Culture media for isolation of *Staphylococcus pseudointermedius* and *Staphylococcus* spp coagulase positive prevalence in domestic animals, veterinary practitioners, veterinary auxiliary workers and environment of a Veterinary Hospital

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**Staphylococcus aureus** and *Staphylococcus pseudointermedius* are well recognized as potential pathogens in both animal and human medicine. In the present study, oral, nasal and skin swabs were collected from 21 dogs and 2 cats attended in a Veterinary Hospital in Porto, Portugal, from veterinary practitioners and auxiliary workers (hands and nose), and from nine different contact surfaces used by veterinary practitioners, auxiliary workers and animals.

Swabs were cultured in Baird Parker – Rabbit Plasma fibrinogen (Biokar) and incubated at 37°C for 36 hours. Subsequently all coagulase-positive *Staphylococcus* were subcultured onto Chromagar STAPlaureus (CHROMAGAR) and then screened for antimicrobial susceptibility. Polymerase Chain Reaction was performed with primers for *S. aureus* (au-F3, and au-much) and *S. pseudointermedius* (pse-F2, pse-R5,) in order to identify the *Staphylococcus* species.

All colonies exhibiting typical *S. aureus* morphology (mauve colour) and all purple-blue coloured colonies were identified as *S. aureus* and *S. pseudointermedius*, respectively. This procedure has been proved to be reliable for *S. pseudointermedius* isolation, being an alternative to the laborious and time consuming biochemical tests.

Among the tested animals, 65.2% (n=15) carried coagulase-positive *Staphylococcus*: 30.4% (n=7) *S. aureus* and 52.2% (n=12) *S. pseudointermedius*. Two dogs (8.7%) carried methicillin resistant *Staphylococcus aureus* (MRSA) and four (17.4%) dogs were colonized with methicillin resistant *Staphylococcus pseudointermedius* (MRSP). Antimicrobial resistances to amoxicillin, thirmeropen-sulphamethoxazole and lomeloxacin were the most common in MRSP carriers. Four animals carried both *S. aureus* and *S. pseudointermedius* from the same swabs. In two animals, MRSP isolates presenting more than one antimicrobial resistance profile were found in the isolation place. Oral and nasal mucosa were the animal locations where more *S. aureus* bacteria were isolated while *S. pseudointermedius* were isolated mostly in oral mucosa and skin.

Among the environment swabs, *S. pseudointermedius* was isolated from the floor of the Hospital recovery area and the computer keyboards, both isolates being MRSP. *S. aureus* was found only in computers keyboards.

Regarding the nine veterinary practitioners and auxiliary workers tested, in all hand samples and in 22.2% of the nasal swabs, *Staphylococcus* displaying coagulase positive activity were isolated. Hand isolates consisted of *S. aureus* in 88.9% (n=8) and *S. pseudointermedius* in 55.6% (n=5), one of which was MRSP. Only 22.2% (n=2) presented *S. aureus* in nose samples and none *S. pseudointermedius* was isolated.

*S. aureus* isolated from computer keyboard and veterinary practitioners displayed the same resistance pattern.

This last fact alerts to the necessity of good hygiene practices such as hand washing, aseptic practices and good surface disinfection during all processes of animal management.

**Keywords**: *Staphylococcus aureus*, *Staphylococcus pseudointermedius*, antibiotic resistance; veterinary practice

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**Detection of resistant mutants within *Pseudomonas aeruginosa* colony morphology variants in lung cystic fibrosis environment**

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Bacterial infections caused mainly by *P. aeruginosa* are typical of cystic fibrosis (CF) lung disease. Despite the long and aggressive antibiotic therapy, CF patients still died because of these chronic infections. The deprived bacterial eradication is mainly due to several strategies adopted by bacteria to achieve CF Airways adaptation and tolerance to antibiotics. Biofilm formation and phenotypic switching are among the most relevant adaptive biological processes. Triggering those processes bacteria have the potential to better survive to CF conditions and antibiotics action. Phenotypic switching provides a source of microbial diversity through switch between two phenotypic states, analogue to a mechanism ON/OFF, without the fitness costs of irreversible mutations. This interchange of states, visible by differential colony morphology, can have serious impact on bacterial virulence, antimicrobial resistance and persistence.

The present work aims to investigate the specific colony variants-forming bacteria responsible by typical CF chronic infections. Through isolation and deep characterization of those colony variants, including discriminatory antibiotic susceptibility profiles and virulence characterization, it is intended to determine the mechanisms underlying the inefficiency of antimicrobial therapies of airway CF.

**Keywords**: cystic fibrosis, antibiotic resistance, colony variants, *P. aeruginosa*, resistant mutants

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