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Prevalence Of Some Pyogenic Organisms In Skin Wound Infections In Sheep And Goat

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Abstract:
Background*: Pyogenic bacterial infections are broadly found worldwide among sheep and goats and are adversely impacting the development of the small ruminants' industry. The study was performed to identify and characterize most prevalent bacteria isolated from skin and wound infections of sheep and goat of various rearing condition in Egypt.*
Materials and Methods*: One hundred and fifteen pus samples (sheep≈70) and (goat≈45) obtained from skin abscesses and pyogenic wounds during the study period 2022-2023. The samples were cultured onto suitable media, microscopically and biochemically tested. The obtained isolates were assessed for their antibiotic susceptibility and subjected to polymerase chain reaction to detect certain genes which responsible for the virulence and antibiotic resistance.*
Results*: Bacterial isolates were recovered from 83 out of the 115 collected pus samples (72.17%) with pure isolation were obtained in 68 cultures. The bacterial isolation was achieved in 88.57% and 46.66% from sheep and goat respectively. Pseudomonas aeruginosa were recovered as 19.35% from sheep and 23.81% from goat while Staphylococcus aureus were isolated as 46.77% and 38.09% from sheep and goat respectively. Regarding Corynebacterium pseudotuberculosis was obtained from 37.09% of sheep and 9.52% of goat samples. P. aeruginosa isolates were highly sensitive to erythromycin, amikacin, ampicillin /sulbactam and cefoxitin and high resistance to amoxicillin /clavulanic acid, clindamycin, and quinolones with 58.82% MDR pattern. S. aureus isolates revealed the high sensetivity to amikacin, imipenem and clindamycin but high resistant to quinolones with 21.62% act as MDR. C. pseudotuberculosis isolates were sensitive to most of tested antibiotics except amoxicillin /clavulanic acid and clindamycin. PCR assays revealed that P. aeruginosa isolates carried toxA and blaOXA-1 genes by 35.29% and 64.70% respectively while S. aureus isolates was found to load eta gene by 13.51% and ermC gene 18.91% and in the same line ten C. pseudotuberculosis was found to harbor pld gene 40%.*
Conclusion: *It was concluded that Pseudomonas aeruginosa, Staphylococcus aureus as well as Corynebacterium pseudotuberculosis were the most isolated bacterial species in skin infection among sheep and goat in Egypt. Majority of the isolates were MDR and highly resistant to β lactams and quinolones but sensitive to amikacin and imipenem. PCR assays revealed that the isolates carried virulence and antibiotic resistance genes in significant incidence.*
Keyword: *Abscess-pyogenic wound- sheep- goats-Corynebacterium pseudotuberculosis- Staphylococcus aureus- Pseudomonas aeruginosa- Egypt.*

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I. Introduction

The bacterial organisms that implemented in skin lesions and hides wounds of domestic animals have been globally surveyed for a long time [1]. Gram positive bacteria such as *Staphylococcus aureus*, *Corynebacterium* spp., and *Streptococcus pyogenes* are of the most common bacteria to cause skin infections while *Pseudomonas aeruginosa* is considered the most prevalent Gram negative bacteria isolated from wound infections [2-4]. All types of skin and hides' wounds frequently happen due to different breach ways; imperfect brand marks, rubbing versus course surfaces, sharp instruments, or during surgical operations. Breaches get contaminated by pyogenic bacteria that induce damage to the leather quality, lead to losses in leather industries

and finally reflect on economy of the country [5]. The study of inducing agents of skin infection helps to protect hides and skin from degradation [6].

P. aeruginosa is usually tends to be a multidrug-resistant bacterium as four distinct resistance mechanisms are determined [7]. One of the mechanisms is the ability to synthetize the extended-spectrum β-lactamases (ESBLs), which play the master role in β-lactam antibiotic resistance; e.g. ampicillin, penicillin and cephalosporins [8, 9]. Interestingly, previous studies have exposed that the *Pseudomonas* strains which express *toxA* gene, reveal antibiotic resistance, including carbapenem resistance in the highest level [7, 10]. The *toxA* gene is a significant virulence determinant in *P. aeruginosa* which is responsible for production of exotoxin A; diphtheria -like toxin, suppressing polypeptide chain elongation in the cell results in its death [11, 12].

The exfoliative toxins which also termed as epidermolytic toxins are group of proteases produced by certain *S. aureus* strains inducing peeling of the skin and pus formation [13]. The exfoliative toxin A (ETA) is coded by the chromosomal *eta* gene loaded on the genome of temperate phage [14]. Resistance to macrolide, including erythromycin is common among *S. aureus* strains and encoded by the *erm* genes family, mostly *ermA* and *ermC*, which responsible for production of proteins causing ribosomal-binding site alteration [15].

Corynebacterium pseudotuberculosis is the causal cause of skin and superficial lymph nodes abscessiation which termed caseous lymphadenitis (CLA) in various small ruminants. It is defined as a significant reason of financial loss to the sheep industry in numerous countries where the illness is endemic [16]. It is known that prime virulence factor is an exotoxin (phospholipase D) that enhances the spread of the pathogen via the induction of the vascular permeabilization, membrane disruption and hemolysis [17]. Generally, data concerning sheep and goat bacterial skin diseases in Egypt is scanty. Dapgh et al. [18] reported isolation of *Pseudomonas aeruginosa* from skin lesion with abscesses of sheep and goats using cultural characterization instead of molecular characterization.

The present study focused on cultural, biochemical and molecular characterization of certain bacterial isolates obtained from skin lesions of sheep and goat reared sporadically or in farms.

II. Material And Methods

Sampling

The current research work was performed considering 115 pus samples (sheep≈70) (goat≈45) obtained from skin abscesses and pyogenic wounds during the study period 2022-2023. Skin swab and pus samples of sheep and goat were aseptically gathered, placed in nutrient broth, kept at 4°C temperature in an ice box and transported to the Laboratory of the Department of bacteriology, Animal Hygiene Research Institute (AHRI), Egypt.

Isolation of Pseudomonas spp., Staphylococcus spp. and Corynebacterium pseudotuberculosis

The enriched cultured skin swabs and pus samples were streaked onto various selective and differential culture media like Blood agar, MacConkey's agar and Mannitol salt agar (Himedia, India) according to Cowan, [19].

Identification of isolates by classical and advanced methods

Identification of the obtained bacterial colonies was relied on colonial appearance, microscopical characteristics as well as biochemical activities [20-22]. For initial identification of obtained isolates; Gram's staining, sugar fermentation test, IMVC reaction, urease, oxidase, catalase and coagulase production tests were carried out.

Further advanced biochemical confirmation was carried out by microbial identification system; Vitek 2 compact system, version: 9.02, (BioMerieux, France). All asserted isolates were kept in Tryptic Soy Broth supplemented with 15% (vol/vol) glycerol and preserved at − 80 °C for possibly future use.

Antibiogram profiles assay

Antibiogram of the obtained isolates was conducted by standard disk diffusion procedure using eighteen variant commercially available antibiotic discs of different classes (Sigma, USA) on Mueller-Hinton agar (HiMedia, India). The selected antibiotics used were mentioned in table 1. The susceptibility was interpreted according to the standard guidelines [23].

Table1: The used antibiotics and concentrations

Antibiotic	Abbreviation	Concentration	Antibiotic	Abbreviation	Concentration
Amikacin	AK	30 µg	Cefuroxime	CXM	30 µg
Amoxicillin/clavulanic acid	AMC	20/10 µg	Ciprofloxacin	CIP	5 µg

Ampicillin /sulbactam	SAM	10/10 µg	Clindamycin	DA	30 µg
Azithromycin	AZM	15 µg	Erythromycin	E	15 µg
Cefixime	CFM	5 µg	Fosfomycin	FF	30 µg
Cefotaxime	CTX	30 µg	Imipenem	IPM	10 µg
Cefoxitin	FOX	30 µg	Levofloxacin	LEV	5 µg
Ceftazidime	CAZ	30 µg	Norfloxacin	NOR	10 µg
Ceftriaxone	CRO	30 µg	Ofloxacin	OFX	5 µg

Molecular detection of Pseudomonas aeruginosa, Staphylococcus aureus and Corynebacterium pseudotuberculosis by PCR assay

Preparation of DNA templates

Extraction of DNA from the isolated species was carried out using extraction kit (QIA amp mini kit, Qiagen), following the kit guidelines.

PCR amplification of tested genes

Details of the oligonucleotide primers (Metabion, Germany) used for the amplification of *toxA* and *blaOXA*-1 genes of *P. aeruginosa*, *etb* and *ermC* genes of *S. aureus* and finally *pld* gene of *C. pseudotuberculosis* and the cycling conditions were summarized in table 2. The PCR reaction mixtures were prepared using Red Mix Master Mix (UK) and the amplification was conducted in T3 Biometra thermocycler. The PCR products were sectioned by electrophoresis on 1.5% agarose gel. After that the gel photographed and analyzed by Alpha Innotech, Biometra, (Jena, Germany).

Table 2: The used primers and cycling conditions.

Tested genes	Primers	Amplifying size bp	Primary denaturation	Secondary denaturation	Annealing	Extension	Final extension	Reference
toxA	GACAACGCCCTCAGCATCAACAGC CGCTGGCCCATTCGCTCCAGC	397	94°C 5min	94°C 30sec	52°C 30sec	72°C 45sec	72°C 7min	[24]
blaOXA-1	ACACAATACATATCAACTTCGC AGTGTGTGTTTAGAATGGTGATC	814	96°C 5min	96°C 30sec	60°C 1min	72°C 1min	72°C 7min	[25]
eta	CGCTGCGGACATTCCTACATGG TACATGCCCGCCACTTGCTTGT	676	94°C 5min	94°C 2 min	57°C 2 min	72°C 1 min	72°C 7min	[26]
ermC	ATCTTTGAAATCGGCTCAGG CAAACCCGTATTCCACGATT	295	94˚C 5 min.	94˚C 30 sec.	55˚C 45 sec.	72˚C 1 min.	72˚C 7 min.	[27]
pld	ATTATGGGGATGCTTC TCACCACGGTTATCCGCT	930	94˚C 5 min.	94˚C 1 min.	55˚C 1 min.	72˚C 1 min.	72˚C 7 min.	[28]

All laboratory procedures were conducted inside biosafety cabinet with application of proper personal protective equipment and hygienic disposal of biological and chemical wastes following the OIE biosafety guidelines [12].

III. Result

Bacteriological results

Our results revealed that bacterial isolates were recovered from 83 out of the 115 collected pus samples (72.17%) with pure isolation were obtained in 68 cultures as shown in table 3.

Table 3: isolation incidence of bacterial isolates

Sheep			Goat		
Pure culture	Mixed culture	Total	Pure culture	Mixed culture	Total
53	9	62 out of 70	15	6	21 out of 45
		88.57%			46.66%

Briefly, *Pseudomonas aeruginosa* were isolated in a number of 12/62 (19.35%) from sheep and 5/21(23.81%) from goat while *Staphylococcus aureus* were obtained from 29/62 (46.77%) and 8/21 (38.09%) from sheep and goat respectively. Regarding *C. pseudotuberculosis* were recovered from 23/62 (37.09%) among sheep and 2/21(9.52%) from goat samples.

Isolation and cultural characterization of Pseudomonas spp. Staphylococcus spp. and C. pseudotuberculosis

The appearance of *Pseudomonas* spp. on MacConkey's agar was presented by non-lactose fermenter, pale, colorless, circular, smooth, mucoid colonies while appeared on blood agar, as white colonies with production of partial hemolysis as shown in figure 1a.

On the other hand, *Staphylococcus* spp. appeared on mannitol salt agar, as circular, smooth, yellowish colonies with the color of the medium changed from bright red to yellow. While, the microorganism appeared on blood agar as white to golden colony with production of β-hemolysis as shown in figure 1b. In similar, *C. pseudotuberculosis* isolates produced β-hemolytic transparent ring on blood agar 1c.

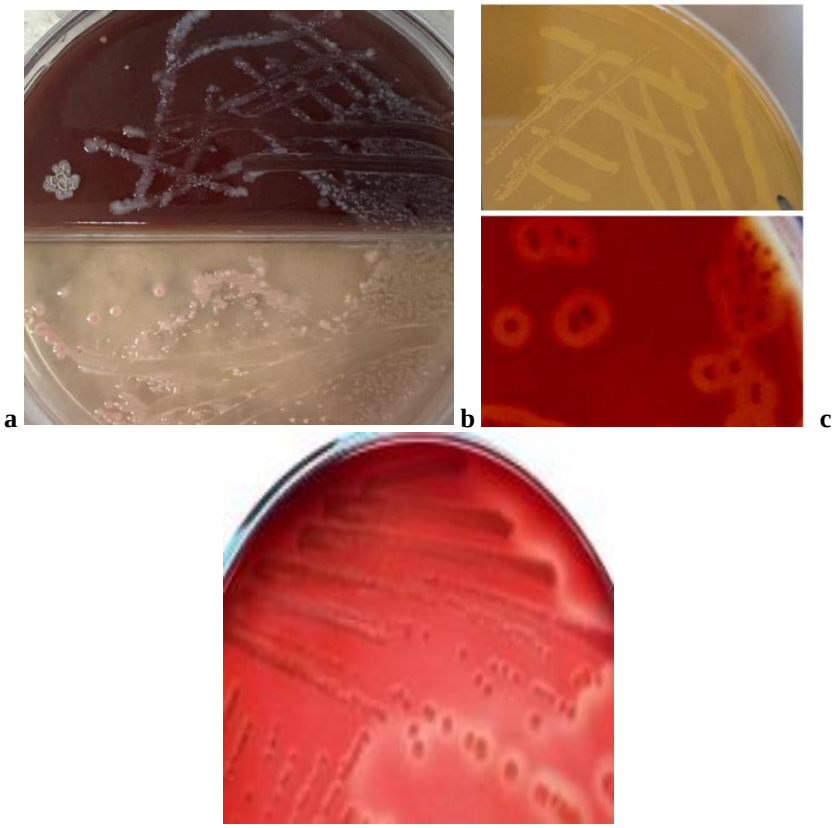


Figure 1: a) Colonial appearance of *Pseudomonas aeruginosa* isolates on MacConkey and blood agar, b) colonial appearance of *Staphylococcus aureus* isolates on Mannitol and blood agar. c) colonial appearance of *C. pseudotuberculosis* isolates on blood agar.

Identification of bacterial isolates by conventional and advanced biochemical methods

Pseudomonas spp. appeared as Gram -ve singly arranged short rod shaped pink colored organisms, glucose fermenter, and positive to oxidase, urease production and citrate utilization and negative to indole test. *Staphylococcus* spp. appeared as Gram +ve grape arranged violet colored, cocci shaped organisms, could ferment dextrose, maltose, lactose, sucrose and mannitol, positive to indole, catalase and coagulase production. Finally *C. pseudotuberculosis* appeared as Gram +ve singly arranged blue-violet rod-shaped, could also ferment dextrose but not ferment maltose, lactose and sucrose with positive production of urease and catalase. Advanced biochemical identification and confirmation of previous bacteria was achieved by Vitek 2 compact system, version: 9.02.

Antibiotic susceptibility testing

Our antibiotic sensitivity of *P. aeruginosa* result revealed that 10 out of 17 (58.82%) exposed multidrug resistance (MDR) pattern. The highest susceptibility was found to erythromycin (88.24%), amikacin (82.35%), 70.59% to ampicillin /sulbactam and cefoxitin. The isolates exposed intermediate sensitivity to azithromycin (52.94%) and levofloxacin (35.29%). The *P. aeruginosa* isolates were showed high resistance to amoxicillin /clavulanic acid and clindamycin (100%), ciprofloxacin (94.12%), norfloxacin and ofloxacin (88.24%) as shown in figures 2 and 3.

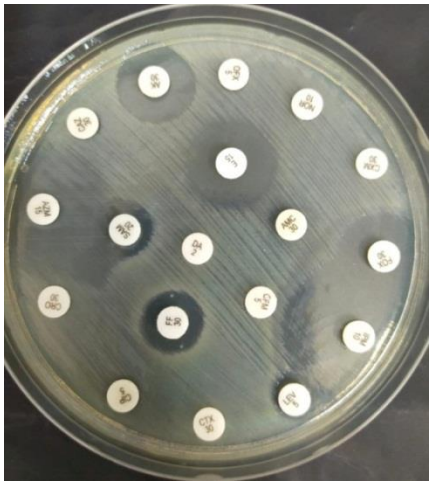


Figure 2: Antibiogram of the obtained *P. aeruginosa* isolates by standard disk diffusion on Mueller-Hinton agar.

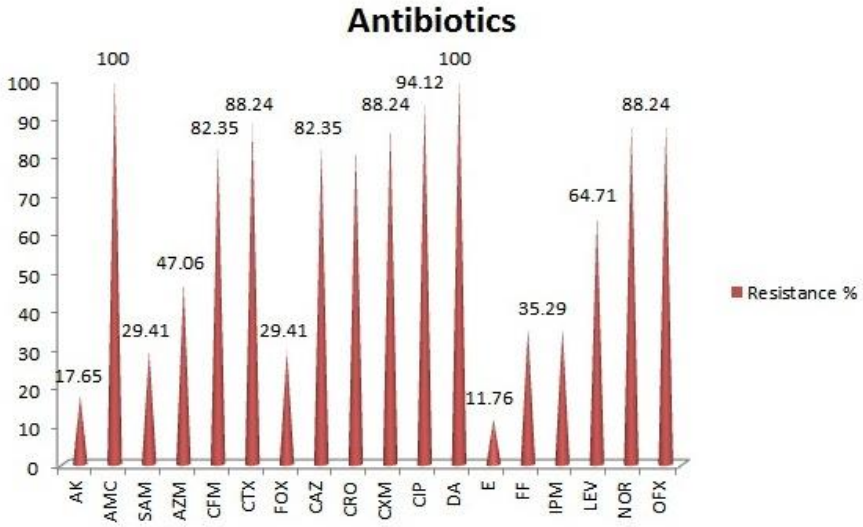


Figure 3: Antibiotic resistance of *P. aeruginosa* isolates

Concerning *S. aureus* isolates, it was found that 8 out of 37 (21.62%) act as MDR. The high sensitivity was determined to amikacin (89.19%) imipenem (81.08%) and clindamycin (78.38%). The intermediate resistance was obtained against cephalosporins by average of 56.76%, then amoxicillin/clavulanic acid (54.05%), azithromycin (44.40%) and erythromycin (35.13%). The highest resistance was observed against quinolones by average (89.19%) and ampicillin /sulbactam (75.67%) as shown in figure 4.

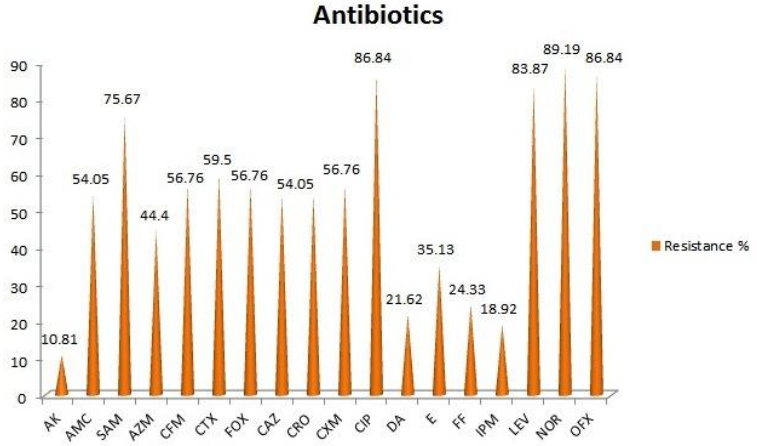
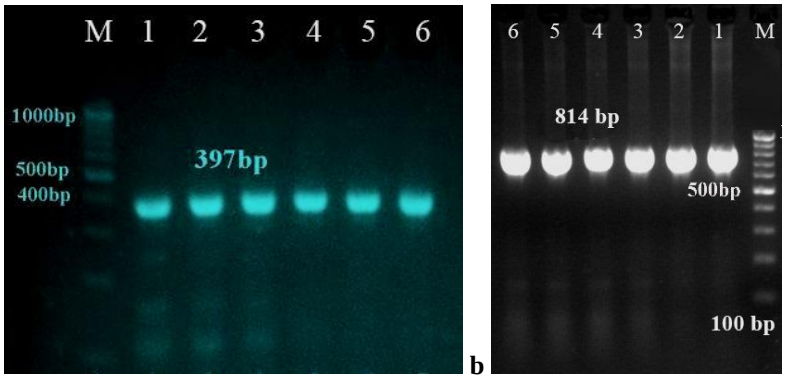


Figure 4: Antibiotic resistance of *S. aureus* isolates

Finally, all twenty five isolates of *C. pseudotuberculosis* were susceptible to the most of tested antibiotics especially cephalosporins (100%) followed by amikacin and ciprofloxacin 84% but moderate resistant to amoxicillin /clavulanic acid and clindamycin 56%.

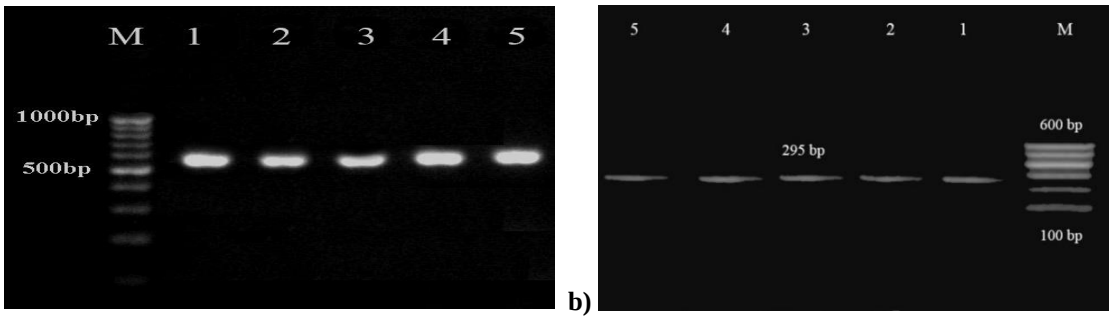
Molecular characterization

The results of our study revealed that five *P. aeruginosa* isolates from sheep and one from goat exposed amplification of virulence *toxA* gene at 397bp as shown in figure 5a, while eleven isolates; eight from sheep and three from goat were found to carry β-lactam resistance gene *blaOXA*-1as noticed in figure 5b. Regarding *S. aureus*; 5 isolates (four from sheep and one from goat) loaded *eta* virulence gene while 7 isolates (five from sheep and two from goat) carried *ermC* gene which responsible for erythromycin resistance as revealed in figure 6a and 6b. Finally it was shown that ten isolates of *C. pseudotuberculosis*; all belonged to sheep only harbored *pld* gene as shown at figure 7.



a b

Figure 5: Uniplex PCR of *P. aeruginosa* isolates a) detection of *toxA* virulence gene; lanes 1-6: positive amplification at 397 bp b) detection of antibiotic-resistance gene *blaOXA*-1; lanes 1-6: positive amplification at 814 bp. M: 100 bp DNA marker.



a) b)

Figure 6: Uniplex PCR of *S. aureus* isolates a) detection of *eta* virulence gene; lanes 1-5: positive amplification at 676 bp b) detection of antibiotic-resistance gene *ermC*; lanes 1-5: positive amplification at 295 bp. M: 100 bp DNA marker.

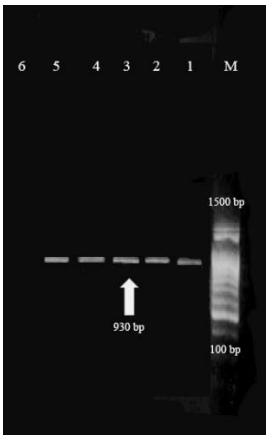


Figure 7: Uniplex PCR of *C. pseudotuberculosis* isolates; lanes 1-5: positive amplification of *pld* virulence gene at 930 bp. M: 100 bp DNA marker.

IV. Discussion

The present study focused on the isolation and identification of some pathogenic microorganisms that implemented in skin abscesses and pyogenic wounds among sheep and goats flocks.

The study was examined 115 pus samples (sheep≈70) (goat≈45) and resulted in successful bacterial isolation in 83 samples (72.17%) with pure cultures were recovered in 68 cultures while mixed insolation was obtained in 15 samples. Bacterial isolates were presented in 62 out of 70 sheep samples (88.57%) while 21 out of 45 goat samples (46.66%) were determined. The result of bacterial isolation was near of that recorded by Al-Harbi [1] who determined bacterial isolates in 80.83% of abscess samples obtained from sheep and goats at Qassim Region, Saudi Arabia. Our study focused on the top three microorganisms isolated.

Pseudomonas aeruginosa were isolated in a number of 12/62 (19.35%) from sheep and 5/21(23.81%) from goat. this results were higher than that obtained by Tapu Kumar et al [29] which revealed isolation of *P. aeruginosa* in 5% skin lesion for sheep and 15% for goat at Bangladesh. Al-Harbi reported an incidence of 6.18%, Also, Alkeshan, [30]**,** recorded an incidence of 6%. Dapgh et al. [18] determined 9.5% in sheep and 6.25% in goat with skin and wound infection in Egypt.

Our results revealed the isolation of *S. aureus* from sheep as 29/62 (46.77%) and goat 8/21 (38.09%). The high incidence of *S. aureus* infection was reported as 66.67% in semi-intensive and 53.33% in intensive farm Tapu Kumar et al [29]. In Khartoum, Sudan, it was found that the prevalence of *S. aureus* in live sheep 6.5% and 10.3% in slaughtered ones [31].

Regarding *C. pseudotuberculosis* were recovered from 23/62 (37.09%) among sheep and 2/21(9.52%) from goat samples. The rate of pure isolation of *C. pseudotuberculosis* was 27.84 % among inspected sheep and goat flocks in Qassim Region, Saudi Arabia [1]. In Ethiopia the incidences of *C. pseudotuberculosis* were 2.7% and 3.1% in young sheep and goats, respectively, while 24.4% and 27.5% in adult sheep and goats respectively [32].

The global concern of antimicrobial resistance was growing in last decades. The *P. aeruginosa* has recently emerged as a significant cause of healthcare-associated infections. The emergence of ESBL particularly Gram negative bacteria as *P. aeruginosa* has become a matter of grave issue for the therapy [33]. Previous studies declared that resistance to various β-lactam cephalosporins and ciprofloxacin was prevalent, while usually exposed sensitivity to amikacin and imipenem [34- 36].

Ferreira et al. [37] observed resistance to the ciprofloxacin and fluoroquinolones (50.0%), erythromycin (44.1%), clindamycin (35.3%) but showed sensitivity to amikacin (91.2%) with 44.1% of the isolates were MDR. Resistance to fluoroquinolones was prevalent (56.4%), intermediate towards ciprofloxacin and (14.5%) resistance to erythromycin and clindamycin [38]. Ramessar et al. [39] mentioned that all obtained *S. aureus* isolates exposed MDR to three or more antibiotic classes. The antibiotic resistance profiles were azithromycin 66.25%, amoxicillin/clavulanic acid 52.50%, erythromycin 40%. Intermediate resistance was noticed for clindamycin (53.75%). The isolates were sensetive to amikacin (95%), ciprofloxacin (91.25%), imipenem (90.00%), norfloxacin (75%).

In case of *C. pseudotuberculosis* susceptibility pattern, El Damaty et al. [40] discussed the antimicrobial susceptibility pattern of 54 *C. pseudotuberculosis* isolates obtained from smallholder sheep and goats suffered from caseous lymphadenitis in Egypt. Their data revealed 100% susceptibility to norfloxacin. The majority of isolates exposed high levels of resistance to erythromycin (92.6%), and cephradine (88.9%). In another study in Korea, it was found that most isolates were sensetive to ciprofloxacin, erythromycin, enrofloxacin, cefoxitin, cephalothin, whereas they were not sensetive to cefotaxime [41].

At molecular base, in detailed, five *P. aeruginosa* isolates from sheep (41.66%) and one from goat (20%) exhibited positive amplification of chromosomal virulence *toxA* gene which is responsible for production of exotoxin1; a potent extracellular virulence factor. Dapgh et al. [18] detected *toxA* gene in 8 isolates (61.5%) of examined sheep and goat, while Ganjo et al. [42] found the gene in 100% of isolates obtained from different clinical sources. It was noticed that *toxA* gene is associated to the highest existence of MDR isolates of *P. aeruginosa* harboring the ESBL genes [43].

Our data exhibited eleven isolates (64.7%); eight from sheep (75%) and three from goat (60%) were found to carry β-lactam resistance gene *blaOXA*-1. The total percentage was harmonized with the finding mentioned by Ganjo et al. [42] (63.8%) in isolates from clinical samples, (68.75%) in isolates from infectious wounds and burn [44], Tarafdar et al. (70.83%) in isolates from wounds [45].

Regarding *S. aureus*; 5 isolates (13.5%); four from sheep (13.8%) and one from goat (12.5%) loaded *eta* virulence gene. The global view exposes that *eta* is diverse and is broadly distributed on multiple genomic backgrounds. Azarian et al. [46] mentioned variant incidences in examined lineages of *S. aureus* isolated from skin infections in different European countries. Forty six out of 260 (17.69%) isolates were found to carry *eta*. Among 139 *S. aureus* isolated from clinical samples a number of 29 carried *eta* gene (20.89%) [47]. A high incidence of *eta* carriage (93.85 %) was reported in among 6145 *S. aureus* which recovered from patients with

Skin and soft tissue infections by Giormezis et al. [48]. In contrast a low incidence of *eta* gene was detected in one isolate (3.8%) among the 26 isolates obtained from skin infected patients in Korea [49]

Erythromycin was disclosed numerous decades ago, it was commonly used due to the great tissue penetration and good oral absorption. However, the resistance to macrolides including erythromycin is rapidly growing globally [50].

Our results revealed that 7 isolates (18.9%); five from sheep (17.24%) and two from goat (25%) carried *ermC* gene which responsible for erythromycin resistance. Many reports mentioned near results; Ferreira et al. [37] detected *ermC* gene in seven out of 34 isolates *S. aureus* recovered from skin and soft tissue infections (SSTIs) in ambulatory patients in Portugal. Another study determined *ermC* gene in fifty-five *S. aureus* in examined SSTIs animals by incidence of (14.5%) [38]. Contrarily, high prevalence of *ermC* gene existence was also reported; an incidence of 62.50% was detected among eighty isolates which obtained from treated wastewater and surface waters in in Durban, South Africa [39].

Finally it was shown that ten isolates; all belonged to sheep only harbored *pld* gene (43.48%). This gene encodes a protein enzyme termed phosphatidylcholine-specific phospholipase which believed to play a main role in *C. pseudotuberculosis* virulence and pathogenesis in the host [51]. Our result was greatly agreed with that mentioned by Magdy Selim et al. [52]; (48.83%) among examined infected sheep in Dakahlia governorate, Egypt. A hundred percent existence of *pld* gene was reported by El Damaty et al. [40] among one hundred and thirty sheep and ninety goats from thirty nine smallholder flocks in Egypt. Also, an incidence of 97.13% (338/348) was determined in clinical isolates of C. *pseudotuberculosis* from different hosts [53].

V. Conclusion

It may be concluded that *Pseudomonas aeruginosa*, *Staphylococcus aureus* as well as *Corynebacterium pseudotuberculosis* frequently implemented in primary and secondary infection of skin in sheep and goat in Egypt. The isolated species were successfully asserted using cultural, conventional and advanced biochemical tests. Antibiogram profile of *P. aeruginosa* isolates exposed the high resistance to amoxicillin /clavulanic acid, clindamycin, and quinolones. The isolates were moderately sensitive to azithromycin. Antibiogram profile of *Staphylococcus aureus* isolates showed the high resistance to quinolones and ampicillin /sulbactam, moderately susceptible to cephalosporins, amoxicillin/clavulanic acid, azithromycin and erythromycin. Regarding *C. pseudotuberculosis* isolates were found to be moderate resistant to amoxicillin /clavulanic acid and clindamycin. Amikacin and imipenem were the most effective antibiotics used against the obtained isolates. PCR assays revealed that the isolates carried virulence and antibiotic resistance genes in considerable percentages.

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