# THE SENSITIVITY TEST AND THE MINIMAL INHIBITORY CONCENTRATIONS OF SELECTED ANTIBIOTICS TO SOME BACTERIAL ISOLATES FROM BUFFALO-SEMEN

M.S. TAWFIC[1], M.A. AZIZ[2], S.M.EL-SHEIKH[3], S.M. EL-AYOUBY[4] AND H.M. HASSAN[1]

*1 Animal Reproduction Research Institute, Agricultural Research Centre, Giza, Egypt.*
*2 Faculty of Veterinary MedicineKafr El Sheikh,Tanta University.*
*3 Faculty of Vetrinary Medicine Zagazig University.*
*4 Vetrinary Serum and VaccineResearch Institute, Agricultural Research Centre, Giza, Egypt.*

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## *Abstract*

A total of 128 semen samples were obtained from buffalo-bulls (3-8 years old) using sterile artificial vagina. The incidence of different microorganisms were : *E.. coli* (16.8%), *Pseudomonas aeruginosa* (15.2%), *Strept.faecalis* (11.6%) ,*Staph. epidermidis* (10.4%), *Staph. aureus* (9.6%), *Coryne. renale* (8.4%), *Proteus vulgaris* (7.6%) *Stept. pyogenes* (7.2%) and *Coryne.pyogenes* (6.8%).

The total bacterial count of buffalo neat semen samples ranged from 3.1 to 5.7 x $10^6$ bacteria /ml. Sensitivity tests of bacterial isolates revealed that Gentamycin, Cephapirin, Amikacin and Doxycycline had more prominent effects than Penicillin and Streptomycin.

## INTRODUCTION

Bacterial contamination in semen may infect the inseminated cow and deteriorate the substrate in semen extenders and affect spermatozoa directly. The prepuce is considered as the main source of contamination for semen from healthy bulls.Also,it is obvious that, bacteria from animal skin and from atmosphere can also contaminate the semen. Similarly, semen can be contaminated by the instruments used for collection and processing.

The insemination of semen contaminated with Pseudomonas aeruginosa caused purulent vaginitis, cervicitis and endometritis (Getty and Elis 1967). Bush (1950) was able to demonstrate a correlation between the fertility rate and initial bacterial content of bull semen. On the same line, Kasda (1963) studied the bacterial flora in semen of bulls and found that *E. coli* had the most marked spermicidal effect. Aleem *et al.* (1988) reported that, the bacterial contaminants of buffalo semen were *E.coli, Pseudomonas aeruginosa, Staph. aureus, Proteus mirabilis* and *Strept. pyogenes.* Ghanam *et al.* (1980) found that, the bacterial count in buffalo-semen was $1.4x10^6$ bacteria/ml. Eaglesome *et al.* (1992) considered that the presence of microorganisms in semen impairs the reproductive functions of the female and, therefore, establishes a limit on the number of non-pathogenic organisms that can be present in bull semen to be used for artificial inseminations. The sensitivity of different bacterial isolates from neat buffalo semen to different antibiotics were studied by Rhman *et al.* (1983), Hassan (1985) and Shin *et al* .(1988). Theyfound that, the bacterial contaminants in semen acquired resistance to pencillin and streptomycin, but they were sensitive to Amikacin,Gentamycin and Doxycycline. The aim of this study was to explore the effect of certain antibiotics on some bacterial isolates from buffalo-semen other than pencillin and streptomycin.

## MATERIALS AND METHODS

In the present study, one hundred and twenty-eight semen samples were obtained from buffalo-bulls 3-8 years old. These animals were raised in different localities of A.R.E. These animals were free from venereal diseases and clinically normal. The semen samples were collected using sterile artificial vagina and kept in sterile vials. After collection, the semen samples were transported to the laboratory in a thermo flasks containing ice.

In the laboratory, semen samples were directly cultivated on nutrient agar, blood agar and MacConkey's agar, and incubated aerobically at 37°C, then, examined after 24-48hours. Different colonies were reconized from their appearance, haemolytic activity,morphologically and identified according to Finegold and Matine (1983).

Semen samples were diluted ten folds in nutrient broth and then , the total bacterial count was determined according to Diliello (1979).Fifteen types of antibi-

otic discs were applied to detect the sensitivity of the different bacterial isolates (Cruickshank *et al.* 1975).

Also, different types of antibiotics were used for determination of the minimal inhibitory concentration (M.I.C.) for bacterial isolates (Elmer 1983).

## RESULTS

The total bacterial count in collected buffalo semen (n = 128) ranged from 3.1 to5.7x10 6 bacteria/ml. The prevalence of different bacteria isolates calculated as percentage are *E. coli* (16.8%) and *Pseudomonas aeruginosa* (15.2%) which were the predominant isolates from buffalo semen, while, *Streptococcus faecalis, Staphylococcus epidermidis* and *Staphlococcus aureus* represented 11.6%, 10.4% and 9.6%, respectively.The other contaminants *(Citrobacter ferundii, Proteus vulgaris, Streptococcus pyogenes, Corynebacterium renale* and *Corynebacterium pyogenes)* were less than 8.5%.

Table 1 shows the results pertaining the sensitivity of buffalo-semen contaminants for different types of antibiotic discs. Regardless to the types of bacterial isolates, Amikacin, Cephapirin, Doxycycline and Gentamycin had the most prominent effect on many of the bacterial isolates from buffalo-semen. The most pronouced effect on *E. coli, Cit. Freundii, Pr. vulgaris* and *Ps. aeuruginosa* was for Amicacin, Gentamycin and Doxycycline (more than 80%). Meanwhile, susceptibility percentage of Staphylococcus group was 91.66% for Cephapirin, 80% for Amikacin, 79.17% for Doxycycline and 75% for Gentamycin were less effective in case of *Strept. Pyogenes* (50% -44.44%, respectively) and *Strept. faecalis* (48.28% - 58.62%, respectively). The most pronounced effect on *C. pyogenes* and *C. renale* was for Cephapirin (82.35 - 85.71%). The other types of antibiotics, especially Pencillin G. and Streptomycin had much lower effect on the different bacterial isolates.

Table 2 shows the minimum inhibitory concentration (MIC) and susceptibility percentage of isolates to different types of antibiotics. MIC was mostly low for Amikacin, Cephapirin, Doxycyline and Gentamycin, while, the susceptibility percentage for isolates were the highest to previous antibiotics. *E. coli* and *Cit. Freundii*

were 100% susceptible for Amikacin while, Streptococcus group was less susceptible to Amikacin and Gentamycin. *Pr. Vulgaris* and *Ps. aeruginosa* were not highly susceptible to Cephapirin. Staphylococcus, Streptococcus and Corynebacterium isolates were highly susceptible to Cephapirin. The susceptibility percentageof isolates to the other types of antibiotics was very low, especially, for Penicillin G. and Streptomycin.

## DISCUSSION

The number of microorganisms in buffalo-semen has been determined by Ghanan *et al.* (1980) to range from 0.82 to 7.48 X $10^6$ bacteria/ml. In the present study, total bacterial count (T.B.C.) in neat semen ranged from 3.1 to 5.7 X $10^6$ bacteria/ml. This difference may be due to the fact that, bacteria encountered in semen are mostly controlled by hygienic housing of bulls and sanitary measures applied in the place of collection (Ghanam *et al.* 1980).

In this study, many bacterial contaminants were isolated including *E.coli* (16.8%), *Pseudomonas aeruginosa* (15.2%), *Staphlococcus aueus* (9.6%), *Corynebacterium renale* (8.4%), *Citrobacter freumdii* (6.4%), *Proteus vulgaris* (7.6%), *Streptococcus pyogenes* (7.2%), *Corynebacterium pyogenes* (6.8%) *Staphylococcus epidermidis* (10.4%) and *Streptoccoccus faecalis* (11.6%). Ghanem et al. (1980), Hassan (1985) and Aleem *et al.* (1988) also, were able to isolate the same types of bacteria wich they recorded as natural saprophytes. It was claimed that, the main source of contamination is the contact of the protruding tip of urethra with prepuce contents (Marinove *et al.* 1966).

There is lack of evidence on the causal relationship between the presence of potentially pathogenic micro-organisms in buffalo-semen and its fertilizing capacity.

The sensivity of bacterial isolates from neat buffalo-semen against 15types of antibiotics, demonstrated in the present work showed that, Amikacin, Cephapirin, Doxycyline and Gentamycin had the most prominant anti bactial effects on the bacterial isolates. This achievement was in accordance with that of Sone *et al.* (1982), Rahman *et al.* (1983) and Hassan (1985). They found that, aminoglycosides such as Gentamycin showed marked antimicrobial activities against several types of semen contaminants.

Amikacin is one of the recently produced antibiotics proved to be effective against a variety of microorganisms including Corynebacterium pyogenes and Pseudomonas aeruginosa (Graber *et al.* 1978). Cephalosporins such as Cephapirin were proved to be effective against Gram-positive and Gram-negative bacteria isolated from bull semen (Bran *et al. 1972).*Doxycyline, a member of tetracyclines, was demonstrated effective against Gram-positive and Gram-negative bacteria (Sande and Mandell, 1980).

However, it is clear apparently that, the contaminants isolated from buffalo-semen showed the highest resistance to Pencillin and Streptomycin. This finding is in agreement with the observetions of Meredith (1985), and Shin *et al.* (1988).

Table 1. Prevalence rate of different bacteria isolated from 128 semen samples.

| Bacterial isolates | Number | Percentage |
|---|---|---|
| *Escherichia coli* | 42 | 16.8 |
| *Pseudomonas aeruginosa* | 38 | 15.2 |
| *Citrobacter freundii* | 16 | 6.4 |
| *Proteus vulgaris* | 19 | 7.6 |
| *Staphylococcus aureus* | 24 | 9.6 |
| *Staphyloccus epidermidis* | 26 | 10.4 |
| *Streptococcus pyogenes* | 18 | 7.2 |
| *Streptococcus faecalis* | 29 | 11.6 |
| *Corynebacterium pyogenes* | 17 | 6.8 |
| *Corynebacterium renale* | 21 | 8.4 |
| Total | 250 | 100.00 |

Table 2. Approximate minimum inhibitory concentration and susceptability percentage of isolated bacteria.

| Antibiotios | E.coli (25) | | Cit.freundii (13) | | P.vulgeris (15) | | Ps.aeruginosa (26) | | Staph.aureus (20) | | Staph.epidermidis (18) | | Staph.pyogenes (15) | | Staph.faecalis (15) | | C.pyogenes (15) | | C. renale (17) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MIC | S,% | MIC | S,% | MIC | S,% | MIC | S,% | MIC | S,% | MIC | S,% | MIC | S,% | MIC | S,% | MIC | S,% | XIC | S,% |
| Amikacin | <16 | 100 | <16 | 100 | <32 | 86.67 | > 1 | 80.0 | <32 | 72.22 | >8 | 53.33 | >4 | 55.00 | <32 | 73.33 | >2 | 76.47 | <16 | 100 |
| Cephapirin | >12 | 80.0 | >32 | 84.62 | <64 | 53.33 | <64 | 90.0 | <8 | 100.0 | <16 | 33.33 | <64 | 70.00 | <16 | 86.57 | <16 | 33.75 | >32 | 84.62 |
| Clindamycin | <16 | 44.0 | <64 | 00.0 | < 8 | 00.0 | <16 | 25.0 | <2 | 22.22 | <16 | 13.33 | <4 | 35.0 | <<32 | 00.0 | <16 | 17.35 | <64 | 00.0 |
| Doxycycline | <16 | 80.0 | <8 | 69.23 | <16 | 73.33 | <8 | 75.0 | <8 | 72.22 | <16 | 80.03 | <64 | 70.00 | <<16 | 73.33 | <8 | 32.35 | <8 | 69.23 |
| Gentamycin | >0.5 | 92.0 | >8 | 84.62 | < 4 | 100.0 | <16 | 75.0 | ,8 | 77.78 | <64 | 53.33 | <64 | 50.02 | <32 | 73.33 | <8 | 32.33 | >8 | 84.62 |
| Kanamycin | >4 | 40.0 | <64 | 38.4 | <16 | 40.0 | <8 | 30.0 | <64 | 22.22 | <32 | 13.33 | <32 | 5.0 | <16 | 40.0 | <64 | 41.13 | <64 | 38.4 |
| Oxacillin | <16 | 36.0 | <16 | 23.08 | <8 | 30.77 | <32 | 50.0 | <8 | 55.55 | <16 | 33.33 | <64 | 30.0 | <16 | 53.33 | <8 | 47.06 | <16 | 23.08 |
| PenicillinG | >128 | 0.0 | >128 | 00.0 | >128 | 00.0 | >128 | 45.0 | <32 | 50.0 | <32 | 20.0 | <16 | 40.0 | <32 | 33.33 | <32 | 35.39 | >128 | 00.0 |
| Streptomycin | <32 | 44.0 | <16 | 30.77 | <64 | 46.67 | <32 | 00.0 | >128 | 0.00 | >128 | 00.00 | >128 | 00.00 | >4 | 13.33 | >16 | 00.0 | <16 | 30.77 |
| Tetracyclin | <16 | 48.0 | <8 | 38.4 | <8 | 46.57 | <16 | 55.0 | <32 | 72.22 | <16 | 46.67 | <8 | 45.00 | <64 | 46.67 | <16 | 53.94 | <8 | 38.4 |

MIC :Minimum inhibitory concentration (mg/ml)
S% :Susceptability percentage.

In the current study, it has been noted that, a highly significant reduction in total bacterial count was recorded under the effect of Cephapirin, Gentamycin and Amikacin. Meanwhile, a slight reduction in total bacterial count was achieved under the effect of Penicillin and streptomycin as based on results of the sensitivity tests.

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# أختبارات الحساسية وأقل تركيز مؤثر لمضادات حيوية مختارة على بعض المعزولات البكتيرية من السائل المنوى الجاموسى

محمود صبرى توفيق [١]، مصطفى عبد العزيز [٢]، سوسن محمد الشيخ [٣]،
صالح محمد صالح [٤] ،،هانى محمد حسن [١]

١ معهدبحوث التناسليات – مركز البحوث الزراعية – الجيزة – مصر
٢ كلية الطب البيطرى – كفرالشيخ – جامعة طنطا
٣ كلية الطب البيطرى – جامعة الزقازيق
٤ معهد بحوث وانتاج الامصال واللقاحات – مركز البحوث الزراعية – الجيزة – مصر.

تم جمع عدد ١٢٨ سائل منوى جاموسى من طلائق عمرها ٣–٨ سنوات باستخدام مهبل صناعى معقم .بالزرع البكتريولوجى تم عزل الميكروب القولونى (١٦.٨٪)، سيدومونس ايروجينوزا (١٥.٢٪)، السبحى البرازى (١١.٦٪)، العنقودى الذهبى (٩.٦٪)، كورينى رينال (٨.٤ ٪)، بروتنيس فالجاريس (٧.٦٪)، السبحى الصديدى (٧.٢٪) ،والكوريني الصديدى (٦.٨ ٪).

تبين من الفحص البكتريولوجى أن اجمالى العد البكتيرى من عينات السائل المنوى الطبيعى الجاموسى تتراوح من ٣.١ الى ٥.٧+ ١٠٦ لكل ملى لتر ومن إختبارات الحساسية تبين ان الميكروبات المعزولة حساسة أكثر للجنتاميسن، سيفابرين، اميكاسلين، دوكسى سيكلين عنها للبنسيلين والستربتوميسين.

كما وجد ان اقل تركيز مؤثر على البكتريا المعزولة هو: للاميكاسين ١–٣٢ ميكروجرام / ملى لتر والسيفابرين ٨–٦٤ ميكروجرام / ملى لتر، والجينتاميسين ٥، – الى ٦٤ ميكروجرام / ملى لتر، دوكس سيكلين ٨–٦٤ ميكروجرام / ملى لتر.