Isolation of *Staphylococcus* spp. and *Pseudomonas* spp from small animals' clinics: A potential hazard for nosocomial infections

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1. Abstract

Veterinary clinic environments contain a variety of pathogens that can cause infection in animals. *Pseudomonas aeruginosa* is one of the most common Gram-negative nosocomial pathogens due to its versatility in nutrients and universal presence. *Staphylococcus pseudintermedius* and *Staphylococcus aureus* usually colonize the skin, mucous membranes, urogenital tract, and occasionally the alimentary tract in dogs and humans. This study was carried out on 250 samples collected from different clinics in Egypt for isolation of *Staphylococcus* and *Pseudomonas* spp. Among 250 samples, 125 samples were used for *Staphylococcus* isolation, revealing 77 samples had staphylococcus, with high count in floor and examination table respectively, while another 125 samples were used for *Pseudomonas* isolation, revealing 80 samples showed growth of *Pseudomonas* with high count in floor and examination table respectively. present especially in the examination table and floor. The improper cleaning program and disinfection lead to high results for isolated *Staphylococcus* and *Pseudomonas* species in the environmental surfaces in small animal's clinics.

Key words: Infection , nosocomial, *Staphylococcus*, *Pseudomonas*, cat, dog.

2. Introduction

Both human and veterinary medicine are increasingly concerned with nosocomial infection. The following bacteria are commonly isolated from dogs and cats that cause nosocomial infections: *Staphylococcus aureus* methicillin resistant MRSA, methicillin resistant *S. pseudintermedius* (MRSP), Pseudomonas *aeruginosa* and multidrug resistant (MDR) [1, 38, 39]

There are numerous pathogens present in the environment of veterinary clinics that may cause infections such as surgical site infections, urinary tract infections, and bloodstream infections in animals from direct contact, personnel, and other animals [28]

In particular, nosocomial pathogens that are resistant to antibiotics are a growing problem around the world, and inappropriate use of antibiotics favors the spread of these bacteria [12] Resulting in longer hospital stays and higher treatment costs [40, 41]

Numerous studies indicate that antibiotic resistance is increasing among nosocomial isolates [13] *Pseudomonas aeruginosa* is one of the more common Gram-negative nosocomial pathogens due to its nutritional versatility and ubiquity as a organism [14] The rod-shaped *Pseudomonas aeruginosa* belongs to the family *Pseudomonadaceae* and is motile and gram-negative . In nature, *P. aeruginosa* can be found in a wide variety of biological samples as well as on different inorganic surfaces [15, 16]

As an opportunistic pathogen of increasing clinical relevance, *P. aeruginosa* causes

chronic and recurrent infections in both humans and animals, particularly in dogs and cats the most common diseases seen are pyoderma and otitis external / media [17, 18] It is the second most common bacteria cause of nosocomial infections, accounting for 21% of cases. It is reported that 16% of nosocomial pneumonia, 12% of urinary tract infections, 17-26% of wound infections, and 10% of septicemia are caused by *P. aeruginosa* [18, 19]

For dogs and humans, *Staphylococcus pseudintermedius and Staphylococcus aureus* usually colonize the skin, mucous membranes, urogenital tract, and occasionally the alimentary tract [20]

Despite *staphylococci* being commensals of the skin, mucous membranes, alimentary tract, and urogenital tract of a wide range of mammals and birds, they have been implicated in human and animal clinical infections [21, 22]

The spread of drug-resistant *staphylococci* in a university clinic was described in an early report in which students, veterinary staff and animal patients were exposed to resistant *staphylococci* [23] Animal clinics often suffer from MRSP outbreaks for both cats and dogs [24] The veterinary environment is an important source of MRSP infections in animal patients, reflecting the importance of veterinary environments because resistance profiles of MRSP isolated from severe clinical cases often reveal limited or even nonexistent chemotherapeutic options, affected animals could suffer indefinitely or have to be euthanized under animal welfare considerations [25]

The objective of our study is to assess the hygienic quality of cat and dog clinics by isolating *Staphylococcus* and *pseudomonas* bacteria from different environmental samples obtained from their clinics.

3. Materials and Methods

The Study area was performed at a veterinary clinic serving dogs and cats in Giza governorate. The veterinary clinic offers services in both internal medicine and surgery.

Samples were taken from 25 clinics located in Giza governorate (5 clinics in Nasr city,5 clinics in Zayed city,6 clinics October city and 2clinics Eldokki, 2clinics in Faisal and 5 clinics in El-Haram areas- Egypt). Five cotton swabs were collected from each clinic from (the hands of workers, instruments, surgical tables, examination tables, and the floor) on 0.9% sterile saline [42] each swab was labeled with name of clinic ,date and surface.

Isolation and identification

The samples were incubated in brain heart infusion broth for 24 hours at 37°C [43], followed by streaking on mannitol salt agar medium (Oxoid Ltd, Hampshire, UK) and incubating at 37°C for 24 hours also on pseudomonas base agar with CFC supplement incubated at 37°C for 24 hours to identify *Pseudomonas aeruginosa*. The presence of green colonies and fluorescence leads to its identification fig.1.[44]

As a result of mannitol fermentation, yellow colonies fig.2 were picked to prepare bacterial films and then Gram's stain was applied to confirm Gram-positive cocci arranged irregularly in clusters. We then tested the cultures for catalase enzyme activity and 5% sheep blood agar medium (Oxoid Ltd, Hampshire, UK) was used to observe haemolytic activity. We performed a tube coagulase test using lyophilized rabbit plasma (BD, MD, USA) on pure β haemolytic colonies identified colonies were confirmed as *S. aureus* when reacted positive with commercial latex agglutination test, Staphaurex® (Remel, Lonex, Kans) isolation and identification of *S. aureus* were performed based on [45].

green colonies and fluorescence were identified by Gram staining, oxidase and catalase testing, sugar fermentation in

Triple Sugar Iron agar (TSI) and Oxidation Fermentation tubes (OF) [46]

4. Results

Among 250 samples, 125 samples were used for *Staphylococcus* isolation, revealing 77 samples had *Staphylococcus*, with high count in floor and examination table (20,18) samples respectively ,while another 125 samples were used for *Pseudomonas* isolation, revealing 80 samples showed growth of *Pseudomonas*, with high count in floor and examination table (22,20) samples respectively.

5. Discussion

Table (1) showed the percentage of isolation of *Staphylococcus* from different environmental samples which was 61.6% , presence of these microorganism in veterinary clinics was already described by several authors [47-49]

The highest percentage of isolated *Staphylococcus* spp. present in examination table and floor (72%, 80%) respectively, High result of *Staphylococcus* spp. among examination tables described in [49, 50] which may be due to direct contact of patients with table with weak disinfection program ,The rate of clinic which receive high number from patients will produce high percentage for isolated microorganism., The examination table should be cleaned and disinfected regularly by strong disinfectants to control nosocomial infection caused by the examination table.

For floor ,high result of *Staphylococcus spp.* may be due to the organism's persistence in an environment with a high level of contact with animals and poor cleaning throughout the day ,these result agreed with [51, 52**]**

Presence *Staphylococcus spp* .on hands of workers indicates lack of or inadequate compliance with hand hygiene protocols by personnel [41, 53] so Hand washing and glove usage must be included in standard operating procedures (SOPs) for regular cleaning and disinfection. The staff should also be strictly monitored for compliance with these procedures. Hand hygiene will have an adverse impact on surgical tables due to contact with their surface during operation preparation.

High results of *Pseudomonas species* in the examination table and floor agreed with [1] who found that higher count may be attributed shortage in cleaning and disinfection program ,so must taking in consideration during choosing disinfectant to make effect on these microorganism.

Presence of *Pseudomonas* species on surgical table indicate bad hygiene inside surgical theatre which affected by contaminated hands of workers and instruments which have percentage (56%-60%) respectively.

6. Conclusion

The present study concluded that *Staphylococcus spp.* and *Pseudomonas* spp. present in small animals clinics especially in the examination table and floor. The improper cleaning program and disinfection lead to high results for isolated *Staphylococcus* and *Pseudomonas* species in the environmental surfaces.

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Table 1: Percentage of the isolated *Staphylococcus* and *Pseudomonas spp*. in this study.

Isolated microorganism	***Staphylococcus spp.***	***Pseudomonas spp.***
Number of samples	125	125
Total positives	77	80
percentage	61.6%	64%

Table 2: Percentage of isolated *Staphylococcus spp*. from different environmental surfaces.

Surface	**Hands of workers**	**Examination table**	**Surgical table**	**Instruments**	**floor**
Total samples	25	25	25	25	25
No. of positive	12	18	13	14	20
Percentage	48%	72%	52%	56%	80%

Table 3: Percentage of isolation of *pseudomonas spp* .from different environmental surfaces.

Surface	**Hands of workers**	**Examination table**	**Instruments**	**Surgical table**	**Flo**
Total sample	25	25	25	25	25
Number of positives	14	20	15	9	22
Percentage	56%	80%	60%	36%	88%

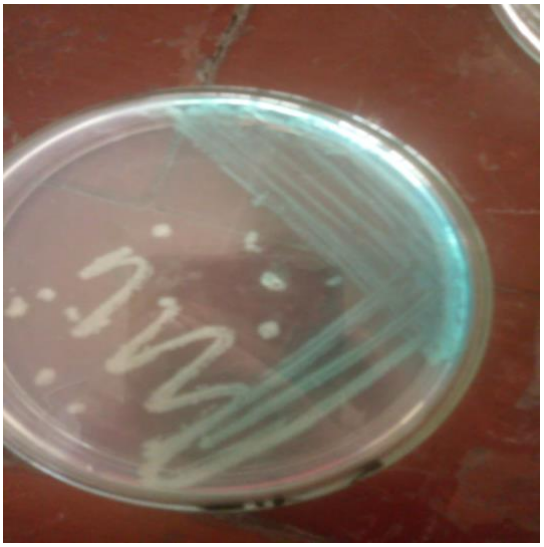

Fig .1: Exopigments of *Pseudomonas aeruginosa* with presence of green colonies and fluorescence

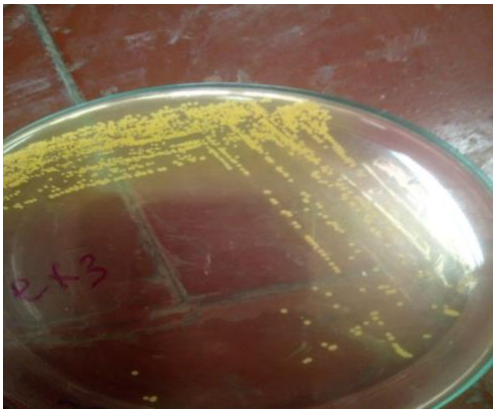

Fig .2: Mannitol fermentation, yellow colonies of *Staphylococcus aureus* on mannitol salt agar