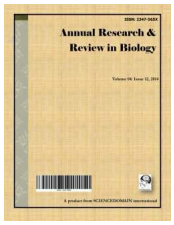

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# Complication of Gynecomastia by Infection with a Novel Resistant *Pseudomonas aeruginosa* Strain in Male Goat

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author NAAH outlined the protocol, contributed to literature search, managed and performed the bacteriological investigation and wrote the initial draft. Author ROR performed the clinical examination, carried out the surgical operation, follow-up and produced photographs. Author FAAH designed the pathological investigation, wrote the pathology report and managed literature search. Author SEB performed histopathological analysis and produced microphotographs. Author AF wrote the protocol, carried out molecular bacteriology investigation and wrote the final draft. All authors read and approved the final manuscript.*

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## ABSTRACT

Gynaecomastia is a glandular tissue proliferation leading to unilateral or bilateral enlargement of male mammary glands of man and animals. The present work describes a rare case of gynecomastia in a four-year-old Nubian buck.
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Clinical examination revealed bilateral unequal enlargement of male rudimentary teats and both testicles were situated caudal to the udder. The animal performed well as a sire and produced liberal amount of normal milk from his udder. It was presented to the Veterinary Clinic for development of chronic mastitis. The case was referred to surgery and the decision was total amputation of the udder.
On microbiological examination, *Pseudomonas aeruginosa* was identified by traditional methods and confirmed by the commercial VITEK 2 technique. Antimicrobial susceptibility test of the strain revealed resistance to common antibiotics in use to treat animals and was sensitive only for Aminoglycosides, Carbepenems, Cephalosporins and Lincosamides antimicrobial groups. Molecular bacteriology analysis by 16S rDNA sequence showed it is *Pseud aeruginosa* with only 99% similarity with the strains S164S, DQ8, PA96, PA1 and PA38182 pointing out it may be a new strain. Mastectomy was performed as radical treatment of gynaecomastia and chronic mastitis developed in the udder of the male goat. The animal performed well after surgery for up to two months follow-up. Histopathological examination demonstrated the udder connective tissue was completely transformed to multiform embryonic connective tissue with fibrosis. This picture is common in chronic infection and may not aid in differential diagnosis of the causes of gynaecomastia. The isolated *Pseud aeruginosa* strain was resistant to common antimicrobial agents with possible production of biosurfactants that induced development of chronic infection.
Implication of gynecomastia in the health and performance of male goats and need to further investigate predisposing factors, are stressed.

*Keywords: Gynaecomastia; male goat; mastitis; Pseudomonas aeruginosa; Saudi Arabia.*

## 1. INTRODUCTION

*Pseudomonas aeruginosa* is a Gram-negative bacterium that is noted for its environmental versatility in many diverse environmental settings like wastewater, soil and rhizosphere, and vegetables [1] ability to cause disease in particular susceptible individuals and its resistance to antibiotics [2]. In man, it is considered to be a serious opportunistic pathogen especially in immune-compromised patients. Based on conventional methods, isolates were identified based on their distinctive grapelike odour of aminoacetophenone, pyocyanin production, and the colonies' structure on agar media [3]. *Pseud aeruginosa* has many strains, including *Pseud aeruginosa* strain PA01, *Pseud aeruginosa* PA7, *Pseud aeruginosa* strain UCBPP-PA14, and *Pseud aeruginosa* strain 2192 [4].

The mammary glands of goats are specialized cutaneous glands related to the sweat and sebaceous glands. The udder of a goat is made up of two mammary glands or "halves." Each half is a functioning unit of its own which operates independently and delivers the milk through its own teat.

In normal conditions, the mammary gland of the male goat is underdeveloped and represented by small nipples. In the female, development of the udder is controlled by estrogen, progesterone and pituitary hormones. Gynaecomastia is a glandular tissue proliferation leading to unilateral or bilateral enlargement of mammary glands with a possible secretory activity [5]. The condition may occasionally be seen in male goats, but, generally reports in animals are meagre. It has been reported in Nubian and Polish White goats [6,7] and in a Sirohi buck [8]. In the Kingdom of Saudi Arabia (KSA), thorough search of the literature showed only one report in goat [9]. In man, gynaecomastia is common being present in 30% to 50% of healthy men [10]. The occurrence of Klinefelter's syndrome (KS) in a patient with gynecomastia from Oman has been reported to aid in differential diagnosis [11]. Although commonly bilateral and symmetric, gynecomastia of different causes may be unilateral or asymmetric. The condition often presents no symptoms and may be diagnosed incidentally on routine examination, but the patient may feel breast pain or tenderness [10]. Histologic studies reveal a proliferation of ductules embedded in a connective tissue stroma; glandular acini are rare. In the early or florid stage, ductal hyperplasia and proliferation are extensive while the stroma is loose and edematous. Usually, over about 12 months, the breast tissue evolves into a quiescent stage, in which the amount of stroma and fibrosis increases and the ductules become less prominent [12,13].

Mastitis is inflammation of the mammary gland that could be subclinical, showing no obvious

signs, clinical acute or chronic. The disease affects milking does and is prevalent among high milking animals during lactation. Subclinical and acute mastitis may proceed to chronicity in case of inadequate management. A multitude of bacterial species namely, *Staphylococcus aureus*, coagulase negative *Staphylococci* [14], *Pseud aeruginosa*, *Mannheimia haemolytica*, *Corynebacterium pseudotuberculosis*, *Streptococcus* spp, *Nocardia* spp and *Brucella melitensis,* has been incriminated in causing caprine mastitis [15]. *Pseud aeruginosa* is most often associated with sporadic clinical mastitis. However, outbreaks and control of mastitis in sheep and goat herds caused by *Pseud aeruginosa*, were described. The morbidity rates in sheep and goat herds were 18.7 and 18.2 per cent, respectively. The unavoidable culling rates of the animals with sub-clinical mastitis correspond to the morbidity rate [16]. Mastitis caused by resistant bacterial strains may pose difficulty in treatment and eventually develop into chronic mastitis that may need surgical interference.

The present article is an investigation into a rare case of chronic mastitis in a functioning udder of a buck, characterization of the causative agent, documentation of tissue reaction and surgical management of the case.

## 2. MATERIALS AND METHODS

Case History: A four-year old Nubian male goat weighing about 50 kilograms was brought to the Veterinary Teaching Hospital, King Faisal University, KSA, with mastitis.

### 2.1 Clinical Findings

Examination showed a bilateral unequal enlargement of male rudimentary teats. Further examination revealed that right teat was slightly globular; a size of football, hard, covered with healthy skin and has a nipple conical in shape. The left teat was elliptical and has elongated conical teat cistern (Fig. 1). Both testicles were situated caudal to the 'udder' (Fig. 2). The owner added that the male goat was producing liberal amount of normal milk over the last 'lactation' season but the milk inclined to abnormality with straw colouration before presentation.

On palpation, the mammary gland was hard with rough skin indicating chronic inflammation with foul-smelling secretions. Decision of the surgeon was total mastectomy.

Specimens consist of sterile swabs of discharge and tissue was sent to the microbiology laboratory and parts of tissue fixed in 10% formalin were sent for histopathology laboratory.

### 2.2 Microbiological Investigation

The specimen was cultured in duplicate onto 5% sheep blood agar, Mac Conkey's agar (Oxoid, Basingstoke, U.K.), Hayflick modified medium and Sabouraud's dextrose agar (Oxoid). Bacterial cultures were incubated at 37℃ aerobically and one blood agar plate anaerobically for 24 – 48 hours. Mycological cultures were incubated at 28 - 30℃ up to 7 days. Presumptive identification was done according to descriptions in textbooks [17]. Confirmation of the identification of isolates was done by VITEK 2 technique (bioMerieux, Marcy L'Etoile, France).

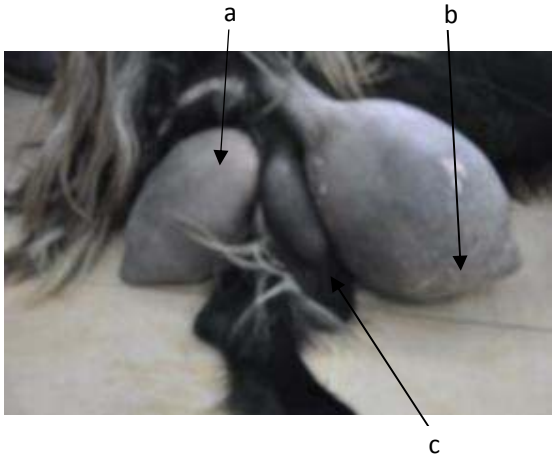


**Fig. 1. Photograph shows a male goat with gynaecomastia, that developed chronic mastitis, placed on its side. (a). Left teat appears elliptical and has elongated conical teat cistern (b). Right teat is slightly globular, a size of football and has a nipple conical in shape. (c). Testicle**

### 2.3 Antimicrobial Susceptibility Test

The isolate was tested by the standard disc diffusion method according to Kirby-Bauer. It was subjected to a susceptibility panel of 16 antibiotics (Oxoid) distributed among 11 groups: Penicillins: Penicillin G (10 IU), Amoxicillin (25 µg); Fluoroquinolones: Ciprofloxacin (5 µg), Flumequine (30 µg); Aminoglycosides: Gentamicin (10 µg), Kanamycin (30 µg), Neomycin (10 µg); Carbepenems: imipenem (10 mcg); Tetracycline: Tetracycline (30 µg); Cephalosporins: Cefotaxime (30 µg);

Lincosamides: Lincomycin (2 µg), Clindamycin (2 µg); Macrolide: Erythromycin (15 µg); Rifamycin: Rifampicin (5 µg); Glycopeptide: Vancomycin (30 µg); Sulfonamide: Trime-sulfamethoxazole 1:19 (25 µg).


**Fig. 2. Animal on standing position showing (a). Both testicles situated caudal to udder (b)**

The test was performed on Mueller-Hinton agar medium (Oxoid) and kept at 37℃ for 24 hours in 10% $CO_2$ enriched incubator. After the incubation period the diameter of zone of inhibition was measured and interpretation of result based on CLSI guidelines was performed by assigning as sensitive (S), intermediately resistant (I) or resistant (R) [18].

## 2.4 Molecular Bacteriology

DNA extraction: Isolates were grown in Luria-Bertani broth at 37℃ for 18 hours. A volume of 1 ml was transferred to micro-centrifuge tubes, centrifuged for 3 min at 3500x g and the supernatant (S/N) was discarded. The pellet was suspended in 200 µl of extraction buffer (0.1 M Tris-HCl pH 7.5, 0.05 M EDTA pH 8.0, 1.25% SDS), mixed and incubated with 20 µl of proteinase K (1 mg/ml) for 35 minutes at 56℃. The tube was brought to room temperature, washed for 3 min at 3500x g and S/N was removed gently to a new tube. An equal volume of absolute ethanol was added to precipitate DNA, washed at 3500 g/ 3 min and dried by air flow.

PCR analysis based on 16S rRNA was utilized. Forward and reverse DNA sequence reactions of PCR amplicon were carried out using 27F and 1492R by Macrogen Inc, company (Seoul, South Korea). The gene sequence was used to carry out BLAST with the 16S r-DNA nucleotide database to detect maximum identity score.

## 2.5 Surgical Procedure

The animal was sedated with xylazine HCl given at the dose of 0.11 mg/Kg body weight intravenously and it was anaesthesized with ketamine HCl given at dose of 3 mg/Kg body weight. The animal was prepared on its back and the ventral abdominal region was approached for aseptic operation (Fig. 3). An elliptical incision was made around each quarter and the latter were removed from the base. The wound was closed in routine manner. Post-operatively, the animal was given broad spectrum antibiotics (oxytetracycline LA), anti-pyretic and analgesic.

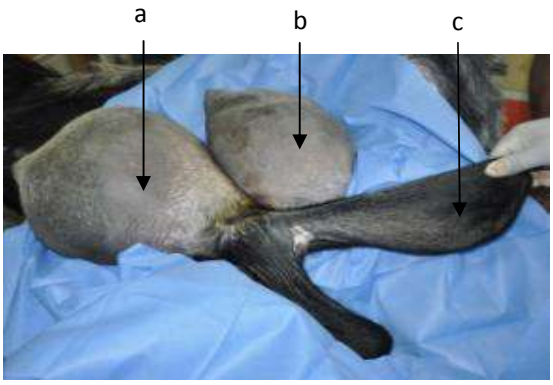


**Fig. 3. Animal is restrained on its back and the ventral abdominal region exposed for surgery. (a). Right, (b) Left halves of udder and (c) Testicle**

## 2.6 Histopathological Examination

Specimens of udder tissue were collected from lesions, fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5µm. and stained with haematoxylin and eosin for histopathological examinations. Histopathological sections were done according to methods described by [19].

## 3. RESULTS

Mastectomy: Clinically the animal appeared in a good condition (Fig. 5) and there were no

complaint or abnormality after follow-up for two months.

Bacteriological examination gave *Pseud aeruginosa* in pure culture and confirmed by Vitek 2 technique (bioMerieux, France).

Cultural features of the isolate on blood agar and nutrient agar showed colonies to be smooth, round, mucoid with scalloped margins and orange in colour. Microscopic examination revealed Gram negative rods, non-spore-forming, motile that measured 0.3 – 0.9 µm in width and 1 – 2 µm length.

Antimicrobial susceptibility test showed the isolate was S to kanamycin, neomycin, imipenem, cefotaxime and lincomycin, I to gentamicin and clindamycin, R to penicillin G, amoxicillin, ciprofloxacin, flumequine, tetracycline, trime-sulfamethoxazole, erythromycin, rifampicin and vancomycin.

Further, molecular characterization by 16S rRNA sequencing showed the isolate to be *Pseud aeruginosa* with partial sequence:

Score = 2724 bits (1475), Expect = 0.0

Identities = 1482/1485 (99%), Gaps = 1/1485 (0%)

d = Plus/Plus

The 16S rDNA sequence of our strain had a similarity of about 99% with those of internal and external isolated *Pseud aeruginosa* strains. Hence, it didn't show a 100% similarity with any of the known sequenced strains. Sequenced strains that produced significant alignments to our strain

*Pseudomonas aeruginosa* strain S164S 16S ribosomal...
*Pseudomonas aeruginosa* strain DQ8 16S ribosomal R...
*Pseudomonas aeruginosa* strain IHB B 6863 16S ribosomal...
*Pseudomonas aeruginosa* PA96 genome
*Pseudomonas aeruginosa* strain S20410 16S ribosomal...
*Pseudomonas aeruginosa* strain C1501 16S ribosomal...
*Pseudomonas aeruginosa* LESlike4 sequence
*Pseudomonas aeruginosa* LESlike1 sequence
*Pseudomonas aeruginosa* LESB65 sequence
*Pseudomonas aeruginosa* LES400 sequence
Mycoplasmas or fungi were not recovered from the specimens.

### 3.1 Pathological Findings

Gross appearance of lesions, in general, included variable sized abscesses, greyish-white nodules and fibrosis in both halves in the infected udders (Fig. 4).

Different histopathological changes were seen in the udder tissue (Fig. 6).

The udder connective tissue was completely transformed to multiform embryonic connective tissue containing stellate, spindle-shaped fibroblasts. Proliferating macrophages, fibroblasts, and loose connective tissue were seen surrounding small blood capillaries.

## 4. DISCUSSION

The present investigation dealt with a condition of gynaecomastia where the owner sought veterinary help to manage mastitis. As described by the owner, it appeared that the patient performed fairly as a sire and concurrently producing milk from his mammary glands. Based on clinical examination, the condition of this male goat was diagnosed as gynaecomastia with chronic mastitis in both halves of the mammary gland. Mastectomy was performed as radical treatment of chronic mastitis, in the present case, with the advantage of reducing potential health problems and utilizing the goat as a stud. The operation has been chosen as gynaecomastia may lead to mammary tumours or reduction in fertility [8]. As well, gynaecomastia grade I-II in 53 human male patients was treated surgically by liposuction combined with subcutaneous resection of the glandular tissue. This surgical technique was reported to be low-impact less invasive with a low rate of complications [20].

By standard laboratory methods, *Pseud aeruginosa* was obtained from the clinical specimens. The isolate was identified by cultural characteristics and confirmed by biochemical tests of the automated system VITEK 2.

*Pseud areuginosa* acts as an opportunist by attacking weak or injured tissues of teats or mammary gland. Animals that are immunologically compromised due to other infectious diseases or nutritional deficiencies are also more susceptible to *Pseud aeruginosa* infections. Contamination of bottles of antibiotics with *Pseud aeruginosa* is quite easy particularly when the same needle is used more than once to draw the drug and treat many animals.

Contaminated antibiotic bottle in this manner will support survival of *Pseud aeruginosa* indefinitely.

Molecular analysis by the 16S rDNA sequence of our strain showed that it had a similarity of above 99% with other *Pseud aeruginosa* strains. Our strain had no 100% similarity with any of the known sequenced *Pseud aeruginosa* strains indicating it is a novel strain that merits further investigation. The strain has the closest genetic relationship with *Pseud aeruginosa* strains S164S, DQ8, PA96, PA1 and PA38182. [21] reported that strain PA1 is a fair rhamnolipid type biosurfactant producer. Biosurfactants are microbial compounds that exert pronounced surface and emulsifying activities and many authors have already described antibiotic effects associated with them. A biosurfactant that demonstrated substantial antimicrobial effect on a multitude of bacterial and fungal species namely, *Bacillus subtilis*, *Staph aureus*, *Proteus vulgaris*, *Strept faecalis*, *Pseud aeruginosa* and Penicillium sp., Alternaria sp., *Gliocadium virens* and *Chaetonium globosum*, was produced by *Pseud aeruginosa* strain LB1 [22]. Whole-genome sequencing of *Pseud aeruginosa* PA96, a clinical multiresistant-to-antibiotics isolate from China, has been completed. Analysis of known core genome virulence factors and resistance genes revealed few differences with other strains [23]. The result of antimicrobial susceptibility test of *Pseud aeruginosa* strain isolated in the present study, showed resistance to antimicrobials from different groups with antibiotics in common veterinary use for treatment in the study area. Sensitivity was recorded only for Aminoglycosides, Carbepenems, Cephalosporins and Lincosamides groups. This finding is, as well, of medical significance as *Pseud aeruginosa* resistant strains pose difficulty in management especially in chronic diseases.


**Fig. 4. Post-operation shows a cross-section of the right half of udder indicating presence of abscesses, nodules and fibrosis of the mammary tissue**

**Fig. 5. Animal on a standing position after mastectomy and recovery from anaesthesia in good condition**

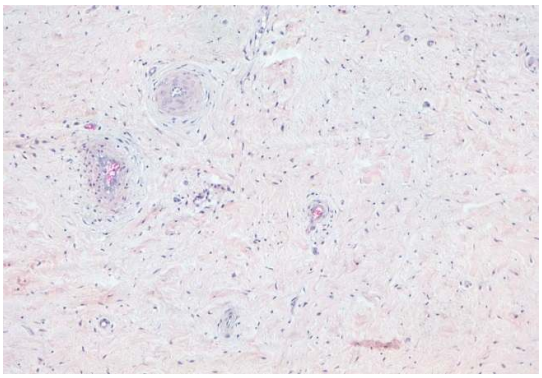

**Fig. 6. Microphotograph of a histopathological section showing the glandular epithelial cells are converted to stellate, ovoid spindle shaped with myxomatous matrix. (H & E, x10)**

The pathogenic mechanism that could account for the inflammatory reactions and cell desquamation associated with mastitis caused by *Pseud aeruginosa* in the present study, is interesting as the affected organ itself was abnormally proliferated. It was suggested that gynaecomastia is congenital that progress by mechanical stimulation and infection [9]. Histopathological evidence, in the present study, showed the udder connective tissue was completely transformed to multiform embryonic tissue with fibrosis which may predispose to malignancy. In man, at the early or florid stage, ductal hyperplasia and proliferation are extensive, while in later stages the amount of stroma and fibrosis increases [12,13]. Histologic findings of the present study, resemble microscopic features of chronic mastitis which are similar regardless of the cause of gynaecomastia, so it may be unimportant diagnostically. More histopathologic studies in natural disease or experimental animals in the early stage of gynaecomastia may be of interest for management in man and animals.

The udder is a complex organ made of several cell types contributing differentially to its immune response [24]. The immune defence in the mammary gland is activated by the invading pathogens leading to a complex process that involves resident and recruited immune cells together with mammary epithelial and endothelial cells. Opportunistic pathogens as *Pseud aeruginosa*, with its broad-spectrum resistance to anti-microbial agents, may cause initially subclinical mastitis that progress to ongoing chronic disease. Some workers suggested that gynaecomastia may predispose the animal to mammary tumours or reduced fertility [8]. Further work is needed to investigate hormonal, cytological and genetic factors that may predispose gynaecomastia in animals which may interest public health and animal health workers.

## 5. CONCLUSION

In a male Nubian breed goat, gynaecomastia was diagnosed after development of chronic mastitis in the proliferated mammary gland. The animal was reported to perform well as a sire and female producing normal milk.

A multi-drug resistant *Pseud aeruginosa* strain was identified from the infected milk. 16S rDNA sequence analysis revealed 99% homology with other sequenced *Pseud aeruginosa* strains with possibility of being a novel strain.

Further work could highlight the role of *Pseud aeruginosa* strains in chronic mastitis and transmission of drug resistance to man.

The case was managed by total mastectomy where the animal returned to function as a male only.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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