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Bacteriological studies on some bacteria causing bovine skin lesions

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Abstract

Skin diseases are very common in bovine globally greatly affect the economy by recorded production and performance of their animals. From buffaloes and cows clinically showing skin lesions in different farms at Giza governorate, a total of 100 samples of hair, pus, exudates and skin scraping scales were collected (50 for each) and subjected to the bacteriological examination. The results showed that the bacterial pathogens isolated from affected buffaloes and cows either in a single or a mixed form included *Staphylococcus aureus* (33.3%), *Coryne bacterium pseudotuberculoses, Dermatophiluscongolensis* and *Pseudomonas aeruginosa*(16.7% of each), *Escherichia coli* (11.1) and *Enterococcus faecalis* (5.5%). Antibiograms of the most commonly isolated bacteria were studied using disc diffusion method and group of antimicrobial agents of the most common used in the fields. The test revealed that, most of the isolates were sensitive to gentamycin, kanamycin, enerofloxacin, erythromycin, amoxicillin/clavulanic acid (AMC) and trimethoprim/sulphamethazole (St) and also most of them were resistant to nalidixic acid (NA), ampicillin, pencillin, streptomycin and tetracyclin. So these results were enhanced by the detection of drug resistance genes to the most common bacterial isolates using polymerase chain reaction test (PCR) to determine and choose optimize antibiotic groups to highlights success therapy with selection of first choice drug. Field recommendations for protection of bovine from skin diseases were pointed out.

Key words: *Skin lesion, Antimicrobial potential, buffaloes and Traditional antibiotic*.

Introduction

Recently, the prevalence of the skin affection has significantly reduced in many developed nations of the world compared to the developing countries due to improved social, economic, health care and hygiene practice factors evident in the former. Skin diseases can be caused by viruses, bacteria, fungi, or parasites.

Herpes simplex is the most common viral skin disease **(Havlickova *et al.,* 2008 and Ilkit, 2010)**.

The bacterial skin affections of animals cause many economic loses among affected animals and may cause zoonotic infection to the contact humans, the most common bacterial skin pathogens are *Staphylococcus aureus* and group Aβ -hemolytic Streptococci, and also *Escherichia coli, Klebsiella, Corynebacterium, Enterococcus* and *Dermatophiluscongolensis* were isolated from cattle suffered from skin affection **(Hassan *et al.*, 2015)**.

Dermatophilus and *staphylococcus* infections affected skin of buffaloes cause skin disease, several factors are involved in their pathogenesis, among them are the mechanical injury to the skin, rainfall, tick infestation, concurrent diseases and or stresses that compromise the hosts immune system **(Amabrose, 1996 and Harman *et al.,* 2001).**

Dermatophilus congolensis is a well-recognized Gram-positive, non-acid-fast, branching filamentous rod. These microorganisms are facultative anaerobic, capnophilic or aerobic tolerant bacteria that belong to the Actinomycetes affecting mainly livestock, occasionally companion animal and wildlife, and rarely human **(Dalis *et al.,* 2014)**. It is an atypical bacterium because it produces infective motile zoospores and aerial mycelia **(Giuffrida, 2016)**.

Corynebacterium pseudotuberculosisis a Gram positive pleomorphic rod-shaped, intracellular, facultative anaerobe with worldwide distribution**.** Infection with *C. pseudotuberculosis* has been reported in sheep, goats, cattle, buffalo, camel, equineand humans. The portal of entry of this soil borne organism is thought to be through abrasions or wounds in the skin or mucous membranes **(Spier, 2006).**

Phospholipase D (Pld) is the most important virulence factor in *C. pseudotuberculosis* **(Hodgson *et al.,* 1999)**. Pldis an exotoxin induces increased vascular permeability through catalyzing sphingomyelin dissociation, resulting in spread and survival of *C. pseudotuberculosis* in cells, and consequently, the invasion of the body and transport by phagocytesto regional lymph nodes **(Corrêa *et al.,* 2018)**. *Pld* gene detection is used as a diagnostic tool for *C. pseudotuberculosis***.**
Staphylococcus aureus is a Gram-positive bacterium that naturally inhabits human and other animals skin and mucous membranes, however, this bacterium can infect other tissues and become an opportunistic pathogen the pathogenic strains produce virulence factors such as potent protein toxins **(Stevens *et al.,* 2005 and Belizário *et al.,* 2015**).

Naturally, *S. aureus* is susceptible to most known antibiotics **(Thomer *et al.,* 2016)**. However, there are antibiotic-resistant strains of *S. aureus***.** The resistance genes expressed by these strains are mainly acquired from external sources; this could be either natural or due to human actions mainly by antimicrobial abuse, misuse and lead to chromosomal mutation **(Montanaro *et al.,* 2016)**. The emergence and worldwide spread of antibiotic-resistant strains of *S. aureus* such as methicillin-resistant *Staphylococcus aureus* (MRSA) is of health and socio-economic importance **(Humphreys *et al.,* 2016)**.

Cows and buffaloes were identified as a potential carrier of MRSA in Bihar (India). The isolation of vancomyc in resistant *Staphylococcus aureus* (VRSA) indicates the emergence of VRSA in animal population which may be transmitted to the human beings working in close contact to the animals **(Kumar *et al.,* 2017).**

Polymerase chain reaction (PCR) test allows the detection and identification of different pathogens and also has been successfully used for rapid identification and evaluation of specific antibiotic resistances genes in bacteria grown in cultures **(Memon *et al.,* 2013).**

Therefore, the present study was undertaken to detect the prevalence rate of some bacterial causes of skin lesions of buffaloes and cows in some farms in Giza governorate, in addition it aimed to study the antibiograms of the most commonly isolated organisms in order to determine the antibiotic resistant genes of this bacteria by PCR test to optimize antibiotic the rapy to high lights success therapy in common generalized bovine skin lesions with selection of first-choice drugs.

Materials and Methods

1. Samples collection and preparation:

A total of 100 samples (hair, pus, exudates and skin scraping scales) were collected from (50) buffaloes and (50) cows clinically showing skin lesions and selected from different farms at Giza governorate for bacteriological examination, the affected skin areas of the animal body were cleaned with 70% alcohol and skin scrapings were taken from the edges of the lesions by scalpel blade and hair as mentioned

by **Refai *et al.* (2014)**. In cases of edematous skin disease in buffalo aspiration of pus from closed sterile lesion was carried out. The samples were collected in clean labeled envelops and sent to the laboratory in an ice box.

2. Isolation and identification of causative bacterial agents:

A. Direct microscopical examination (Quinn *et al.,* 2002)

Small pieces were taken from the underside of the scab and softened in few drops of distilled water on a clean microscope slide; a smear was made and stained by Loffler's methylene blue and Gram's stains.

B. Isolation and identification of causative agent:

A part of samples were inoculated into the test tubes containing nutrient broth and were incubated at 37˚C for 24 hours aerobically. The subcultures were also made on nutrient agar, 5% sheep blood, eosin methylene blue (EMB), brain heart infusion (BHI) and Baird Parker (BP) agar plates and incubated aerobically at 37˚C for overnight. Based on morphological and staining characteristics, hemolytic activities on blood agar and biochemical characters, the growing surface colonies were identified according to **Quinn *et al.* (2002) and Anon (2007**). Coagulase test was applied for staphylococcus isolates as recommended by **Flandrois and Carret (1981),** also they were tested using the numerically API Kits (bioMerieux, Marcy-l'Etoile, France) and the results were interpreted according to **Finegold and Baron (1986).**

C. Isolation and identification of dermatophilus species (Haalstra, 1965):

A small amount of scab material was ground up, placed in a screw capped bottle, moistened with one ml sterilized distilled water and allowed to stand open for 3 and half hours on the bench. Then the opened bottle transferred to candle jar with a candle was burned within the jar to obtain 10 – 20% CO2 tension (so the motile zoospores were chemo-tactically attracted to the CO enhanced atmosphere and move to the surface of distilled water). After 15 minutes, the bottle was carefully removed and a drop was taken from the water surface with a bacteriological loop and cultivated on brain heart infusion agar plates which were incubated at 37˚C in 20% CO2 tension for 24 to 48 hours. The suspected colonies were identified as recommended by **Silva *et al.* (2003) and Anon (2007).**

D. Antibiotic sensitivity test:

According to clinical and laboratory standard institute **(CLSI, 2008),** bacterial strains having high incidence rate in the study and historically play an important role in the disease occurrence were subjected to antimicrobial susceptibility testing by disc diffusion method using a group of common used antibiotics in the field was determined on Muellar-Hinton agar medium (Oxoid) supplemented with 5 % blood as described. Antibiotic discs were ampicillin (25µg), gentamycin (30µg), enerofloxacin (10µg), streptomycin (30µg), amoxicillin clavulanic acid AMC (30µg), nalidixicacidNA (30µg), trimethoprim/sulphamethazole St (25µg) and penicillin (10IU), tetracycline (25µg), kanamycin (15µg) and erythromycin (15µg).

E. PCR detection of antibiotic resistant genes:

DNA extraction: DNA extraction from some isolate samples resisted to antibiotics by disc diffusion method was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH). Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteins K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer.

Oligonucleotide Primer: Primers used were supplied from Metabion (Germany) are listed in Table (1).

PCR amplification: Primers was performed in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20pmolconcentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied bios stem 2720 thermal cycler.

Analysis of the PCR Products: The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the products was loaded in each gel slot. Gelpilot 100 bp, 100 bp plus DNA Ladders (Qiagen, Germany, GmbH) and generuler 100 bp ladder (Fermentas, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (AlphaInnotech, Biometra) and the data was analyzed through computer software.

Table (1): Primers sequences, target genes, amplicon sizes and cycling conditions:

Target agent	Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
					Secondary denaturation	Annealing	Extension		
Coryne	*mefA*	AGTATCATTAATCACTAGTGC TTCTTCTGGTACTAAAAGTGG	345	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	Morvan *et al*., 2010
	ermX	GAGATCGGRCCAGGAAGC GTGTGCACCATCGCCTGA	488	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Klima *et al*., 2014
	bla	ATGAAAGAAGTTCAAAAATATTTAGA G TTAGTGCCAATTGTTCATGATGG	780	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Catalán *et al*., 2010
	Aada2	TGTTGGTTACTGTGGCCGTA GATCTCGCCTTTCACAAAGC	622	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Walker *et al*., 2001
Staph	*blaZ*	TACAACTGTAATATCGGAGGG CATTACACTCTTGGCGGTTTC	833	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Bagcigil *et al.* 2012
	mphC	GAGACTACCAAGAAGACCTGACG CATACGCCGATTCTCCTGAT	722	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Schlegelova *et al*., 2008
	msrA	GCAAATGGTGTAGGTAAGACAACT ATCATGTGATGTAAACAAAAT	400	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	
	norA	TTCACCAAGCCATCAAAAAG CTTGCCTTTCTCCAGCAATA	620	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Pourmand *et al*., 2014
P.aerogenosa	*mphA*	GTGAGGAGGAGCTTCGCGAG TGCCGCAGGACTCGGAGGTC	403	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	Nguyen *et al*., 2009
	blaIMP	CATGGTTTGGTGGTTCTTGT ATAATTTGGCGGACTTTGGC	488	94˚C 5 min.	94˚C 30 sec.	53˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Xia *et al*., 2012
	blaVIM	AGTGGTGAGTATCCGACA ATGAAAGTGCGTGGAGAC	280	94˚C 5 min.	94˚C 30 sec.	53˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	
	qnrS	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	417	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Robicsek*et al*., 2006

Results

Table (2). Results of bacteriological examination of the collected samples from bovine skin lesions:

Animal Species	Total number of examined samples	Positive Samples		Negative samples	
		No.	%	No.	%
Cow	50	33	*66	17	*34
Buffaloes	50	29	*58	21	*42
Total	100	62	**62	38	**38

*% was calculated according to the number of examined samples from each species (50).
**% calculated according to the total number of examined samples (100).

Table (3). Prevalence rate of bacterial isolates recovered from examined bovine skin lesion samples:

Bacterial isolates	Buffaloes		Cows	
	No. (50)	*%	No. (50)	*%
Single infections:				
Staphylococcus aureus	4	8	6	12
Corynebacterium pseudotuberculoses	12	24	3	6
Dermatophilus congolensis	2	4	9	18
Mixed infections:				
S. aureus + E. fecalis	1	2	2	4
S. aureus + P. aeruginosa	5	10	6	12
S. aureus + E. coli	2	4	4	8
E. fecalis+P.aeruginosa	1	2	1	2
D. congolensis + P. aeruginosa + E. coli	1	2	1	2
E. coli + D. congolensis.	1	2	1	2
Total	29	58	33	66

*% was calculated according to the total number of samples examined from each species.

Table (4). Number and percentages of bacterial species isolated from examined skin lesion (single and mixed infection):

Bacterial isolates	No.	%
S. aureus	30	33.3
C. pseudotuberculosis	15	16.7
D. congolensis	15	16.7
P. aeruginosa	15	16.7
E. Coli	10	11.1
E. fecalis	5	5.50
Total isolates	90	100

*% was calculated according to the total number of isolates (90).

Table (5). Antimicrobial susceptibility pattern in the isolates of bovine's skin:

Anitmicrobial drugs	*C. pseudotuberculosis* N (15)				*D. congolensis* N (15)				*S. aureus* N (30)				*P. aeruginosa* N (15)			
	Senstive		Resistant		Senstive		Resistant		Senstive		Resistant		Senstive		Resistat	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Trimethoprime/SulphamethazoleST 25 µg	15	100	0	0	15	100	0	0	30	100	0	0	0	0	15	100
Genamycin CN10 µg	10	66	5	44	9	60	6	40	25	82.5	5	17.5	9	60	6	40
Nalidixic acid NA 30µg	0	0	15	100	12	80	3	20	30	100	0	0	11	73.3	4	30.8
Amoxicillin/Clavulanic acid 30µg	15	100	0	0	10	66	5	34	0	0	30	100	9	60	6	40
Erythromycin E 15 µg	11	73.6	4	26.4	10	66	5	34	26	85.8	4	14.2	0	0	15	100
Enrofloxacin ENR 15 µg	11	73.6	4	26.4	15	100	0	0	25	82.5	5	17.5	10	66.7	5	33.3
Ampicillin AM 10 µg	0	0	15	100	9	60	6	40	0	0	30	100	0	0	15	100
Penicillin G 10 IU	6	40	9	60	5	34	10	66	6	20	24	80	9	60	6	40
Tetracycline	5	34	10	66	6	40	9	60	14	47	16	53	9	60	6	40
Kanamycin	12	77	3	23	11	73	4	27	25	82.5	5	14.5	12	80	3	20
Streptomycin	3	20	12	80	5	34	10	66	4	13	26	87	3	20	12	80

Table (6). Detection of the antibiotic resistance genes in some bacterial isolates

Bacterial Isolates	Antibiotic resistance genes	Results
C. pseudotuberculosis	*MefA* *ermX* *bla* *Aada2*	+ + + +
S. aureus	*blaZ* *norA* *mphC* *msrA*	+ + +
P. aeruginosa	*blavVIM* *blaIMP* *qnrs* *mphA*	– – + + +

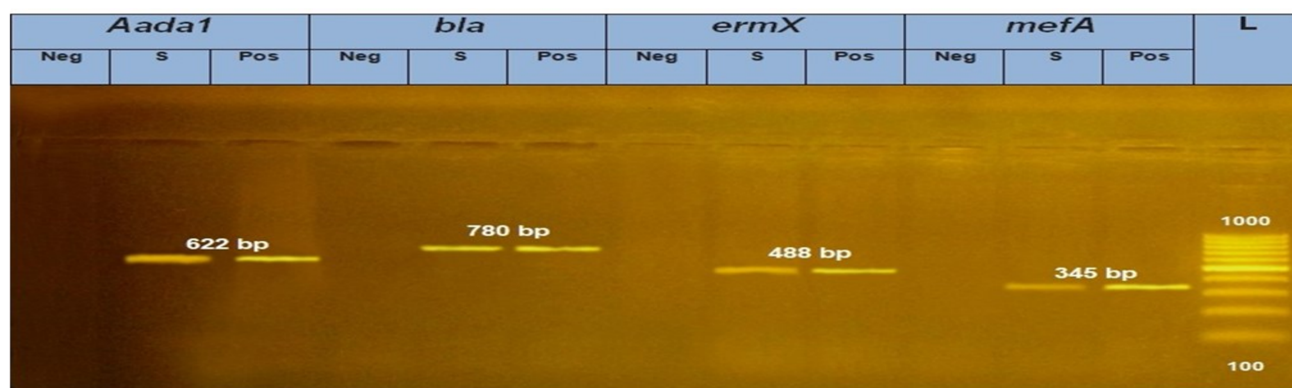

Fig. (1): Detection of antibiotic resistance genes (*Aada1, bla, ermX and mefA*) of *C. pseudotuberculosis* by polymerase chain reaction (PCR).L. - DNA ladder (1000 -100 bp.). Pos. - Positive control. Neg.-Nigative control. S.- sample Lanes arrangement from the left to right showing (*Aada, bla,ermXandmefA*) genes amplifications (622 bp,780 bp,488 bp and 345 bp) respectively .

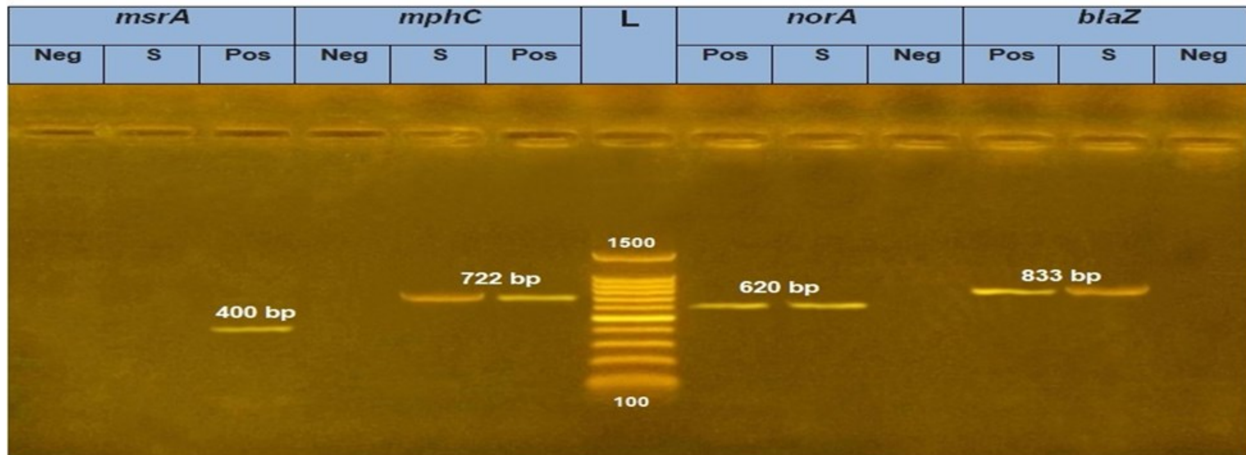

Fig. (2): Detection of antibiotic resistance genes (*msrA, mphc, norAb* and *blaZ*) of *S. aureus* by polymerase chain reaction (PCR). L. - DNA ladder (1500-100 bp.). Pos. - Positive control. Neg. -Nigative control. S. – sample Lane of *mphc* gene amplification showing 722 bp.Lane of gene amplification showing 620 bp. Lane of *blaZ* gene amplification showing 833 bp.

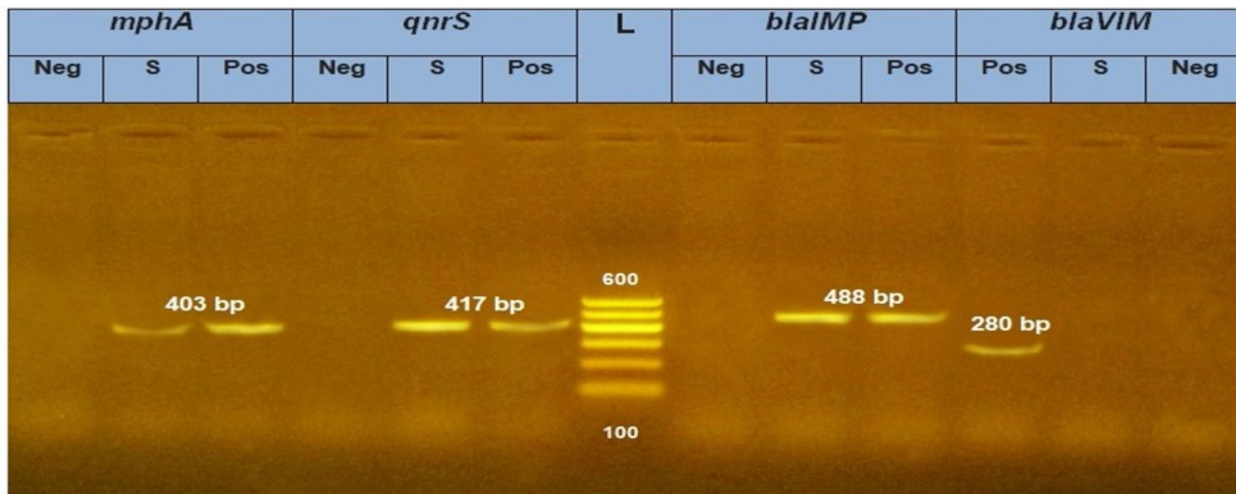

Fig. (3): Detection of antibiotic resistance genes (*mphA, qnrS, blaIMP* and *blaVIM*) of *P. eruginosa* by polymerase chain reaction (PCR): L. - DNA ladder (600 -100 bp.). Pos. - Positive control. Neg.-Nigative control. S. – sample Lane of *mphA* gene amplification showing 403 bp. Lane of *qnrS* gene amplification showing 417 bp. Lane of *blaIMP* gene amplification showing 488 bp

Discussion

The skin provides a remarkably good barrier against bacterial infections. Although many bacteria come in contact with or reside on the skin, they are normally unable to establish an infection. The emerging infectious diseases and the development of drug resistance in the pathogenic bacteria at an alarming rate is a matter of serious concern. Despite the increased knowledge of microbial pathogenesis and application of modern therapeutics, the morbidity and mortality associated with the microbial infections still remains high **(Kolar *et al*., 2001).**

The infection with *D. congolensis* occurs when the integrity of the skin is impaired, as in case of long exposure to rain or traumatic injuries resulting from arthropod bites which serve as mechanical transmitters of microorganisms into epidermal layers, where germination zoospores takes places. The high relative humidity has a significant influence on the maturation and motility of the infective zoospores and it has been claimed **(Zaria, 1993 and Burd**

***et al.,* 2007).**

In the present study Table (2)illustrated that, out of (50) samples examined from buffaloes skin lesions 29 (58%) were positive for bacteriological examination while 21(42%) were negative. On the other hand out of (50) samples examined from cow skin lesions 33 (66%) were positive for bacteriological examination and 17 (28%) were negative.

Table (3) showed that, *C. pseudotuberculosis, S. aureus*, *D. congolensis* which considered the main causes of skin lesions in bovine were isolated in a sigle form from buffalo skin lesions in a percentage of (24), (8) and (4) respectively, while in cow their percentage of isolation were (6), (12) and (18). The mixed infections in both animal were (*S. aureus*+ *E. feacalis*), (*S. aureus* + *P. aeruginosa)*, (*S. aureus* + *E. coli), (E. feacalis*+ *P. aeruginosa), (D. congolensis* + *P. aeruginosa*+ *E. coli*) and (*E. coli* +*D. congolensis*) in percentage of (2), (10), (4), (2), (2) and (2) in buffaloes and (2), (12), (8), (2), (2) and (2) in cow respectively. These results revealed that buffaloes were more susceptible for infection with *C. pseudotuberculosis* which causes edematous skin disease (OSD), that affect buffaloes mainly and characterized with its special lesion. These results agreed with **Sohier *et al.* (2013)** who isolated *C. pseudotuberculosis* from buffalo OSD at the rate of (25%). The presence of *E. Feacalis* and *E. coli* contamination which not recorded as causes to skin lesions affections is not an unexpected finding. It has been suggested that their found in the skin lesion probably represent its normal inhabit in environment and widely distributed in soil, faces, water and in plant as well as in the intestine of animals and human as reported in clinical veterinary microbiology book **(Quien *et al.,* 2002)**.These results also agree with **Awadalla *et al.* (2015)** and **Jiang and Zhang (2013)** who isolated *E. coli* from diarrheic calves. Table (3) also revealed that, cows were more susceptible for infection with *D. congolensis* which was the main cause of skin lesions in cow called dermatophilosis, we were in agreement with **Mannan *et al.* (2009)** who isolated *D. congolensis* from 11% of cow skin lesions, also **Hassan *et al.* (2014**) isolated 6 species of bacteria namely *Staphylococcus, E. coli, Klebsiela, Corynebacterium, Enterococcus* and *Streptococcus* species from diseased buffaloes and also **Hassan *et al.* (2015)** recovered *D. congolensis* from cow suffered from skin affection.

Table (4) showed that, the incidence of total number of isolates for both single and mixed infection were 33.3%, 16.7%, 16.7%, 16.7, 11.1% and 5.5% for *S. aureus, C. pseudotuberculosis, D. congolensis, P. aeruginosa, E. coli* and *E. faecalis* respectively. These results agreed with **Hassan *et al.* (2015)** that isolated *S. aureus* and *D. congolensis* at an incidence of (15% and 13%) from hairs and (17% and 14%) from scales of skin of buffaloes.

Table (5) showed that, *C. pseudotuberculosis* resist ampicillin 25µg and nalidixic acid 30µg , but sensitive for enerofloxacin 10µg, erythromycin 15µg, amoxycillinclavulanic acid 30µg, gentamycin 30µg and trimethoprim sulphmethazole 25µg. This table also showed that *D. congolensis*resist nalidixic acid 30µg and sentive for all the rest in the table, so the treatment in cases of dermatophilosis was simple just accurate diagnosis was needed to avoid false diagnosis with some fungi these results were agreed with **Mannan *et al.* (2009)** and **Aires *et al.* (2017)** who used different protocols for the in vivo treatment of bovine dermatophilosis and they reported that, although beta-lactams penicillin, aminoglycosides streptomycin, and tetracyclines were recorded as the first-line antimicrobials to the treatment of localized cutaneous lesions of dermatophilosis among livestock **(Giuffrida, 2016).** Also the result in the table revealed that, *S. aureus* resist ampicillin 25µg penicillin and nalidixic acid 30µg and sensitive for the other drugs, these results agreed with the results of **Awadalla *et al.* (2016**). *P. aerogenosa* were resist ampicillin 25µg, trimethoprim sulphamethazole 25µg and erythromycin 15µg but sensitive for the rest of drugs under testing. On the other hand

most of strains were more resistance to penicillin (10IU).

Table (6) showed that, the *C. pseudotuberculosis* was positive for the presence of antibiotic resistance genes (*mefA* gene, *erXm* gene, *bla* gene and *Aada2* gene), meanwhile *S. aureus* were positive for the presence of antibiotic resistance genes (*blaZ gene, norA gene* and *mphC gene*) and were negative for the presence of antibiotic resistance gene *msrA*. On the other hand *P. aeruginosa* was negative for the presence of antibiotic resistance gene *blaVIM but* positive for the presence of antibiotic resistance genes (*blaIMP* gene, *qnrS*gene and *mphA* gene). Resistance against beta-lactams and presence of *blaZ*gene in our isolates is in agreement with **Green and Bradley (2004)** who reported that, *S. aureus* resistance to beta lactams is due to production of beta-lactamase. **Olsen *et al.* (2006)** found that, in Brazilian buffalo, *bla*Z (39.6%) gene was detected in *Staphylococcus* species isolates using PCR, at rate of (39.6%). **Memon *et al.* (2013)** determined the antimicrobial resistance traits of 34 *S. aureus* isolated from subclinical mastitis in Eastern China, and found that, *blaZ*gene were detected in 82% isolates. While **Awadalla *et al.* (2015**) detected *blaZ gene* inmost of strains of *S. aureus* isolated from buffalo milk samples, also **Otarigho *et al.* (2018)** improved analysis of antibiotics resistant genes in different strains of *S. aureus*.

The periodical examination of animals for skin affections pathogens should be undertaken. All environmental factors which predispose for these affections must be under hygeinic measures and good control. Our study proved that, buffaloes are more susceptible to infection with *C. pseudotuberculosis*, while cows are more susceptible to infection with *D. congolensis*, both organisms together with *S. aureus* are major causes of bovine skin disease, also there is no standard procedure for in vitro antimicrobial susceptibility tests for some bacteria caused skin lesion. So our results were improve dusing PCR test which revealed that *S. aureus*, *C. pseudotuberculosis* and *P. aeruginosa* were interconnected in function and become more resist to some drugs when one or more other genes are expressed although trimethoprim/ sulphamethazole, aminoglycosides (gentamycin) and macrolide (erythromycin) are reported as the first-line antimicrobials to the effective therapy of generalized bovine skin infection based on modified in vitro disk diffusion method.

Recommendations

Strict hygienic measures should be applied in animal farms by periodical application of effective disinfectants against bacteria and other pathogenic agents for protection of animals.

Avoid miss use of antibiotics and use of them according to the results of laboratory because it resulted in increased resistance of bacteria to the antibiotics.

Prevention and control of viral skin diseases as lumpy skin disease and herps as it cause invitation to bacterial infection and usually accompanied with it and this can occur by vaccination program against viral diseases.

Rapid diagnosis of skin diseases affected animals with application as soon as possible of effective treatment either locally or systemic.

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