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# Effectiveness of Disinfectants on Environmental Multidrug Resistance Contaminants Causing Skin Abscess in Farm Animals

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**Abstract:** Resistance to antimicrobial agents is recognized as a growing problem for different farms and infection of farm animals with abscesses is detrimental to livestock due to the enormous economic losses. This study aimed to find out the bacterial infection of some cases in farm animals suffering from wounds and abscesses in Fayoum Governorate, Egypt and Related Antimicrobial Resistance (AMR) procedures and technical investigations focused on disinfectant effects or the effects of AMR on bacterial persistence. The samples were collected from 130 different animals (24 buffaloes, 6 cows, 35 sheep, 40 goats, 10 camels and 15 donkeys). By bacteriology examination, eight isolates (33.3%) of *Corynebacterium pseudotuberculosis biovarequi* (*C. equi*) was isolated only from buffalo with edematous skin lesions (OSD), while *Corynebacterium pseudotuberculosis biovar ovis* (*C. ovis*) were isolated from sheep and goats with caseous lymphadenitis lesions in a rate 28.6% and 27.5% respectively. On susceptibility test against antimicrobial agents. Pipracillin-tazablam was the best of choice for *Pseudomonas aeruginosa* and *C. equi* (20%).The result showed the bacterial growth at OD595 after treatment with Povidone-iodine (PI) 10% at different time intervals, where all types of isolates showed the lowest growth after 24 h, *C. ovis* showed the lowest bacterial growth, while the *C. equi* showed the lowest bacterial growth with $H_2O_2$1%. Conclusion: Caseous lymphadenitis remains an important challenge for the sheep and goat industries. *C. equi* also plays a role in buffalo raring and caused edematous skin disease which affects milk and meat production. The high resistance of both *C. ovis and C. equi* to antibiotics and disinfectants reflected the necessity to adopt control measures. MRSA, as well as PDR *Pseudomonas aeruginosa*, were observed animal wounds which prove the bad hygiene. Increasing contact time is more effective against microbes.

**Keywords:** Environmental Contamination, Abscess, Farm Animals, Multidrug Resistance Bacteria, Disinfectants

## Introduction

Infection of farm animals with abscesses is detrimental to livestock due to the enormous economic losses to animals, meat, milk production, skin and wool production accompanying this affection. Mostly, the distribution of wound is the normal continuity of body structures by physical means (Devrajani *et al*., 2010). Wounds were identified into three types: 1- Incision wounds, which occur due to sharp tools and if there is accompanying tissue that tears the wound, it is known as a torn wound.2- Abrasions, which are defined as damage to the surface of the skin due to friction and are characterized by superficial bruises and a loss of varying thickness of the superficial and subcutaneous layers3- Puncture wounds, which are open injuries in which foreign substances and organisms are likely to enter deep into the underlying tissues. Timothy and Bruno (2021) classified wounds to four classes according to the cleanliness and condition of wounds class (1) wounds are clean, class (2) wounds are

clean and contaminated, class (3) wounds are be contaminated and class (4) dirty infected wounds. They often engage in long hours and when released, they are left to surf and feast on the less nutritious litter. This has been linked to a potential negative impact, particularly on their well-being and health (Mekuria and Abebe, 2010; Bogale *et al*., 2012)

Donkeys (Equusasinus) are among the early domesticated equines that have been a heavy beast for thousands of years. There are currently about 44 million donkeys distributed all over the world. In Africa, the donkey population is considered by 13.7 million (Gichure *et al*., 2020). Despite increasing mechanization worldwide, donkeys still feature prominently in the farming systems of numerous developing countries. (Shrikhande *et al*. 2009; Molla and Gondar 2015). The donkey are still one of the most underappreciated animals and an important project, as it plays a major role in the agricultural economy of the developing world involving Africa (Ahmed *et al*., 2008). Donkey skin wounds are a proper habitat for the growth of microorganisms especially in saddle ulcers because they accelerate the risk of infection by providing ideal conditions for the growth and reproduction of some microorganisms (Devrajani *et al*., 2010). Caseous lymphadenitis in sheep and goats is a chronic and subclinical disease all over the world, presenting high animal and flock prevalence's. Pseudo cystic tuberculosis, affects sheep and goats and can also infect cattle and horses and rarely in humans; so, it is considered an occupational animal disease. The microorganism was isolated from other species, including pigs, buffaloes, deer, porcupines, llamas, camels and laboratory animals (Dorella *et al*., 2006. In Assiut Governorate-Egypt, *C. equi*and *C. ovis* were isolated by 72% and 28% respectively from buffaloe and cows (Arafa *et al*., 2019).

Corynebacterium pseudotuberculosis is categorized by two biovars (Sellyei, *et al*., 2017) the biovar Ovis, which at most affects goats and sheep, causes superficial and visceral abscesses, mainly affects horses, causes ulcerative lymphangitis of the abdominal abscesses in the chest and abdomen, distal extremities and hematomas. The presence of these two types of biovar has been assured by bimolecular techniques (Connor *et al*., 2007). Caring for animals in veterinary clinics leads to an increased risk rate of MDR-organisms (MDROs) (Schmidt *et al*., 2020)

Antimicrobial resistance is an increasing problem for both humans and veterinarians and the issue that needs to be addressed in both related areas is a current policy priority. Attempts to decrease Antimicrobial Resistance (AMR) on farms have yet centered on controlling the supply and utilization of antimicrobial drugs. (Robert and Andrew, 2019).

The aim of this study is to find out the bacterial infection of some cases in farm animals suffering from wounds and abscesses in Fayoum Governorate, Egypt and related Antimicrobial Resistance (AMR) procedures and technical investigations focused on disinfectant effects or the effects of AMR on bacterial persistence

## Materials and Methods

### *Samples and Sampling*

The study was carried out in villages of Fayoum Governorate, Egypt during the period from march 2020 to march 2021. The owners of the studied animals are individual farmers every farmer had one or more species from cows, buffaloes, sheep, goats, donkeys and camel. The farmers are mainly keeping their animals with each other in the back yard of their house.

The samples were collected from 130 different animals (24 buffaloes, 6 cows,35 sheep, 40 goats, 10 camels and 15 donkeys). The sample from buffaloes were collected from edematous skin, 6 swabs from cows with eye pus, that from sheep, goats and camels were collected from abcessiated and caseating lymph nodes and that from donkeys were obtained from wounds at necks.

The study was designed as follow:

a. Swabs samples were collected from the studied farm animals suffer from abscesses and wound infection
b. All samples were submitted for isolation of bacteria followed by a susceptibility test for different antimicrobial agents
c. Study the effect of the used two disinfectants (Povidone-iodine (PI) 10 and $H_2O_2$ 1%) on bacterial isolates and evaluation of disinfectant activity on Each Test Isolate by Time Killing assa

### *Bacteriology Examination*

All samples from lymph nodes and edematous skin lesions were inoculated on trypticase soy broth (TSB) and incubated for 37C for 24 h (Jones and Collins, 1986).

All samples were cultured on duplicated blood agar (incubation at 37؛C for 24 to 48h in complete aerobic conditions supported with 10% $CO_2$), MacConkey agar, Pseudomonas agar, mannitol salt agar (incubation for 24h at 37°C) (Quinne *et al*., 2002)

All suspected colonies were put forward for Gram stainingand different biochemical identification tests (Quinne *et al*., 2002).

β hemolytic colonies with golden yellow or pink on mannitol, Gram-positive grapes like cocci were examined for catalase test, oxidase and confirmed the identification by using S.R.O GP24 for identification of *Staphylococcus* spp. and *Streptococcus* spp.

**Table 1:** List of antimicrobial disks used

| Name of antibiotic | Code of disc | Conc/µg |
|---|---|---|
| Penicillin | P | 10 |
| Oxacillin | OX | 1 |
| Ampicillin | AMP | 10 |
| Ampicillin-sulbactam | SAM | 20 |
| Ampicillin -clavulanic acid | AMC | 30 |
| Piperacillin-tazobactam | TZP | 110 |
| Cephalexin | CL | 30 |
| Cephradine | CE | 30 |
| Cefotaxime | CTX | 30 |
| Cefoperazone | CFP | 75 |
| Meropenem | MEM | 10 |
| Aztreonam | ATM | 300 |
| Clarithromycin | CLR | 10 |
| Erythromycin | E | 15 |
| Oxytetracycline | OT | 30 |
| Chloramphenicol | C | 30 |
| Norfloxacin | NO | 10 |
| Ofloxacin | OFX | 5 |
| Lomefloxacin | LOM | 10 |
| Kanamycin | K | 30 |
| Novobiocin | NV | 30 |
| Amikacin | AK | 30 |
| Linezolid | LZD | 30 |
| Clindamycin | DA | 2 |

Hemolytic colonies and non-hemolytic creamy in color on blood agar and in trypticase soy broth, it forms a surface film, though the culture remains clear; this film is broken by agitation, forming flakes were submitted for catalase, oxidase and confirmed for Corynebacterium species by S.R.O GP24.

Blueish green colonies on *P. eudomonas* agar, Gram-negative small bacilli, were examined for oxidase test and confirmed identification for *P. aeruginosa* by using S.R.O. GN24.

### *Susceptibility Disc Diffusion Test Against Different Antimicrobial Agents*

In relation to the characters of antimicrobial agent used were listed in Table (1). Each culture tested is plotted on a non-inhibitory agar medium (brain heart infusion agar, blood agar or try ptone soy agar) After incubation at 35°C overnight, 4 or 5 well-isolated colonies were selected with an inoculating needle or loop and Transfer the broth and vortex Mueller-Hinton completely, incubate the broth at 35°C until turbid, then adjust the turbidity to the appropriate density by comparing the standard 0.5 McFarland tube. During 15 min after checking the turbidity of the inoculum suspension,

A sterile cotton swab dipped in suspension. Pressing firmly on the inner wall of the tube just above the liquid level. The swab was trimmed over the entire surface of the medium three times, rotating the plate about 60° after each application to ensure even distribution of the inoculum.

Antimicrobial discs were placed on plates as soon as possible, but not more than 15 minutes after inoculation. Place the discs individually with sterile forceps, then gently tap the agar. After the plate was inverted and incubated at 35°C for 16 to 18 h (CLSI, 2012). The diameter of the zones of complete inhibition was measured (including the diameter of the disk) and recorded in millimeters. The measurements were made with a ruler on the undersurface of the plate without opening the lid according to CLSI (2017).

### *Evaluation of Disinfectant Activity on Each Test Isolate by Time Killing Assay*

Ten of each isolate except *C. equi* (8 isolates) working inoculum was prepared by diluting overnight cultures with sterile water to match their turbidity to the 0.5 McFarland standard (*A*595 0.06–0.08) which contained approximately 1-2 × *$10^8$ CFU/$mL^{-1}$ (NCLS 1996, Jorgensen *at al*., 1999). The McFarland 0.5-turbidity bacterial suspensions were additional diluted with Muller Hinton Broth (MHB) to obtain 1 × *$10^6$for antibacterial assays. Povidone-iodine(PI) 10% as a broad antiseptic for external use of animals and $H_2O_2$ 1% in water for routine use used in this study.

An aliquot of bacterial suspensions (1 mL) prepared as described above (1 × *$10^6$CFU/mL) was incubated with 1mL of the disinfectant agents alone (PI and $H_2O_2$), at 37°C. A sample of 100 µL was taken every 4 h up to 24 h and subsequently measured for the bacterial growth by reading the OD595 nm (Chusri *et al*. 2014).

## Results and Discussion

Eight isolates (33.3%) of *C. equi*was isolated only from buffalo with edematous skin lesions (OSD), while *C. ovis*were isolated from sheep and goats with caseouslymphadenitis lesions at a rate of 28.6% and 27.5% respectively (Table 2). The isolates were nonhemolytic cream-colored colonies on sheep blood agar, in trypticase soya broth, the surface film is formed, though the culture still clear; this film is broken by agitation, forming flakes, the isolates were catalase positive and oxidase negative and confirmed identified by S.R.O. GP24.

In Egypt, (Arafa *et al*., 2019) showed that serotype II (*C. equi*) was the cause of OSD in buffalo. The reason why buffalo is not affected by the nitrate-negative serotype I*(C. ovis)* organism under natural conditions is unknown.

*Corynebacterium pseudotuberculosis* (*C. ovis*) is a common cause of the bacterial disease caseous lymphadenitis which occurs in goats, sheep, cattle, buffalo and horses. The morphological and biochemical characteristics Nitrate reductase production was determined the identification of *C. pseudotuberculosis* was used by Sellyei *et al*. (2017) to distinguish the equi biovar (isolated from horses and cattle; nitrate reduction

positive) from the ovis biovar (isolated from sheep and goats; nitrate reduction negative), Dorella *et al*. (2006).

The occurrence of caseous lymphadenitis in Egypt can increase the participation of goats and sheep in the national animal husbandry and its relationship to the effect of this disease. The economic losses include low milk production, loss weight gain, decreased value of skins due to scarring and the high value of treatment of superficial abscesses. When animals affected in critical areas of lymph nodes (jaw, groin area, udder) the losses were increased (Guimarães *et al*., 2011; Osman *et al*., 2018) .

The highest rate of *Staphylococcus aureus (S. aureus*) isolates was observed in the wound of donkeys and eye lesion of cows (66.7%) followed by camel 60%; goats 22.5% and it was nearly similar in sheep and buffalo (8.6 and 8.3% respectively) Tiwari, (2016) recorded the highest rate of *S. aureus* isolation was from equine wound followed by cattle and buffalo. The isolates were characterized by β hemolysis on blood agar, golden - yellow colonies on mannitol agar, Gram-positive grapes like colonies and confirmed by S.R.O. GP24.

According to the above definitions, S. aureus can be classified as an opportunistic disease for both human and animals. The composition of natural flora depends on the organism on the species, forage and the environment, including population density. Nevertheless, *S. aureus* is the most frequently isolated coagulase-positive Staphylococcus (CPS) from the anterior nares and temporarily from the skin of humans and animals. (Bierowiec *et al*., 2016).

On the other hand, the total isolates of *P. aeruginosa* were 32 (24.6%) this result is nearly similar to Tiwari (2016). *P. aeruginosa* was highly isolated from donkeys followed by sheep, cows, goats, camel and buffalo in a rate of 80; 33.3; 31.4; 255; 20 and 8.3% respectively. The isolates were bluish-green on pseudomonas specific media, oxidase-positive and confirmed by S.R.O. GP24. Saha *et al*. (2019) reported the rate of *P. aeruginosa*was 15% for cattle and goats and 5% for sheep. Devrajanid *et al*. (2010) isolated *P. aeruginosa* and *S. aureus* from wounds in camel at a rate of 10.52%.

On a susceptibility test against antimicrobial agents (Table 3), Piperacillin-tazablam was the best of choice for *P. aeruginosa* and *C. equi* (20%). Many of the members of the Enterobacteriaceae, including Klebsiella spp. and Pseudomonas Are Inhibited by Piperacillin. It is given by intramuscular or intravenous injection and is widely distributed in body fluids and tissues. Like other newer penicillins, it's been accredited for serious infection caused by susceptible strains of specific organisms in intra-abdominal, urinary tract, gynecologic, decrease respiratory tract, skin structure, bone and joint and gonococcal infections and septicemia (Young and Plosker, 2001). It was found that *P. aeruginosa*is resistant to the rest of the antibacterial agents. *P. aeruginosa* is intrinsically resistant to many antimicrobial classes because of the presence of numerous efflux pumps in its cell wall and cell membrane. Upregulation of those efflux pumps results in resistance to the limited range of effective agents; *P. aeruginosa* is widely recognized for its capacity to become resistant during treatment. It also can become resistant to β-lactams viaporin loss and the purchase of β-lactamases (AURA, 2019).

Methicillin-resistant *S. aureus* (MRSA) isolates that showed resistance to multiple drug resistance to a high number of antibacterial agents as penicillins, methicillin (oxacillin), β-lactam; macrolides and aminoglycosides antibiotics. In the present study, *S. aureus* was resistant to all classes of antibiotic classes used except phencols and fluoroquinolones were they sensitive at a rate of 13.3% to lomefloxacin and 6.7% to chloramphenicol (Table 3). Chloramphenicol is an artificial broad-spectrum antibioticIt is a rarely used drug for its known severe adverse effects and it was indicated to be used superficially as bacterial conjunctivitis (Oong and Tadi 2020). Tiwari *et al*. (2016) reported that MRSA was isolated from wounds of farm animals and accomplice animals.

The Povidone iodine is a beneficial preoperative decolonizing agent for the prevention of S. aureus infections which includes MRSA. Lepelletier *et al*. (2020). Iodine and iodophorsarea highly virucidal, bactericidal, tuberculocidal, sporicidal and fungicidal. Although aqueous or alcoholic (tincture)solutions of iodine are related to irritation and excessivestaining and aqueous solutions are generally unstable. Overcome these problems by developing iodine carriers. Iodophors Consists of complexes of iodine and a solubilizing agent, which acts as a pool of the active “free” iodine (Hobosyan *et al*., 2012). Hydrogen peroxide ($H_2O_2$) is an external antiseptic used in wound cleaning which kills pathogens by oxidation eruption and local oxygen production Zhu *et al*. (2017).

In the present study, Fig. (1) showed the bacterial growth at OD595 after treatment with PI 10% at different time intervals, all types of isolates showed the lowest growth after 24 h, *C. ovis* showed the lowest bacterial growth. Figure (2) showed the bacterial growth at OD595 after treatment with $H_2O_2$1% at different time intervals, also types of isolates showed the lowest growth after 24 h, *C. equi* showed the lowest bacterial growth.

The presence of Bacterial growth in all treated isolates till exposure to treatment for 24 h proved the tolerance of these clinical isolates which ranged from 75-30% in treatment with PI and from70-50 in treatment with $H_2O_2$

(Table 4). Show *C. equi and C. ovis* highest for tolerance to PI (75&0% respectively), while *S. aureus* showed the highest rate of tolerance to $H_2O_2$ (70%). These results agree with Aksoy *et al*. (2020). Who reported that $H_2O_2$ is the strongest disinfectant followed by glutaraldehyde and iodine when exposed to 3 types of salmonella. The killing activity of disinfectants is depends upon 3 factors including the concentration of treatment, temperature and treatment period also, from our opinion concentration of clinical isolates plays a role.

Antibiotic resistances are classified into Multidrug - Resistant (MDR) which isn't susceptible to at least one representative from every of three classes of selected antimicrobial compound families (El Zowalaty *et al*. 2015; Fodor *et al*., 2020). Extreme or extensively Drug-Resistant (XDR) is not susceptible to at least a single representative of all but very few classes of antimicrobial compound families. Pan-drug resistant (PDR) is not susceptible to any of the tested or empirical representatives of all acknowledged antimicrobial compound families (El Zowalaty *et al*., 2015). MDR and PDR isolates are inconsistent in medical literature, disqualifying reliable comparison of data. To reach a standardized definition, we applied the multidrug resistance definition from human medicine (Magiorakos *et al*., 2012). This adaption was limited by the unattainability of certain susceptibility results and differing antimicrobial agents in human and veterinary medicine. Therefore, the establishment of a standard definition of MDR bacteria in veterinary medicine should be supported.

The prevalence, phenotypic resistance pattern and diversity of the four clinical isolates (randomly 10 isolates of each strain except 8 *C. equi* isolates were used) are recorded in (Table 5). They were tested for their resistance phenotypic profile against 24 antibiotics representing 9 classes. This diversity of both Gram-ve bacteria and Gram +ve bacteria isolated from skin lesions reflect the capacity of AMR revealed the haphazard and extensive use of antibiotics which has led to the emergence and extent spread of resistant pathogenic bacteria (Wolska *et al*. 2012; Garza-Cervantes *et al*., 2020). Also, Table (5) showed the tolerance of each isolate to PI and H2O2, where most PDR isolates showed tolerance to both PI and H2O2. The risk of MDR and PDR strains on antiseptic and disinfectants effect was observed in this study (Fig. 3). Although antiseptics have broader spectrums of antimicrobial activity than antibiotics and a much lower risk of selecting bacterial resistance and Lachapelle *et al*. (2013) pronounced that antiseptics are consequently suitable alternatives to antibiotics for the control of localized superficial skin infections. George (1996) provides numerous examples of MDR systems in which an operon or gene is associated with changes in antiseptic or disinfectant susceptibility. Also, Mycock (1985) said that MRSA strains showed a remarkable increase in intolerance (at least 5,000-fold) to povidone-iodine.

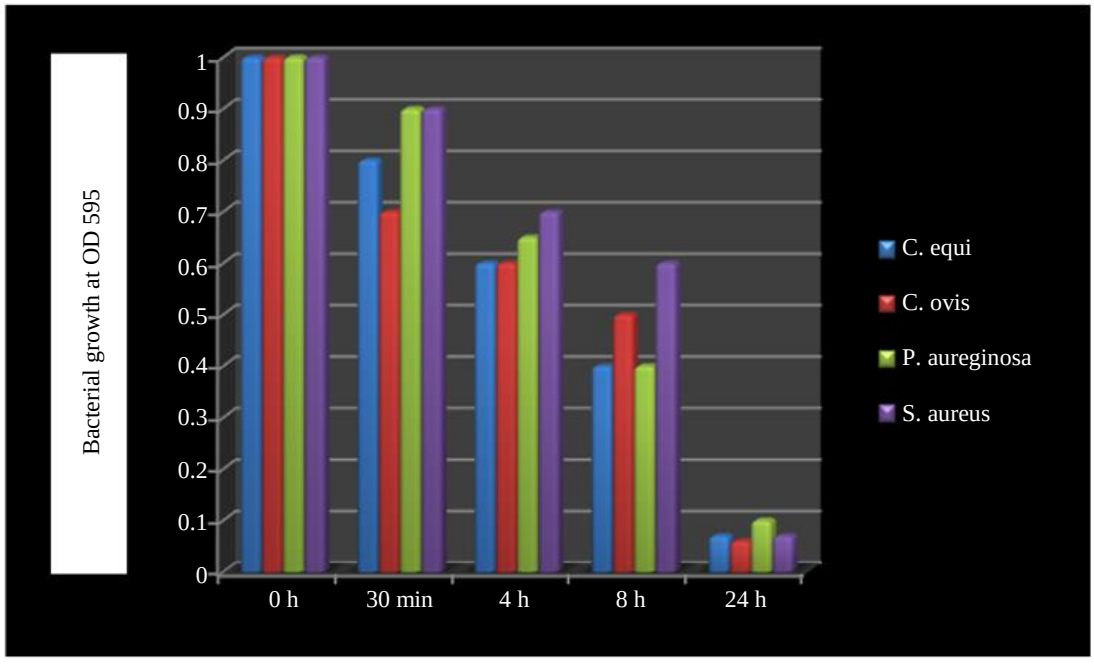


**Fig. 1:** Bacterial growth of isolates after treatment with PI 10% using time killing assay

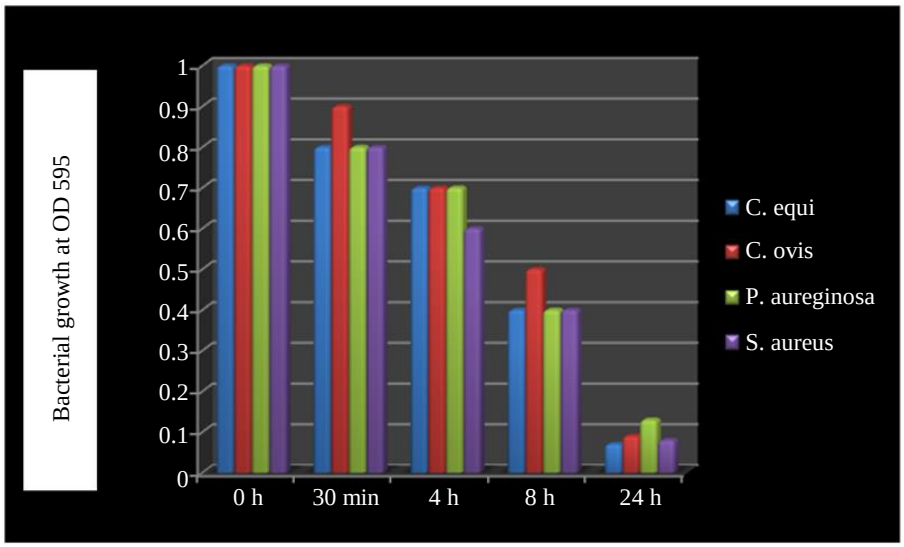


**Fig. 2:** Bacterial growth of isolates after treatment with H2O2 1% using time killing assay

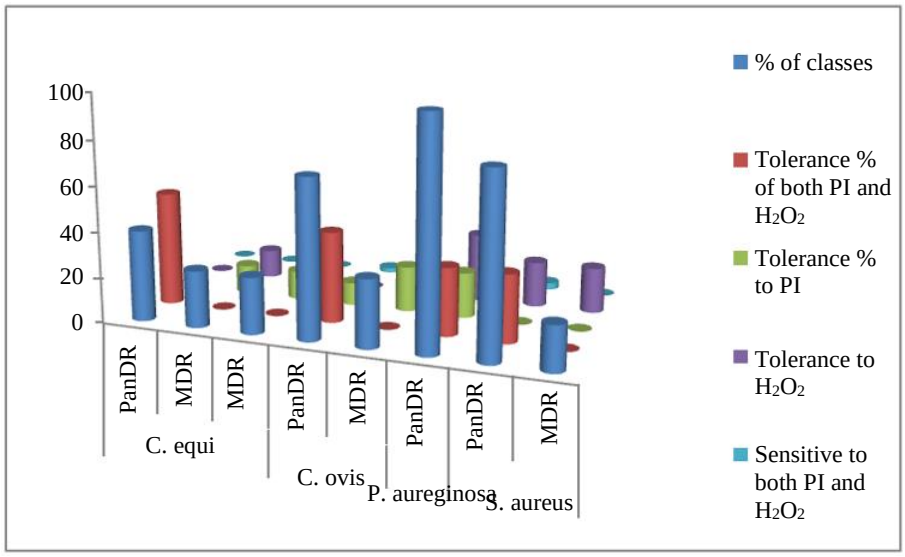


**Fig. 3:** The relationship between type of antimicrobial resistance patterns and tolerance to PI and $H_2O_2$

**Table 2:** Rate of different isolates obtained from different animal species

| Source of samples | Type of samples | No. of samples | Type of isolates *C. equi* No. | *C. equi* % | *C. ovis* No | *C. ovis* % | *S. aureus* No. | *S. aureus* % | *P. aerugenosa* No. | *P. aerugenosa* % |
|---|---|---|---|---|---|---|---|---|---|---|
| Buffalo | Edematous skin | 24 | 8 | 33.3 | 0 | 0 | 2 | 8.3 | 2 | 8.3 |
| Cows | Eye swab | 6 | 0 | 0 | 0 | 0 | 4 | 66.7 | 2 | 33.3 |
| Sheep | L.n | 35 | 0 | 0 | 10 | 28.6 | 3 | 8.6 | 11 | 31.4 |
| Goat | L.n | 40 | 0 | 0 | 11 | 27.5 | 9 | 22.5 | 10 | 25 |
| Camel | L.n | 10 | 0 | 0 | 2 | 20 | 6 | 60 | 2 | 20 |
| Donkeys | neck wounds | 15 | 0 | 0 | 0 | 0 | 10 | 66.7 | 5 | 80 |
| | Total | 130 | 8 | 6.2 | 23 | 17.7 | 34 | 26.2 | 32 | 24.6 |

**Table 3:** Susceptibility test results against different antibiotic agents

| | *P.aeruginosa* * | | | *S.aureus* | | | *C.equi* | | | *C.ovis* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Antibacterial agents | S | I | R | S | I | R | S | I | R | S | I | R |
| Penicillins | | | | | | | | | | | | |
| Penicillin | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 25 | 75 | 26.7 | 0 | 73.3 |
| Oxacillin | 0 | 0 | 100 | 0 | 0 | 100 | - | - | - | - | - | - |
| Ampicillin | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 |
| β-Lactam/β-Lactamase Inhibitor Combinations | | | | | | | | | | | | |
| Ampicillin-subactam | 0 | 26.7 | 73.3 | 0 | 26.7 | 73.3 | 0 | 0 | 100 | 0 | 0 | 100 |
| Ampicillin -clavulanic acid | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 |
| Pipracillin-tazablam | 20 | 0 | 80 | 0 | 0 | 100 | 20 | 0 | 80 | 0 | 0 | 100 |
| Cephems | | | | | | | | | | | | |
| Cephalexin | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 |
| Cephradine | 0 | 20 | 80 | 20 | 0 | 80 | 0 | 0 | 100 | 0 | 0 | 100 |
| Cefotaxam | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 |
| Cefoperazon | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 |
| Cefquinome | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 |
| *Monobactam* | | | | | | | | | | | | |
| Meropenem | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 |
| Aztreonam | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 |
| Macrolides | | | | | | | | | | | | |
| Clarithromycin | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 |
| Erythromycin | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 |
| Tetracyclines | | | | | | | | | | | | |
| Oxytetracyclin | 0 | 0 | 100 | 0 | 26.7 | 73.3 | 8.3 | 0 | 91.7 | 0 | 0 | 100 |
| Phenicols | | | | | | | | | | | | |
| Chloramphenicol | 0 | 0 | 100 | 6.7 | 0 | 93.3 | 0 | 0 | 100 | 0 | 0 | 100 |
| Fluoroquinolones | | | | | | | | | | | | |
| Norfloxacin | 0 | 0 | 100 | 0 | 26.7 | 73.3 | 0 | 0 | 100 | 0 | 0 | 100 |
| Ofloxacin | 0 | 0 | 100 | 0 | 26.7 | 73.3 | 0 | 0 | 100 | 0 | 0 | 100 |
| Lomofloxacin | 0 | 0 | 100 | 13.3 | 0 | 86.7 | 0 | 0 | 100 | 0 | 0 | 100 |
| Aminoglycosides | | | | | | | | | | | | |
| Kanamycin | 0 | 0 | 100 | 0 | 20 | 80 | 0 | 0 | 100 | 0 | 0 | 100 |
| Novobiocin | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 |
| Streptomycin | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 |
| Neomycin | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 100 |
| Amykacin | 0 | 0 | 100 | 0 | 26.7 | 73.3 | 0 | 0 | 100 | 0 | 0 | 100 |
| Oxazolidinones | | | | | | | | | | | | |
| Linezolid | 0 | 0 | 100 | 0 | 0 | 100 | 33.3 | 0 | 66.6 | 40 | 0 | 60 |
| Lincosamides | | | | | | | | | | | | |
| Clindamycin | 0 | 0 | 100 | 0 | 6.7 | 93.3 | 0 | 0 | 100 | 0 | 0 | 100 |

*Results were recorded in percentage (-): Not applied S: Sensitive I: Inermediete R: rESISTENCE

**Table 4:** The rate of tolerance of different isolates to disinfectants

| | *C. equi* | | *C. ovis* | | *P. aeruginosa* | | *S. aureus* | |
|---|---|---|---|---|---|---|---|---|
| Disinfectant | No. | % | No. | % | No. | % | No. | % |
| PI 10% | 6 | 75 | 7 | 70 | 5 | 50 | 3 | 30 |
| $H_2O_2$ 1% | 6 | 60 | 5 | 50 | 6 | 60 | 7 | 70 |

**Table 5:** Risk antimicrobial resistance types with tolerance to disinfectants

| Strain | No. of isolate | No. of Resistance of different antibiotic | Antimicrobial resistance | No. of classes (9) | Type of resistance | Tolerance to PI | Tolerance to $H_2O_2$ |
|---|---|---|---|---|---|---|---|
| *C.equi* | 1 | 24 | P,0X.AMP. SAM.AMC,TZP, CL,CE,CTX , CFP, MEM,AZ, CLR,E,OT, C,NO,OF, LOM,K,NOV, AK,LIN,CD | 9 | PDR | + | + |
| | 2 | 22 | P,0X.AMP. SAM.AMC,CL, CE,CTX,CFP, , MEM,AZ,CLR, E,C,NOR, OF,LOM,K, NOV,AK,LIN, CD | 8 | MDR | - | + |
| | 3 | 23 | P,0X.AMP,SAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK, CD | 8 | MDR | + | - |
| | 4 | 24 | P,0X.AMP. SAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK,LIN,CD | 9 | PDR | + | + |
| | 5 | 24 | P,0X.AMPSAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK,LIN,CD | 9 | PDR | + | + |
| | 6 | 20 | P,0X.AMP .SAMAMC,CT, CR,CF,CQ, M,AZ,CLR, E,C,NOR, OF,LOM,K, NOV,AK | 7 | MDR | - | + |
| | 7 | 20 | P,0X.AMP. SAM.AMC,CT, CR,CF,CQ, M,AZ,CLR, E,C,NOR, OF, LOM,K, NOV,AK | 7 | MDR | + | - |
| | 8 | 22 | P,0X.AMP. SAM.AMC,PIP/TAZ, CT,CR,CF, C, CLR,E, OXT,C,NOR, OF,LOM,K, NOV,AK,LIN, CD | 9 | PDR | + | + |
| *C.ovis* | 1 | 24 | P,0X.AMP.SAM.AMC,PIP/TAZ,CT,CR,CF,CQ,M,AZ, CLR,E,OXT,C,NOR,OF,LOM,K,NOV,AK,LIN,CD | 9 | PAN | + | - |
| | 2 | 24 | P,0X.AMPSAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK,LIN,CD | 8 | MDR | + | - |
| | 3 | 24 | P,0X.AMP. SAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK,LIN,CD | 9 | PDR | + | + |
| | 4 | 23 | P,0X.AMP. SAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK, ,CD | 8 | MDR | + | - |
| | 5 | 24 | P,0X.AMP. SAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK,LIN,CD | 9 | PDR | + | + |
| | 6 | 24 | P,0X.AMP. SAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK,LIN,CD | 9 | PDR | - | - |
| | 7 | 24 | P,0X.AMP. SAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK,LIN,CD | 9 | PDR | + | + |
| | 8 | 24 | P,0X.AMP. SAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK,LIN,CD | 9 | PDR | + | + |
| | 9 | 23 | P,0X.AMP. SAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK, ,CD | 8 | MDR | - | - |
| | 10 | 24 | P,0X.AMP. SAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK,LIN,CD | 9 | PDR | - | - |
| *P.aeruginosa* | 1 | 24 | P,0X.AMP. SAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK,LIN,CD | 9 | PDR | - | + |
| | 2 | 24 | P,0X.AMP,SAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK,LIN,CD | 9 | PDR | + | + |
| | 3 | 23 | P,0X.AMP. SAM.AMC,CT, CR,CF,CQ, M,AZ,CL, E,OXT,C, NOR,OF,LOM, K,NOV,AK, LIN,CD | 9 | PDR | - | - |
| | 4 | 24 | P,0X.AMP. SAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK,LIN,CD | 9 | PDR | - | + |
| | 5 | 23 | P,0X.AMP. SAM.AMC,CT, CR,CF,CQ, M,AZ,CLR, E,OXT,C, NOR,OF,LOM, K,NOV,AK, LIN,CD | 9 | PDR | + | - |
| | 6 | 23 | P,0X.AMP. SAM.AMC,CT ,CR,CF,CQ, M,AZ,CLR, E,OXT,C, NOR,OF,LOM, K,NOV,AK, LIN,CD | 9 | PDR | - | - |
| | 7 | 24 | P,0X.AMP. SAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM, K,NOV, AK,LIN,CD | 9 | PDR | + | + |
| | 8 | 24 | P,0X.AMP. SAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK,LIN,CD | 9 | PDR | - | + |
| | 9 | 24 | P,0X.AMP. SAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK,LIN,CD | 9 | PDR | + | - |
| | 10 | 24 | P,0X.AMPSAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK,LIN,CD | 9 | PDR | + | + |
| *S.aureus* | 1 | 24 | P,0X.AMP. SAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK,LIN,CD | 9 | PDR | - | - |
| | 2 | 24 | P,0X.AMP. SAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF ,LOM,K,NOV, AK,LIN,CD | 9 | PDR | + | + |

**Table 5:** Continue

| | | | | | | |
|---|---|---|---|---|---|---|
| 3 | 24 | P,0X.AMP. SAM.AMC,PIP/TAZ, CT,CR,CF, CQ,M,AZ, CLR,E,OXT, C,NOR,OF, LOM,K,NOV, AK,LIN,CD | 9 | PDR | + | + |
| 4 | 24 | P,0X.AMP. SAM,AMC,TZP, CL,CE,CEP, CEQ,MEM,AZ, CLR,E,OT, C,NO,OFX, LOM,K,NV, AK,LIN,DA | 9 | PDR | + | + |
| 5 | 23 | P,0X.AMP. SAM.AMC,TZP, CL,,CE,CEQ, MEM,AZ,CLR, E,OT,C, NO,OFX,LOM ,K,NV,AK, LIN,DA | 9 | PDR | - | + |
| 6 | 23 | P,0X.AMP. SAM.AMC,TZP, CL,CE,CTX CEQ,MEM,AZ, LR,E,OT, C,NO,OFX, K,NV,AK, LIN,DA | 9 | PDR | - | - |
| 7 | 23 | P,0X.AMP. SAM.AMC,TZPZ, CL,CE,CTX, CEQ,MEM,AZ, CLR,E,OT, C,NO,OFX, K,NV,AK, LIN,DA | 9 | PDR | - | + |
| 8 | 22 | P,0X.AMP. SAM.AMC,TZPZ, CL,CTX,CEQ, M,AZ,CLR, E,OT,C, NO,OF, K, NV,AK,LIN, DA | 7 | MDR | - | + |
| 9 | 23 | P,0X.AMP, SAM.AMC,TZP, CL,CE,CTX, CEQ,MEM,AZ, CLR,E,OT, C,NO,OFX, K,NV,AK, LIN,CD | 9 | PDR | - | - |
| 10 | 22 | P,0X.AMP. SAM.AMC,TZP, CTX,CF,CEQ, MEM,AZ,CLR, E,OT,C, NO,OFX, K, NV,AK,LIN, DA | 7 | MDR | - | + |

CL= Cephalexin , 0X = oxacillin, SAM = ampicillin/sulactam, AMP = ampicillin, AMC = Apmpicillin/clavulinic acid, TZP = Pipracillin-tazablam,, P = penicillin, CTX = Cefotaxam, CE = Cephradine, CEQ = Cefquinome, CEP = Cefoperazone MEM = Meropenem, CLR = Clarithromycin, AZ = Aztreonam, E = Erythromycin, C = Chloramphenicol, OT = Oxytetracycline,OFX = Ofloxacin, NO = Norfloxacin, K = kanamycin, LOM = Lomefloxacin,AK = Amikacin, NV = Novobiocin,DA = Clindamycin LIN = Linezolid

## Conclusion

Caseous lymphadenitis is still an important challenge for sheep and goat industries, limiting their productivity. The forceful market and movement of small ruminants, without the necessary biosecurity measures, are important problems to the control of caseous lymphadenitis, maintaining its frequency at high levels; *C. equi* also plays a role in buffalo raring as causing edematous skin disease which affects milk and meat production. as well as the high resistance of both *C. ovis and C. equi* to antibiotics and disinfectants which indicates that specific control measures should be adopted. Thus, great efforts need to be made by all responsible persons in sheep and goat industries and buffalo raring to control these terrible diseases. MRSA, as well as PDR *Pseudomonas aeruginosa*, were observed animal wounds which prove the bad hygiene. Increasing contact time is more effective against the microbe.

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## Author's Contributions

**Sultan Farag Nagati:** Sample collection, bacteriological examination, writing the paper, editing, revising the manuscript and Corresponding Author.

**Soumaya, El Shafii**: Bacteriological examination and data analysis- writing the paper.

**Shaimaa Abd ElMawgoud:** Bacteriological examination and data analysis

## Ethics Approval

The research protocol has been approved by Ethics Committee of experimental and clinical studies at Animal Health Research Institute (AHRI), Egypt. Approval number (167638).

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