml) was added and mixture was sterilized by autoclaving at 121°C for 15 min. The mixture was allowed to cool to about 60°C, then appropriate sterile carbohydrate solution was added to give final concentration of 0.5 – 1 %, mixed and distributed aseptically into sterile test tubes and allowed to solidify in slope position.

2.5.11 Nutrient broth.

This medium contained, beef extract, peptone and sodium chloride. Thirteen grams of nutrient broth (Oxiod) were added to one liter of distilled water, mixed well and distributed in 3 ml amount into clean test tubes, then sterilized by autoclaving at 121°C for 15 minutes.

2.5.12 Peptone water.

This medium contained peptone and sodium chloride. It was prepared according to Barrow and Feltham (1993) by dissolving 10 grams of peptone and 5 grams sodium chloride in one liter of distilled water, mixed well, distributed in 3 ml amount into clean test tubes and sterilized by autoclaving at 121°C.

2.5.13 Peptone water sugar.

Peptone water sugar medium was prepared as described by Barrow and Feltham (1993). It contained peptone water 900 ml, Andrade's indicator 10 ml, sugar 10 grams and distilled water 90 ml. The pH of peptone water was adjusted to 7.1-7.3 before the addition of Andrade´s indicator. The sugar was added to the mixture of peptone water and indicator, mixed well,

and then distributed into portion of 2 ml into sterile test tubes containing inverted Durham's tubes, covered with metal caps and sterilized by autoclaving at 115°C (101b/inch^2) for 10 minutes and kept at 4°C until used.

2.5.14 Robertson's cooked meat medium.

The medium was prepared according to Barrow and Feltham (1993). One kilogram minced meat was added to one liter of alkali solution (0.05N-NaOH), mixed well, heated to boiling, simmered for 20 minutes with frequent stirring. The pH was adjusted to 7.5, strained through gauze and dried. It was distributed in 5g amount into screw capped containers; sufficient nutrient broth was added and then sterilized by autoclaving at 121°C for 20 min.

2.5.15 Glucose phosphate (MR – VP) medium.

This medium was prepared according to Barrrow and Feltham (1993). Peptone 5g and 5g of phosphate buffer (K_2HO_4) were added to one liter of distilled water, steamed till dissolved, filtered and pH was adjusted to 7.5. Then five grams of glucose were added, mixed well, distributed into clean test tubes and sterilized by autoclaving at 115 °C for 15 min.

2.5.16 Nitrate broth medium.

This medium was prepared according to Barrow and Feltham (1993). Potassium nitrate 1 gram was dissolved in nutrient broth 1 liter, distributed into clean test tubes and sterilized by autoclaving at 115 °C for 20 minutes.

2.5.17 Nutrient gelatin medium.

One hundred and twenty eight grams of nutrient gelatin (Oxoid) CM132, were hydrated in a liter of distilled water, steamed to dissolve, pH was adjusted to 6.8, distributed in screw-capped bottles and autoclaved at 121 °C for 15 minutes.

2.6 Collection of samples.

Specimens used in this study were collected from Wadmadni slaughterhouse, Attra warehouse for hides and skins and Gezira tannery, in central Sudan.

a – Samples for bacteriological examination.

A total of 160 samples were collected for bacteriological examination during period from January 2004 to December 2004. These samples were consisting of 80 cattle hides and 80 sheep skins. The samples were collected form raw hides and skins in slaughterhouse and from salted or unsalted hides and skin in warehouse and tannery. The number and source of samples are shown in table 1 and 2.

Table (4): Sources and types of cattle hides sample collected in Gezeria state.

locality	Stage of leather processing	Time of collection	Number of sample
Wadmadni Slaughterhouse	Fresh hide	4 hours after flaying	10
Wadmadni Slaughterhouse	Washed hide	2 hours after washing	10
Attra Warehouse	Immediately salted hide	6 hours after salting	10
Attra Warehouse	Traditional salted hide	24 hours after salting	20
Attra Warehouse	Dried hide	24 hours after drying	10
Gezeria Tannery	Delivered hide	24 hours after flaying	20

Table (5): Sources and types of sheep skins sample collected in Gezeria state.

Locality	Stage of Leather processing	Time of collection	Number of Samples
Wadmadni Slaughterhouse	Fresh skin	4 hours after flaying	10
Wadmadni Slaughterhouse	Washed skin	2 hours after washing	10
Attra Warehouse	Immediately salted skin	6 hours after salting	10
Attra Warehouse	Traditional salted skin	24 hours after salting	20
Attra Warehouse	Dried skin	24 hours after drying	10
Gezeria Tannery	Delivered skin	24 hours after flaying	20

b- Samples for histopathological examination.

A total of 12 samples consisting 6 cattle hides and 6 sheep skins were collected for histopathological examination.

2-6-1 Sources of samples

Samples for bacteriological and histopathological examination were collected from Wadmadni slaughterhouse, Attra warehouse of hides and skins and Gezeria tannery.

2.6.2 Collection of samples

i- Samples for bacteriological

Sterilized swabs were used for collection of samples. They were rubbed on chosen site on flesh site (butt) of cattle hides and sheep skins. The swabs were then placed in tubes.

ii- Samples for histopathological.

Pieces of approximately 3×3 cm thick were cut from butt of hide and skin lesion with scissors and forceps and then placed into 10% formalin.

2.6.3 Transport of samples.

All swabs collected from cattle hides and sheep skins were labeled and placed immediately on ice in thermo- flask and then taken to laboratory for bacteriological examination.

2.7 Bacteriological examination.

2.7.1 Primary isolation.

All samples were cultured within two hours of collection. Isolation attempts were made on all samples at Medical Laboratory, University of Geizeria. All samples were cultured onto blood agar and MaConkey agar.

a- Blood Agar.

It was used as an enriched, non-inhibiting medium for primary isolation of bacteria and for determination of colonial morphology and hemolytic activity.

b- MacConkey Agar.

This medium was used for isolation of coliform.

2.7.2 Isolation:

The swabs were inoculated onto 10% defibrinated sheep blood agar and MacConkey agar. The inoculated plates were then incubated aerobically at 37°C for 24 hours as described by Barrow and Feltham, (1993). Further incubation was continued for another 24 h and if no growth was evident, then the plates were discarded as negative.

2.7.3 Cultural characteristics.

2.8 Purification.

All bacteria isolated were purified by several subculturing from single well-separated colony on separate blood agar plates and then examined for purity microscopically as described later. Each of purified isolates was inoculated into Bijou bottle containing sterile Robertson's cooked meat medium, allowed to grow and then the bottles were placed on ice in thermos-flask and transferred to the laboratory of the department of Microbiology, Faculty of Veterinary Medicine, University of Khartoum for identification.

2.9 Preservation.

The isolates were preserved for further studies to determine their cultural and biochemical characterizations. Preservation was made by subculturing the purified isolates on fresh sheep blood agar plates weakly. Cultures were kept between successive transfers at 4°C. All isolates were also stored in Robertson's cooked meat medium at 4°C and subcultured every three months.

2.10 Microscopic examination:

Smears were made form purified colonies, fixed by heating and stained by Gram stain method of Barrow and Feltham (1993). Then examined microscopically for cell morphology and arrangement, staining reaction. Gram stain was also used to check the purity.

2.11 Biochemical tests:

2.11.1 Sugar fermentation test.

The test was carried out as described by Barrow and Feltham (1993). The peptone water sugar was inoculated with organism under the test, incubated at 37°C and then examined daily for several days. Acid production was indicated by appearance of reddish color, while gas production was indicated by presence of an empty space in the inverted Durham's tubes.

2.11.2 Oxidase test:

The method of Barrow and Feltham (1993) was followed. Strips of filter paper was soaked in 1% solution of tetramethyl-p-phenylenediamine

dihydrochloride and dried in hotair oven and then placed on clean glass slide by sterile forceps. A fresh young tested culture on nutrient agar was picked off with sterile glass rod and rubbed on the filter paper strip. If a purple color developed within 5-10 seconds, the reaction was considered positive.

2.11.3 Catalase test:

The test was carried out as described by Barrow and Feltham (1993). A drop of 3% aqueous solution of hydrogen peroxide was placed in a clean glass slide. A colony of test culture on nutrient agar was picked off and put on drop of hydrogen peroxide. Evolution of gas and appearance of bubbles indicated positive test.

2.11.4 Coagulase test.

The test was performed as described by Barrow and Feltham (1993). To 0.5 ml of 1:10 dilution of human plasma in saline, 0.1 ml of an 18-24 h old broth culture of tested organism was added, then incubated at 37°C and examined after 6-24 hr for coagulation. Definite clot formation indicated positive result.

The test was also performed as described by Barrow and Feltham (1993) on slide. Two colonies of tested culture were placed on a clean glass slide, emulsified in a drop of normal saline and then a loopful of human plasma was added to the drop of bacterial suspension. Appearance of coarse visible clump was recorded as positive result.

2.11.5 The Oxidation-fermentation (O/F) test.

The test was carried out as described by Barrow and Feltham (1993). Duplicate test tubes of Hugh and Leifeson´s medium were inoculated by tested organism with straight wire. To one of the test tube a layer of sterile melted soft paraffin oil was added to depth of 3 cm above the medium to seal it from air. The inoculated tubes were incubated at 37°C and examined daily for fourteen days. Yellow color in open tube only indicated oxidation of glucose, yellow color in both tubes showed fermentation reaction and blue or green color in open tube and green color in the sealed tube indicated production of alkali.

2.11.6 Indole production test.

Indole production test was carried out as described by Barrow and Feltham (1993). The tested organism was inoculated into peptone water and incubated at 37°C for 48 h. One milliliter of the Kovac´s reagent was run down along side of test tube. Appearance of pink color within a minute indicated positive reaction.

2.11.7 Methyl red (MR) test.

Methyl red test was carried out as described by Barrow and Feltham (1993). The tested organism was inoculated into glucose phosphate medium (MR-VP) then incubated at 37°C for 48 h. Two drops of methyl red reagent were added, shaken well and examined. Appearance of red color indicated positive reaction, whereas orange or yellow color indicated negative reaction.

2.11.8 Voges-Proskaur (VP) test.

The test was performed as described by Barrow and Feltham (1993). The tested culture was inoculated into glucose phosphate medium (MR-VP) then incubated at 37°C for 48 h. Three milliliter of 5% alpha-naphthol solution and one milliliter of 40% potassium hydroxide were added. When bright pink color developed within 30 minutes, the reaction was regarded as positive.

2.11.9 Nitrate reduction.

The nitrate test was carried out as described by Barrow and Feltham (1993).The tested culture was lightly inoculated into nitrate broth and incubated at 37°C for two days. Then 1 ml of solution A followed by 1 ml of solution B of nitrite test reagent were added. Red color indicated positive reaction that showed nitrate in the medium had been reduced. If red color did not develop, powdered zinc was added to see whether there was residual nitrate or not. Red color development indicated that nitrate in medium had been reduced to nitrite by zinc but not by organism, whereas unchanged color indicated nitrate in original medium had been reduced completely and nitrite was further broken down by organism.

2.11.10 Urease activity tests.

The test was carried out as described by Barrow and Feltham (1993).

The tested organism was inoculated heavily onto slope urea agar medium and then incubated at 37°C for two days. Appearance of red color indicated positive reaction.

2.11.11 Citrate utilization:

The test was performed as described by Barrow and Feltham (1993). The tested culture was inoculated as a single streak over the surface of slope of Simon's citrate medium and examined daily for 7 days. Growth of the organism and changed color to pink indicated positive test.

2.11.12 Hydrogen sulphide (H_2S) production.

The method of Barrow and Feltham (1993) was followed. The tested culture was inoculated into nutrient broth; filter paper impregnated with 10% lead acetate solution was placed in the neck of the tube and incubated at 37ºC for two days. Brown or black color of the paper indicated positive reaction.

2.11.13 Ammonium salt sugar test.

The test was performed as described by Barrow and Feltham (1993). The tested organism was inoculated onto slope of ASS medium and incubated at 37°C for up to 7 days. The medium was examined on alternative days for growth and acid production.

2.11.14 Gelatin hydrolysis:

Gelatin hydrolysis test was carried out as described by Barrow and Feltham (1993). The tested culture was stabbed into nutrient gelatin and was incubated at 37ºC for up to 14 days. The inoculated tube was placed in refrigerator for 2 hours every 2-3 days and was examined. The liquefaction of gelatin indicated positive test.

2.12 Motility test.

The Craigi tube in semi-solid nutrient agar prepared as described by Cruckshank *et al*. (1975) was inoculated by straight wire. A small piece of the colony of the bacterium under test was picked by the end of the straight wire and stabbed in the center of semi solid agar in the Craigi tube and then incubated at 37°C overnight. The organism was considered motile if there was turbidity in the medium in/outside the Craigi tube.

2.13 Preparation samples for histopathological examination.

All preparations were carried out as described by Carteton´s ( ).

2.13.1.1 Fixation.

Neutral formalin 10% was used as fixatives. Samples were fixed for 48 hours or more.

2.13.1.2 Dehydration

First the tissues were cut (trimmed) into small squire pieces about one cubic cm, and labeled with a pencil, then washed in running tap water for 15 min to remove fixing agent. The dehydration was carried out by passing the samples though increasing concentration of alcohol 60%, 70%, 80%, 90%, and 100%.

2.13.1.3 Cleaning.

Clearing was carried out by chloroform, zylene, benzene, and cedar wool oil.

2.13.1.4 Impregnation.

Melted paraffin wax (two changes) was used to remove the clearing agent from the tissue and penetrate the tissue to fill the intracellular spaces

2.13.1.5 Blocking

Tissues were blocked in melted paraffin wax and quickly cooled.

2.13.1.6 Section Cutting.

Sections 5-6 microns thick were cut with rotary microtome.

2.13.2 Fixing section to slide.

The sections were floated in warm water bath 50-60ºC containing amount of gelatin powder the section is transferred and left to float, then fixed to the slide glass and then incubated for 30 min at 60ºC to dry.

2.13.3 Staining.

Routine stain, haematoxyline and eosin was used. Section were stained in heamatoxyline for 10 min, washed, differentiate in 1% acid alcohol, in running tap water for 10 min, counter stained with eosin 2-3 min, rinse quickly in water and dehydrated in 70%, 90% absolute alcohol. Sections were cleared in zylene.

2.13.4 Mounting.

The section was covered with cover glass using a suitable mounting medium Canada balsam. After overnight drying at room temperature, sections were examined microscopically.

CHAPTER THREE

3. RESULTS

3.1. Isolation of organism:

Bacteriological findings in this study were based on the isolation and identification of aerobic bacteria of cattle hides and sheep skins. Ten fresh skins, 10 washed skins, 10 immediately salted skins, 20 traditional salted skins, 10 dried skins, 20 delivered without treatment skins, 10 fresh hides, 10 washed hides, 10 immediately salted hides, 20 traditional salted hides, 10 dried hides and 20 delivered without treatment hides, were sampled by cotton wool swabs.

A total of 414 organisms were isolated from 160 cattle hides and sheep skins swab samples. Three hundred and seventy nine were Gram positive isolates (236.87%) and 35 isolates were Gram negative bacteria (21.87%).

3.2. Isolation of bacteria from cattle hides:

A total of 206 organisms (257.5%) were isolated from 80 cattle hides. One hundred and eighty three were Gram-positive isolates (228.75%) and 23 isolates (28.75%) were Gram-negative. The distribution of these organisms among different type of sample is shown in table (6).

Table (6). Gram-positive and Gram-negative aerobic bacteria isolated from fresh hides, washed hides, immediately salted hides, traditionally salted hides, dried hides and delivered without treatment hides swab samples.

Type of samples	No. of sample collected	No. of total isolates (percent)	No. of Gram positive isolates (percent)	No. of Gram negative isolates (percent)
Fresh hides	10	37 (370%)	32 (325%)	5 (50%)
Washed hides	10	33 (330%)	24 (240%)	9 (90%)
Immediately salted hides	10	18 (180%)	18 (180%)	0 (0%)
Traditionally salted hides	20	52 (260%)	49 (245%)	3 (15%)
Dried hides	10	11 (110%)	11 (110%)	0 (0%)
Delivered without treatment hides	20	55 (275%)	49 (245%)	6 (17.5%)
Total	80	206 (257.75%)	183 (228.25%)	23 (28.75%)

3.2.1. Bacteria species isolated from hides samples collected at slaughterhouse:

3.2.1.1. Bacteria species isolated from fresh hides.

A total of 37 (370%) bacteria were isolated from 10 fresh hides. Thirty-two (50%) were Gram-positive and five were Gram-negative bacteria**.** The aerobic Gram-positive bacteria isolated included Staphylococcus, Micrococcus, Gamella, Corynebacterium, Stomococcus, Aerococcus and Entrococcus. The species isolated of these genera are shown in tables 7-8.

The Gram-negative bacteria isolated from 10 fresh hides were *Protus vulgaris biogroup* and *Pseudomonas aruginosa* (Table 9)

3.2.1.2**.** Bacteria species isolated from washed hides**:**

A total of 33 (330%) bacteria species were isolated from 10 washed hides. Twenty–four were Gram positive and nine were Gram negative. The aerobic Gram-positive bacteria isolated included Staphylococcus, Micrococcus, Corynebacterium, Enterococcus and Listeria. The species isolated of these genera are shown in tables 7-8.

The Gram-negative bacteria isolated from 10 fresh hides were *Protus vulgaris biogroup, Echerichia coli* and *Pseudomonas pseudoalcaligenes* (Table 9)

3.2.2. Bacteria species isolated from salted and dried hides in warehouse:

3.2.2.1 Aerobic bacteria isolated from immediately salted hides**.**

Eighteen organisms were isolated and all of them were Gram-positive. These isolated Gram-positive bacteria included, *Staphylococcus sacchrolyticus, Staphylococcus capitis, Staphylococcus hyicus, Micrococcus lylate*, *Corynebacterium bovis*, *Corynebacterium xerosis, Lactobacillus jensenii*, *Bacillus cerus* and *Bacillus amylogliguesta*

3.2.2.2. Aerobic bacteria isolated from traditionally salted hides.

A total of 52 bacteria were isolated from 20 traditional salted hides, 49 were Gram-positive bacteria and 3 were Gram-negative bacteria. The aerobic Gram-positive bacteria isolated included Staphylococcus, Micrococcus, Corynebacterium, Stomococcus, Aerococcus, Bacillus, and Entrococcus. The species isolated of these genera are shown in tables 7-8.

The Gram-negative bacteria isolated from 20 traditionally salted were *Pseudomonas pseudoalcaligene*s and *Protus penneri* (Table 9).

3.2.2.3. Bacteria species isolated from dried hides:

Eleven bacteria strains were isolated from 10 dried hides and all of them were Gram-positive bacteria. These isolated bacteria were *Staphylococcus chromogenes*, *Staphylococcus xylosus, Staphylococcus kloosii* and *Bacillus mycoides* (Tables 7-8)

3.2.3. Bacteria species isolated from hides in the tannery:

3.2.3.1. Aerobic bacteria isolated from delivered without treatment hides.

A total of 55 organisms were isolated from 20 delivered without treatment hides, 51 were Gram–positive bacteria and 4 were Gram–negative bacteria. The aerobic Gram-positive bacteria isolated included Staphylococcus, Micrococcus, Corynebacterium, Stomococcus, Lactobacillus and Bacillus. The species isolated of these genera are shown in tables 7-8.

The Gram-negative bacteria isolated from hides delivered without treatment were *Pseudomonas areuginosa*, *Eschericha coli* and *Morxella bovis* (Table 9).

Table (7). *Staphylococcus spp* isolated from cattle hides at different stages of processing before tanning.

Bacteria species	Number of strain isolated from (persent)					
	Fresh Hides	Washed Hides	Immediate Salted hides	Traditional Salted hides	Dry Hides	Delivery without treatment hides
Staphylococcus caprae	2(20%)	4 (40%)	_	_	_	_
Staphylococcus epidermidis	3 (30%)	3 (30%)	_	2 (10%)	_	3 (15%)
Staphylococcus intermedius	_	_	_	_	_	2 (10%)
Staphylococcus sciuri	3 (30%)	_	_	1 (5%)	_	_
Staphylococcus hyicus	_	2(20%)	1 (10%)	2 (10%)	_	3 (15%)
Staphylococcus lentus	2 (20%)	3 (30%)	_	_	_	4 (20%)
Staphylococcus saprophyticus	1 (10%)	_	_	2 (10%)	_	_
Staphylococcus auricularis	4 (40%)	2 (20%)	_	3 (15%)		3 (15%)
Staphylococcus xylosus	_	_	1 (10%)	9 (45%)	3 (30%)	_
Staphylococcus capitis	_	_	2 (20%)	1 (1.92%)	_	_
Staphylococcus chromogens	_	_	_	1 (5%)	4 (40%)	5 (25%)
Staphylococcus gallinarum	_	1 (10%)	_	_	_	_
Staphylococcus schleferi	1 (10%)	_	_	_	_	1 (5%)
Staphylococcus haemolyticus	_	_	_	_	_	3 (15%)
Staphylococcus caseolysticus	5 (50%)	_	_	-	_	3 (15%)
Staphylococcus kloosii	_	_	_	_	2 (20%)	_
Staphylococcus sacchrolyticus	_	_	3 (30%)	_	_	_

Table (8) Gram-positive bacteria species other than Staphylococcus isolated from cattle hides at different stages of processing before tanning

	Number of strain isolated from (persent)					
	Fresh Hides	Washed Hides	Immediate Salted hides	Traditional Salted hides	Dry Hides	Delivery without treatment hides
Micrococcus lylae	2 (20%)	_	2 (20%)	4 (20%)	_	4 (20%)
Micrococcus luteus	_	_	_	5 (25%)	_	3 (15%)
Micrococcus varians	_	1 (10%)	_	3 (15%)	_	2 (10%)
Micrococcus sedentaricus	_	1 (10%)	_	_	_	_
Micrococcus agilis	_	_	_	1 (5%)	_	_
Streptococcus bovis	_	_	_	_	_	1 (5%)
Lactobacillus jensennii	_	_	3 (30%)	_	_	1 (5%)
Aerococcus viridans	1 (10%)	_	_	_	_	_
Stomatococcus mucilaginosus	1 (2.70%)	_	_	2 (1.92%)	_	1 (1.81%)
Enterococcus casselifarus	1 (10%)	2 (20%)	_	_	_	_
Enterococcus faecalis	_	_	_	2 (10%)	_	_
Gamella haemolysan	1(10%)	_	_	_	_	_
Corynebacterium jieikeium	_	2 (20%)	_	2 (10%)	_	_
Corynebacterium bovis	1 (10%)	_	3 (30%)	3 (15%)	_	3 (15%)
Corynebacterium pseudodiphthenticum	_	_	_	_	_	1 (5%)
Corynebacterium xerosis	_	_	1 (10%)	_	_	_
Corynebacterium minutissium	_	_	_	_	_	1 (5%)
Gardnerella vaginalis	_	_	_	_	_	2 (10%)
Bacillus amylogliquesta	_	_	1 (10%)	_	_	3 (15%)
Bacillus sphaericus	_	_	_	1 (5%)	_	_
Bacillus mycoides	_	_	_	_	2 (20%)	_
Bacillus circulans	_	_	_	1 (5%)	_	_
Bacillus magatarium	_	_	_	_	_	3 (15%)
Bacillus cereus	_	_	2 (20%)	2(20%)	_	2 (10%)
Listeria monocytogenes	_	2 (20%)	_	1 (5%)	_	_

Table (9) Gram-negative bacteria species isolated from cattle hides at different stages of processing before tanning

Bacteria species	Number of strain isolated from (persent)					
	Fresh Hides	Washed Hides	Immediate Salted hides	Traditional Salted hides	Dry Hides	Delivery without treatment hides
Protus vulgaris bigroup II	3(30%)	2 (20%)	_	_	_	_
Escherichia coli	_	5 (50%)	_	_	_	1 (5%)
Pseudomonas aruginosa	1 (20%)	_	_	_	_	1 (5%)
Pseudomonas pseudoalcaligen	_	1 (20%)	_	1 (5%)	_	_
Morexella bovis	_	_	_	_	_	4 (20%)
Vibro alginolyltium	_	_	_	2 (10%)	_	_

3.3. Isolation of bacteria from sheep skins

A total of 208 organisms (260%) were isolated from 80 sheep skins, 196 (245%) were Gram positive bacteria and 12 (15%) were Gram negative. The distribution of these organisms among different type of sample is shown in table (10).

3.3.1. Bacteria species isolated from skins samples collected at slaughterhouse:

3.3.1.1. Bacteria species isolated from fresh skins:

A total of 36 bacteria strains were isolated from 10 fresh skins. Thirty three isolates were Gram positive bacteria and three were Gram negative bacteria. The aerobic Gram-positive bacteria isolated included Staphylococcus, Micrococcus, Corynebacterium, Stomococcus and Areococcus. The species isolated of these genera are shown in tables 11-12.

The Gram-negative bacteria Isolated from 10 fresh skins were *Protus vulgaris Salmonella typhi* (Table 13)

3.3.1.2. Bacteria species isolated from washed skins**.**

A total of 28 bacteria strains were isolated from 10 washed skins. Twenty four were Gram positive bacteria and five were Gram negative bacteria. The aerobic Gram-positive bacteria isolated included Staphylococcus, Micrococcus, Corynebacterium, Stomococcu,

Table (10) Gram-positive and Gram-negative aerobic bacteria isolated from fresh skins, washed skins, immediately salted skins, traditionally salted skins, dried skins and delivered without treatment skins swab samples.

Type of samples	No. of sample collected	No. of total isolates (percent)	No. of Gram positive isolates (percent)	No. of Gram negative isolates (percent)
Fresh skins	10	36 (360%)	33 (330%)	3 (30%)
Washed skins	10	28 (280%)	24 (240%)	5 (50%)
Immediately salted skins	10	21 (210%)	21 (210%)	0 (0%)
Traditionally salted skins	20	48 (240%)	46 (230%)	2 (10%)
Dried skins	10	13 (130%)	13 (130%)	0 (0%)
Delivered without treatment skins	20	62 (310%)	59 (295%)	3 (3.75%)
Total	80	208 (260%)	196 (245%)	12 (15%)

Entrococcus and Areococcus. The species isolated of these genera are shown in tables 11-12.

The Gram-negative bacteria isolated from 10 washed skins were *Eschericha coli* and *Protus vulgaris* (Table 13).

3.3.2. Bacteria species isolated from skins samples collected at warehouse:

3.3.2.1. Bacteria species isolated from immediately salted skins.

Twenty one bacteria strains were isolated from 10 immediately salted skins and all of them were Gram positive bacteria. The isolated bacteria were *Staphylococcus intermedius, Staphylococcus saprophyticus, Staphylococcus auricularis*, *Staphylococcus hominis*, *Staphylococcus capitis, Staphylococcus xylosus*, *Micrococcus lylae*, *Micrococcus varinas, Micrococcus lentus, Corynebacterium bovis* (Tables 11-12)

3.3.2.2. Bacteria species isolated from traditionally salted skins.

A total of 48 bacteria strains were isolated from 10 traditionally salted skins, 45 were Gram positive bacteria and three were Gram negative bacteria. The aerobic Gram-positive bacteria isolated included Staphylococcus, Micrococcus, Streptococcus, Corynebacterium, Stomococcus, and Bacillus. The species isolated of these genera are shown in tables 11-12.

The Gram-negative bacteria strains were isolated from 10 traditionally salted skins. These isolates were *Pseudomonas aerugnosa* and *Morexella bovis* (Table 13).

3.3.2.3. Bacteria species isolated from dried skins.

Thirteen bacteria strains were isolated from 10 dried skins and all of them were Gram positive bacteria. These isolated bacteria were *Staphylococcus xylosus, Staphylococcus equorum, Staphylococcus saprophyticus, Staphylococcus kloosii*, *Staphylococcus chromogens*, *Micrococcus lylae* and *Dermacoccus nishinomiyaenesis* (Tables 11-12).

3.3.3. Bacteria species isolated from skins samples collected at tannery:

3.3.3.1. Bacteria species isolated from skins delivered without treatment:

A total of 62 bacteria were isolated from 20 skins delivered without treatment, 59 strains were Gram positive bacteria and three strains were Gram negative bacteria. The aerobic Gram-positive bacteria isolated included Staphylococcus, Micrococcus, Streptococcus, Corynebacterium, Aerococcus, Gardnerella and Bacillus. The species isolated of these genera are shown in tables 11-12.

The Gram-negative bacteria strains were isolated from skins delivered without treatment. These isolated strains were *Pseudomonas pseudoalcaligenes* and *Morexella bovis* (Table 13).

`Table (11). Staphylococci isolated from sheep skins at different stage of preparation before tanning.

Bacteria species	Number of strains isolated from (%)					
	Fresh skins	Washed skins	Immediate salted	Traditional salted	Dried skins	Delivery without treatment skins
Staphylococcus caprae	1 (10%)	2 (20%)	_	_	_	2 (10%)
Staphylococcus epidermidis	5 (50%)	3 (30%)	_	2 (10%)	_	4 (20%)
Staphylococcus intermedius	2 (20%)	_	3 (30%)	4 (20%)	_	_
Staphylococcus sciuri	_	2 (20%)	_	_	_	2 (10%)
Staphylococcus hyicus	2 (20%)	2 (20%)	_	_	_	_
Staphylococcus lentus	2 (20%)	_	_	_	_	3 (4.33%)
Staphylococcus saprophytics	_	_	5 (50%)	6 (30%)	2 (20%)	_
Staphylococcus auricularis	3 (30%)	3 (30%)	_	2 (10%)	_	11 (55%)
Staphylococcus xylosus	_	_	3 (30%)	3 (15%)	3 (30%)	_
Staphylococcus capitis	_	1 (10%)	2 (20%)	2 (10%)	_	3 (15%)
Staphylococcus chromogens	_	1 (10%)	_	_	_	_
Staphylococcus hominis	1 (10%)	_	2 (20%)	_	_	_
Staphylococcus caseolysticus	1 (10%)	_	_	_	_	_
Staphylococcus kloosii	_	_	_	3 (15%)	1 (10%)	_
Staphylococcus sacchrolyticus	_	1 (10%)	_	_	_	_
Staphylococcus simulans	1 (10%)	_	_	_	_	_
Staphylococcus equorum	1 (10%)	_	_	_	2 (20%)	_

Table (12) Gram-positive bacteria species other than Staphylococcus isolated from sheep skins at different stages of processing before tanning

Bacteria species	Number of strains isolated from (%)					
	Fresh skins	Washed skins	Immediate salted	Traditional salted	Dried skins	Delivery without treatment skins
Micrococcus lylae	1 (10%)	2 (20%)	1 (10%)	3 (15%)	2 (20%)	5 (25%)
Micrococcus luteus	2 (20%)	3 (30%)	2 (20%)	2 (10%)	_	4 (20%)
Micrococcus varians	2 (20%)	_	1 (10%)	4 (20%)	_	3 (15%)
Micrococcus nishinomiyaensis	_	_	_	2 (10%)	1 (10%)	_
Micrococcus sedentaricus	1 (10%)	_	_	_	_	_
Micrococcus agilis	_	_	_	_	_	1 (5%)
Streptococcus agalactiae	_	_	_	_	_	1 (5%)
Streptococcus faecalis	_	_	_	2 (10%)	_	2 (10%)
Streptococcus bovis	_	_	_	_	_	3 (15%)
Aerococcus homorri	1 (10%)	1 (10%)	_	_	_	_
Stomatococcus mucilaginosus	1 (10%)	_	_	3 (15%)	_	3 (15%)
Enterococcus faecalis	_	2 (20%)	_	1 (10%)	_	_
Corynebacterium jieikeium	_	1 (10%)	_	2 (10%)	_	_
Corynebacterium bovis	1 (10%)	1 (10%)	2 (20%)	3 (15%)	_	3 (15%)
Corynebacterium pseudodiphthenticum	1 (10%)	_	_	_	_	4(20%)
Corynebacterium minutissium	2 (20%)	_	_	_	_	_
Gardnerella vaginalis	_	_	_	_	_	2 (10%)
Bacillus sphaericus	_	_	_	_	_	2 (10%)
Bacillus cerus	_	_	_	3 (15%)	_	_

Table (13) Gram-negative bacteria species isolated from sheep skins at different stages of processing before tanning

Bacteria species	Number of strains isolated from (%)					
	Fresh skins	Washed skins	Immediate salted	Traditional salted	Dried skins	Delivery without treatment skins
Protus vulgaris bigroup II	2 (20%)	3 (30%)	_	_	_	_
Escherichia coli	_	2 (20%)	_	_	_	_
Salmonella typhi	1 (10%)	_	_	_	_	_
Pseudomonas aruginosa	_	_	_	1 (5%)	_	_
Pseudomonas pseudoalcaligen	_	_	_	_	_	1 (5%)
Morexell bovis	_	_	_	2 (10%)	_	2 (10%)

3.4. Gram positive bacteria:

Total of 379 isolates were characterized as Gram positive bacteria they included eleven genera which were *Staphylococcus* 206 (128.75%), *Micrococcus* 70 (43.75%), *Streptococcus* 9 (5.63%), *Areococcus* 3 (1.88%), *Stomatococcus* 10 (6.25%), *Enterococcus* 8 (5%), *Gemella* 1 (0.63%), *Corynebacterium* 37 (23.13%), *Bacillus* 22 (13.75%), *Listeria* 3 (1.88%), *Lactobacillus* 4 (2.5%), and *Gardnerella* 4 (2.5%).

3.4.1. Staphylococcus.

Staphylococcus represented the highest percentage of the total Gram positive bacteria isolated 206 (128.75%). The isolated Staphylococcus were Gram – positive spherical cells of varying size occurring in groups or clusters; they were non – motile, non-spore forming, aerobic and facultative aerobic and were catalase – positive.

Staphylococcus isolates were identified by their morphology, growth characteristics and their biochemical reaction; they were all identified as coogulase – negative staphylococci, as shown in table (14).

3.4.2. Micrococcus.

Seventy-nine isolates of genus Micrococcus were obtained; these represented 43.75% of the total Gram positive isolated bacteria.

Micrococci isolated in this study were Gram– positive cocci resembled staphylococci morphologically but differed biochemically. In contrast to staphylococcus pigmentation among Micrococci was stable and was important differential character. Biochemical characteristic used for identification of *Micrococcus spp* are listed in table (15).

3.4.3. Streptococcus.

Only nine strains of streptococci were isolated in this study. Four isolates were identified as *Streptococcus faecalis;* only one isolate was

identified as *Streptococcus bovis;* and also only one isolate was identified as *Streptococcus agalactiae*.

Streptococcus spp. were identified by their morphology, growth characteristic and by their biochemical reaction as shown in table 16. They were bile soluble.

3.4.4. Corynebacterium.

A total of 39 strains of corynebacterium were isolated in this study. Biochemical tests and others characters were used for identification corynebacterium as shown in table (16).

3.4.5. Bacillus.

Twenty–two isolates were identified as species of genus *Bacillus*. These were Gram positive rods in young culture, motile non-acid fast and produced spores, they were aerobic and facultative anaerobic, catalse–positive, oxidase–variable and varied in manner by which they attacked sugars. *Bacillus amyloliquefaciens* was positive to the gelatin liquefaction test, other bacillus were negative. Biochemical tests and others characters were used for identification corynebacterium as shown in table (17).

3.5. Gram-negative Bacteria:

Twenty–nine isolates of Gram–negative bacteria were obtained in this investigation. They included five genera which were Protus, Escherichia, Pseudomonas, Morexella, *Salmonella*

3.5.1. Proteus:

Eight – isolates were identified in genus proteus, and all of them were identified as *Proteus valgaris* biogroup II. The isolates were Gram-negative straight rods, motile, and non-capsulated. On blood and nutrient agar and other solid media the isolates produced characteristics swarming over the surface of the media which was inhibited on MacConkey agar and produced individual pale yellow, non lactose fermenting colonies

after overnight incubation at 37° C. It rapidly hydrolyzed urea, are shown in table 18.

3.5.2. Escherichia:

Four isolates were identified as *Escherichia coli*. This bacterium was medium sized rods, motile and grew on ordinary isolation medium. The colonies were circular, smooth with entire edges. On MacConkey agar *E.coli* fermented lactose producing pink colonies. It was oxidase-negative and catalase – positive as illustrated in table 18.

3.5.3. Pseudomonas:

Eight isolates were identified in the genus pseudomonas out of these five were identified as *Pseudomonas aruginosa* and three strains were identified as *Pseudomonas pseudoalcaligenes*. Biochemical tests and other characters used for differentiation between *Pseudomonas spp* are shown in table 18.

3.5.4. Morexella:

Six isolates were identified in the genus Morexella. All of them were identified as *Morxella bovis* (3.75%). They were isolated from hides and skins delivered without treatment. Biochemical tests and others characters were used for identification of *Morxella bovis* as shown in table 18.

3.5.5. Salmonella:

Three isolates were identified as *Salmonella typhi* (0.63%).They were isolated from washed skins.

Table (14) Gram-stain reaction and biochemical properties of Staphylococci isolated from hides and skins.

character	*Staphylococcus intermedius*	*Staphylococcus lentus*	*Staphylococcus hyicus*	*Staphylococcus saprophytics*	*Staphylococcus epidermidis*	*Staphylococcus caprae*	*Staphylococcus auricularis*
Gram – reaction	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-
Catalase	+	+	+	+	+		+
Oxidation Fermentation	F	F	F	F	F	F	F
Coagulase	-	-	-	-	-	-	+
Glucose	+	+	+	+	+	+	-
Lactose	+	+	+	-	-	-	-
Maltose	-	+	-	+	-	-	+

Table (14) continue

character	*Staphylococcus intermedius*	*Staphylococcus lentus*	*Staphylococcus hyicus*	*Staphylococcus saprophytics*	*Staphylococcus epidermidis*	*Staphylococcus caprae*	*Staphylococcus auricularis*
Mannitol	+	+	-	-	+	-	+
Sucrose	+	+	+	+	+	-	+
Xylose	-	-	-	-	-	-	-
Trehalose	+	+	+	+	+	+	+
Nitrate	+	+	+	-	+	+	+
VP	-	-	-	+	+	+	-
Urease	+	-	+	+	-	+	-
Indole	-	-	-	-	-	-	-
Gelatin Liquefaction	-	-	-	-	-	-	-

- = Negative; + = Positive; F = fermentative

Table (14) continue

character	*Staphylococcus sciuri*	*Staphylococcus xylosus*	*Staphylococcus capitis*	*Staphylococcus chromogens*	*Staphylococcus schleferi*	*Staphylococcus sacchrolyticus*
Gram – reaction	+	+	+	+	+	+
Oxidase	+	-	-	-	-	-
Catalase	+	+	+	+	+	+
Oxidation Fermentation	F	F	F	F	F	F
Coagulase	-	-	-	-	-	-
Glucose	+	+	+	+	+	+
Lactose	-	+	-	+	-	+
Maltose	+	+	-	+	-	+
Mannitol	+	+	+	-	-	+
Sucrose	+	+	+	+	-	+
Xylose	-	+	-	-	-	+
Trehalose	+	+	+	+	-	+
Nitrate	+	+	+	+	+	-
Urease	-	+	-	+	-	-
VP	-	-	+	-	+	-
Gelatin Liquefaction	-	-	-	-	-	-

- = Negative; + = Positive; F = fermentative

Table (15): Gram-stain reaction and biochemical properties of Micrococci isolated from hides and skins.

character	*Micrococcus lylae*	*Micrococcus luteus*	*Micrococcus roseus*	*Micrococcus varians*	*Micrococcus agilis*	*Micrococcus nishinomiyaensis*	*Micrococcus sedentaricus*	*Stomatococcus mucilaginosus*
Gram – reaction	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+
Oxidation Fermentation	-ve	- ve	O	O	- ve	O	- ve	O
Glucose	-	-	+	+	-	+	-	+
Sucrose	-	-	+	+	-	+	-	+
Nitrate	-	-	+	+	-	+	-	+
VP	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-
Gelatin Liquefaction	-	-	-	-	-	-	-	-

- = Negative; + = Positive; F = fermentive O = Oxidative

Table (16); Gram-stain reaction and biochemical properties of Gram-positive bacteria species other than Staphylococcus and Micrococcus isolated from hides and skins.

character	*Aerococcus viridans*	*Enterococcus faecalis*	*Enterococcus mundtii*	*Corynebacterium bovis*	*Corynebacterium jieikeium*	*Corynebacterium pseudodiphthenticum*	*Corynebacterium exposés*	*Corynebacterium striatum*
Gram – reaction	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-
Catalase	-	-	-	+	+	+	+	+
Oxidation Fermentation	F	F	F	- ve	- ve	- ve	F	F
Glucose	+	+	+	-	-	-	+	+
Maltose	ND	+	+	-	-	-	+	+
Mannitol	-	+	+	-	-	-	-	+
Xylose	ND	ND	ND	-	-	-	+	+
Lactose	+	+	+	-	-	-	-	-
Sucrose	+	ND	ND	ND	ND	ND	ND	-
Urease	ND	ND	ND	-	-	+	-	-
Nitrate	ND	ND	ND	-	-	+	+	-
VP	+	+	+	-	-	-	-	-
Gelatin Liquefaction	-	-	-	-	-	-	-	-

- = Negative; + = Positive; F = fermentive ND = Not done

Table (16); continine.

character	*Corynebacterium bovis*	*Corynebacterium jieikeium*	*Corynebacterium pseudodiphthenticum*	*Corynebacterium xerosis*	*Corynebacterium striatum*	*Gardnerella vaginalis*	*Listeria monocytogenes*	*Lactobacillus jensennii*
Gram – reaction	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	-	+	-
Oxidation Fermentation	- ve	- ve	- ve	F	F	F	F	F
Glucose	-	-	-	+	+	+	+	+
Maltose	-	-	-	+	+	+	ND	-
Mannitol	-	-	-	-	+	-	+	+
Xylose	-	-	-	+	+	-	+	-
Lactose	-	-	-	-	-	-	-	+
Sucrose	ND	ND	ND	ND	-	-	-	-
Urease	-	-	+	-	-	-	+	-
Nitrate	-	-	+	+	-	-	+	-
VP	-	-	-	-	-	-	+	ND
Gelatin Liquefaction	-	-	-	-	-	-	-	-

- = Negative; + = Positive; F = fermentive ND = Not done

Table (17) Gram-stain reaction and biochemical properties of Bacillus isolated from hides and skins

character	*Bacillus mycoides*	*Bacillus sphaericus*	*Bacillus magatarium*	*Bacillus cereus*	*Bacillus circulans*	*Bacillus amylogliquesta*
Gram – reaction	+	+	+	+	+	+
Spore shape	X	X	X	X	X	X
Spore position	S	T	T	T	C	C
Oxidase	-	-	-	+	-	-
Catalase	+	-	+	+	+	+
Oxidation Fermentation	F	- ve	F	F	F	F
Glucose	+	-	+	+	+	+
Mannitol	-	-	-	-	-	ND
Raffinose	-	-	-	-	+	+
Lactose	-	-	-	-	-	-
Sucrose	+	-	ND	ND	-	-
Xylose	-	-	+	+	+	-
Salicin	ND	ND	+	+	+	+
Nitrate	+	+	+	+	-	+
Urease	-	-	-	-	-	+
Citrate	ND	ND	+	+	-	ND
VP	+	-	-	-	W+	+
Indole	-	-	-	-	+	-
Gelatin Liquefaction	-	-	+	+	-	+

- = Negative; + = Positive; F = fermentive ND Notdone

x = Oval C = Central S = Subterminal W = Week reaction

Table (18); Gram-stain reaction and biochemical properties of some Gram-negative bacteria isolated from hides and skins.

character	*Morxell bovis*	*Pseudomonas pseudoalcaligen*	*Protus penneri*	*Protus vulgaris* bigroup II	*Escherichia coli*	*Vibro alginolytium*	*Salmonella typhi*
Gram – reaction	-	-	-	-	-	_	_
Oxidase	+	+	-	-	-		
Oxidation Fermentation	-	-	F	F	F		
Glucose	-	-	+G	+G	+G		
Maltose	-	-	+	+	+		
Mannitol	ND	-	-	-	+		
Lactose	-	-	-	-	-		
Xylose	-	-	+	+	+		
Sucrose	-	-	+	+	-		
Trehalose	ND	-	+	+	+		
Citrate	-	+	-	-	-		
Urease	+	-	+	+	-		
Nitrate	-	-	ND	+	+		
MR	ND	+	-	+	+		
H_2S	ND	ND	-	+	-		
Indole	ND	-	ND	+	+		
Gelatin Liquefaction	+	-	+	+	-		

- = Negative; + = Positive; F = fermentive ND =Notdone G= Gas formation

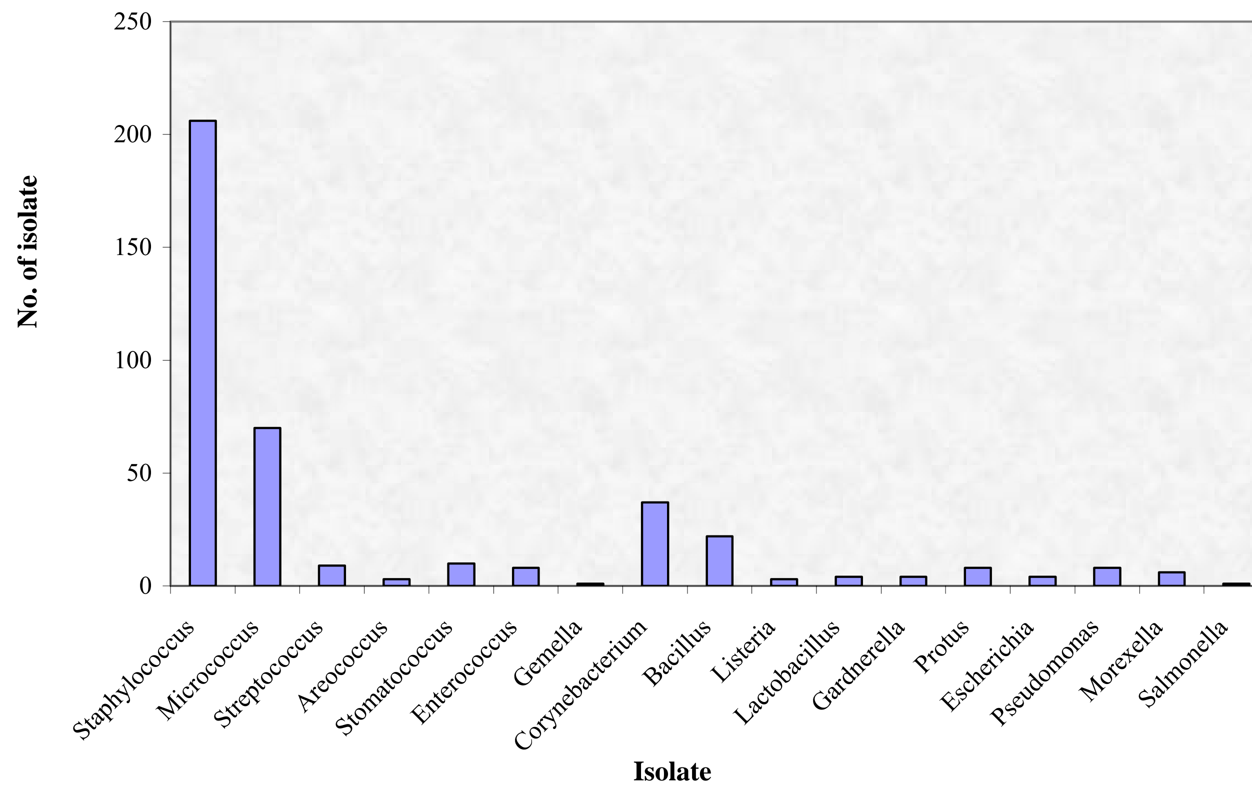

Fig. (1) Number of bacteria species isolated from hides and skins Gram - positive and Grame - negative bacteria

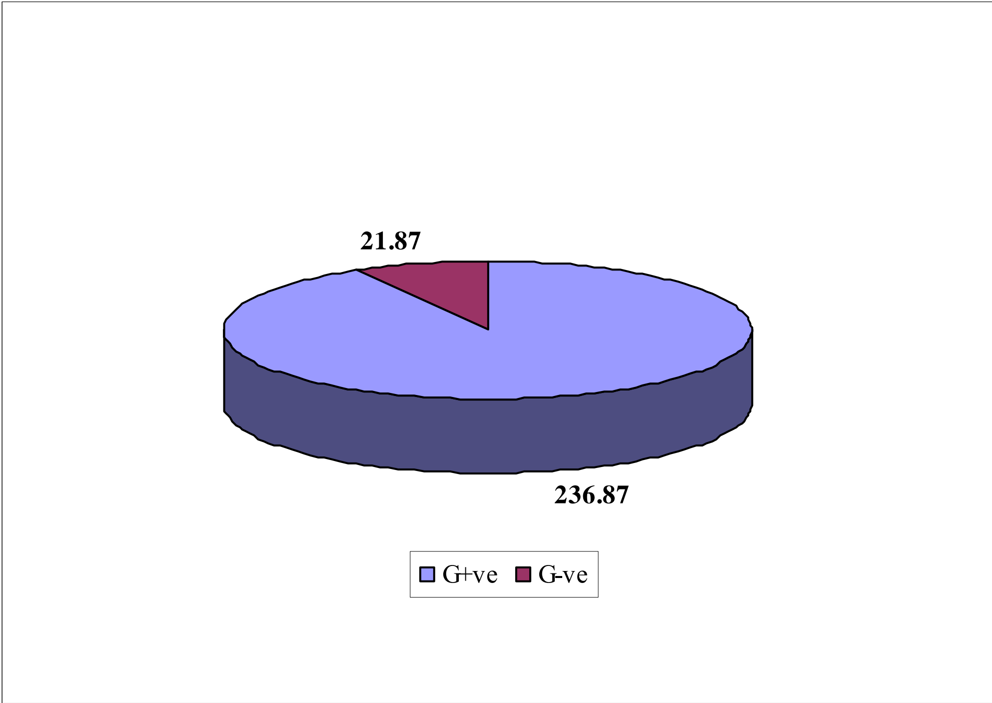

Fig. (2) Bacteria species isolated from hides and skins

Plate (1) Bacterial damage-wet blue hides

Plate (2) Putrification -wet blue hides

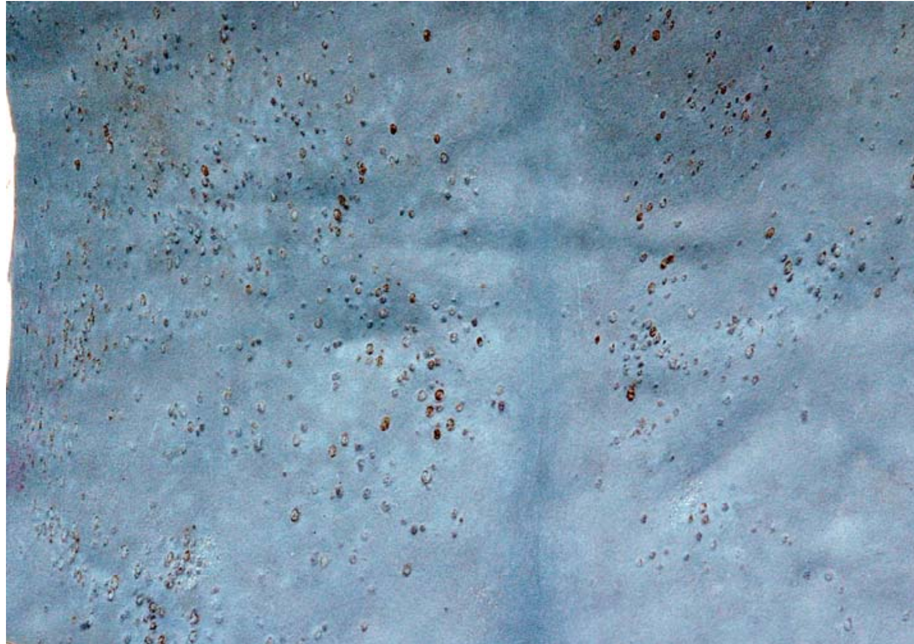

Plate (3) Putrification -wet blue hides

Plate (4) Putrification -wet blue hides

CHAPTER FOUR

4. DISCUSSION

Over the few last years great attention was given to hides and skins as they are processed into leather and subsequently manufactured into different finished leather products such as shoes , hand bags etc .There has been recently an expansion in the leather industry and many tanneries and leather workshop have been established in the country to promote leather industry.

In addition, hides and skins are exported in a great numbers from Sudan and recently become a source of foreign currency.

It is widely realized that improvement of hides and skins quality can be achieved only if quality grading norms are applied. The primary producer as well as the whole chain of related services, including flaying, handling and storing should be rewarded by better price for improved quality

(UNIDO, 1988).

It is unfortunate that Sudan hides and skins are invariably damaged and majority rather severely , damaged and defects peculiar to the Sudan are numerous and can be divided into three categories each one of being of interest to the cattle owner, the butcher or producer and exporter respectively (Knew, 1952).

The major problem which faces the progress of this industry is damage to hides and skins caused by putrefaction bacteria.

In most cases of bacterial damage of raw hides and skins is serious established problem and no previous attempts were made to study this problem in the Sudan.

This study was carried out to isolate and identify aerobic bacteria associated damage of hides and skins and see the infection in tissue of hides and skins (Histopathology).

In this study investigation were done in Gezira region (central Sudan) that populated density with raw hides and skins. Samples were collected were fresh hides, salted hides, dried hides and delivered without treatment hides, and fresh skins, washed skins, salted skins, dried skins, and delivered without treatment skins

Samples in this study were collected in different places: slaughterhouse, warehouses and tanneries. The study showed variation of the incidence the putrefaction.

Slaughterhouse was chosen because bacterial multiplication usually starts after death of animal .Composite samples from fresh hides and skins wash after 4 hrs to avoid incomplete bleeding, moisture, dirty.

In warehouse were collected from immediately salted, traditional salted and dried hides and skins, the case of salting method must be quickly and to isolated bacteria resistant to salt. In tannery were collected new hides and skins without any treatment so as to mention purification hides and skins are a good media for growth of bacteria.

In this study it was observed that raw hides and skins poor general condition were more susceptible to have purification of bacteria so as

delivery without treatment (28.26 %) and poor treatment, traditional salted ( ), dirt and warmth are factor which favor the multiplication of organisms , that lead to purification of hides and skins (knew, 1952). Salting (24 %) the high representage, this may be due to late of treated so as bacteria growth in law salting, this fact is in agreement with that

Bacteriological finding in this study were based on isolation and identification of aerobic bacteria from different stage of raw hides and skins. From 160 samples collected 414 bacterial isolates were obtained and were identified by conventional bacteriological methods.

The results showed presence of both Gram positive (92.3%) and Gram negative (7.8 %) bacteria. Gram positive were represented the majority of bacteria isolated (206) this was the same as observed by different workers (Gmeiner,1908; Bergey›s, 1974; Ruhrmann, 1987; Ibrahim, 1989; Keiri, 2001).

The organisms isolated in this study were *Staphylococci spp.* (53.12%), *Micrococcus spp*. (23.05 %), *Streptococcus spp*. (1.5 %) , *Aerococcus* (1.30%) , *Stomatococcus* (1.81%), *Enterococcus* (2.07%) *Gemella* (0.26%) , *Corynebacterium* (10.10%), *Bacillus* (4.66%) , *Listeria* (0.78 5) , *Lactobacillus* (0.52 %) , *Gardnerella* (1.04 %), *Protus* ( ) , *Escherichia coli* ( ) , *Pseudomonas* ( ) , *Morxella* ( ) and *Salmonella* ( ) .

Samples collected from delivered raw hides and skins putrefied, 117 bacteria were isolated and they constituted the largest number of isolation. Bacteria isolated from samples taken after hours consisted of (26.57 %). Gram positive bacteria and (1.69 %) Gram negative bacteria. The higher rate of isolation of gram positive organisms indicated that these organisms

were more active in causing putrefaction. the putrefaction was clear in these samples as shown by offensive odor and hair slipping , this confirm with (kiri, 2001) were isolate *Stahpylococcus* , *Micrococcus* , *Bacillus* and *Corynebacterium* were predominate bacteria isolated from samples after 11 hours among latter isolated was *Morxella bovis* and *Erwinia herbicola* which are gelatinic bacteria this aggress with isolated from hides and skins during present work . And also Ibrahim (1989) work in bacterial putrefaction of hides and skins isolated *staphylococcus albus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Corynebacterium pyogens*.

All swabs collected from traditional salting hides and skins in this study showed bacterial growth because it is not treated quickly, 100 bacteria were isolated (24 %) Gram positive bacteria and ( ) Gram negative bacteria.

The large number of microorganisms obtained in the present study from traditional salting was resistant to salt. Lesions observed were red areas (red heat) seeing figure (1). This finding are similar to that found (Bergey 's ,1974) , halophillic bacteria grow in 7% salt while extremely grow in 20% concentration and higher, Staphylococcus and Micrococcus grow in 5-15% salt tolerance of Bacillus range from 2-25% Nacl, also (Cowan, 1993 and Khiri,2002) reported in this isolation from salting of hides and skins putrefaction, the results in tables 4-5 showed that large variety of traditional salt and immediately salt from both raw hides and skins in warehouse . One hundred isolated were obtained from traditional salt while 39 strains were isolated from immediately salted. The results

show much difference between isolates from traditional and immediately salted hides and skins as expected. This was probably due to time of curing, the used of small amount of salt, or application of used salt Wood, Atkinson, Cooper and Gulloway (1969) reported that increased concentration of salt adversely affected the bacterial count. The rate of isolation from samples taken after drying hides and skins in warehouse was lower than salting (24 species) FAO (1955), if drying is too slaw the bacterial activity will start before the moisture content has been reduced sufficiently, on other hand if dry occur too quickly the middle of hides or skins will begin to gelatinized by bacterial activity Maarzo (1995).

Bacteria isolated from fresh hides and skins in slaughterhouse after 4 hours of slaughtering consisted 73 isolated. Bacteria isolated from both fresh and wash hides and skins represented (32.46 %) of total bacteria isolated , this agree with Jemmi (1990); Rei, et al (2002) and Ruhrmann (1987).

Ruhrmann 1987 isolated most important organisms involved in hides and skins in slaughterhouse , the main micro flora of the hides was primarily Gram positive , non spore forming rod and Gram positive cocci were *Staphylococcus* and *Micrococcus* . The commonest Staphylococcus was *Staph. xylosus*, *Staph. sciuri, Staph. cohnii, Staph. simulans, Staph. hycus, Staph. epidermidis, Staph. saprophytics, Staph. hominis, Staph. warneri, Staph. aureus* and *Staph. haemolyticus.* The commonest Micrococcus was *Micrococcus varians*. Major of these organisms were

isolated from hides and skins during the present work. These results support the report by Khiri (2002).

In this study the bacteriological results are correlated with leather decay grading and it appeared that the bacterial activity on hides tissue , the effects of (20 samples)(histopathology test) different concentration of growth and gelatinolytic activity have been studied Schmitt and Deasy, (1963) ; Cooper *et al.* (1973); Wood *et al.* (1970); Veis (1964) : Wood *et al.* (1971) and Waldo *et al.* (1986).

This confirms with reported FAO (1995) that bacteria as result effect of hide and skin fibers in the area of destroyed. The period of delayed curing can extend for as much 6-12 hours after salting hide for stack-salting. This due to fact that salt has to penetrate into the grain layer of hide. Halophillic organisms isolated damage the grain layer of brine cured hide and so lower the value of the leather , Dvid *et al.* (1996) many explain that a number of bacteria were isolated in this study made damage in fibers of hides and skins that showed lesions in putrefied hides and skins that Figure (2) .

In this study observed that not all bacteria occur on hides are necessarily responsible for decomposing the collagen , this fact is in agreement with that stated by Veis (1964) and Wood et al. (1970) who mentioned relationship between some spices and collagenolysis in raw hides bacteria showed higher rate of collagenolysis than with cured hides. The collagenolysis was highest at salt concentration below 7.0 % this agreement with Wood *et al.* (1971).

Staphylococcus spp. Represent 53.12 % of the total Gram positive bacteria isolated, all of them were identified as co-agulase negative.

Out of 35 from fresh hides and skins, 27 isolates from wash hides and skins, 74 isolates from salted hides and skins 10 isolates from dried hides and skins and 50 isolates from delivered without treatment these the higher rate of isolation of staphylococci indicated that these organisms were more active in causing purification hides and skins and they were isolated and they were isolated alone or in mix infection with other organism , they were exhibited an extensive damage in hides and skins the same organism was reported by different authors (Bergey 's, 1974) ; Esuruoso and unworth, 1946; Ruhmann, 1987; Ibrahim, 1989; Khiri ,2000 and Gihering et al.,2003).

Micrococcus spp. represent 23.05 % of total Gram positive bacteria isolates , 15 from fresh hide and skins ,13 from wash hides and skins , 32 from salted hides and skins three dried , 22 delivered without treatment hides and skins. The same microorganism was isolated from purification of hides and skins by various workers (Bergey 's, 1974 ; Ruhrmann, 1987; Gihering et al., 2003; Khiri, 2001).

In this investigation, corynebacterium represents 10.10% of total Gram positive bacteria isolated. Seven from fresh, four from wash, 15 from salted, 12 from delivered hides and skins, this confirm what was reported by (Bergey 's, 1954; Barrow and Felthman, 1993 ; Ibrahim, 1989; and Khiri, 2001).

Bacillus spp. represent 4.66 % of total Gram positive isolates, none isolates from wash and fresh hides and skins, seven from salted, eight from delivered, *B. amylolquefaciens* causes purification of hides and skins and isolated in study that was due to gelatinolytic activity of bacteria these results support the report by Waldo, et al. (1936); Cooper, (1973) ; Ibrahim (1989); Khiri (2001).

Six isolates of *Morxella* all of them were identified as *Morxella bovis* in the present study they represent 20.69 % of the total Gram negative bacteria isolated, tow from salted and tow from delivered without treatment hides and skins. This bacterium was activity for putrefaction of hides and skins, gelatnic bacteria they were also isolated from hides and skins damage by other *workers* Bergey, s (1974); Barrow and Feltham (1993).

Escherichia coli represent 13.79 % of the total Gram negative isolates. Four isolates from wash hides and skins, the same organism was reported in slaughterhouse by different authors Waldo *et al.* (1986); Donkersgoed *et al* (1997).

The contamination bacteria isolates from slaughterhouse and warehouse in flesh side and wash were *Listeria monocytogenes*, *Salmonella typhi*, *Pseudomonas auraginosa*, Pseudomonas *pseudolaaligen*. This microorganism were suggested to be part of normal micro flora in the cattle hides and sheep skins were the main source of contamination Gobat and Jemmi (1990); Rei *et al* (2002).

Eight organism of *Protus vulgaris* biogroub 1II were represent 27.59 % of Gram negative bacteria isolated, four from fresh hides and skins

, three from wash in slaughterhouse and one from delivered cattle hides and sheep skins in tannery . These isolated by Kallenberger et al (1986).

Lactobacillus jennii, *Gardnerella vaginatis* and *vibro alginolytium*. These were isolated from putrefied hides and skin. In that the damage of hide and skins was due to gelatinlytic activity of bacteria these report of presence of these bacteria in salted and delivered hide and skins reported here might be localize one.

Eight isolates of *Enterococci spp* , these represent (2.07%) of the total Gram positive isolated , one from fresh , four from wash, three from salted hides and skins . This results confirm what was reported by Bargey's, (1974); Kallenberger *et al*.(1986) ; Barrow and feltham, (1993).

CONCLUSION AND RECOMMENDATION

It is widely realized that Improvement of hides and skins quality can be achieved if several measures should be taken to protect against bacterial putrefaction as a pre-quiste to production of high quality leather

From the findings of the present study it can be start at time to slaughter and include the Following:

1. Concern of Animal husbandry including herd and disease management, feed quality availability, which affect quality of hides and skins.
2. Concern of slaughtering and flaying techniques, practices and Facilities (organized slaughter houses vs. scattered slaughtering), which cause damage to hides and skins
3. Concern of techniques and procedures used at various stages in the processing chain from slaughtering to finished products that affects.
4. Hides and skins should be washed in clean place immediately after flaying to remove blood and dirt.
5. Salting and air drying of hides and skins should be done as soon as possible and bactericidal agent added to salt.
6. The correct amount of salt should be used and the salt should be used once only.
7. Transportation of hides to tanneries and warehouse should be done in clean trucks and as soon as possible.

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