



Keywords

Biofilm, methicillin-resistant,
Staphylococcus
pseudintermedius, *icaA*, *icaD*.

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Biofilm-forming ability and virulence factors of methicillin-resistant *Staphylococcus pseudintermedius* from canine pyoderma.

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Staphylococcus pseudintermedius (SP) has been associated with high antibiotic-resistance rates (e.g. methicillin) in European countries (Flemming and Wingender., 2010; Osland et al., 2010; Bardiau et al., 2013). This condition could be also related to the ability to produce biofilm (Flemming and Wingender., 2010). The aim of this study was to investigate the presence of methicillin-resistant SP strains and determine their ability to produce biofilm and some crucial virulence factors.

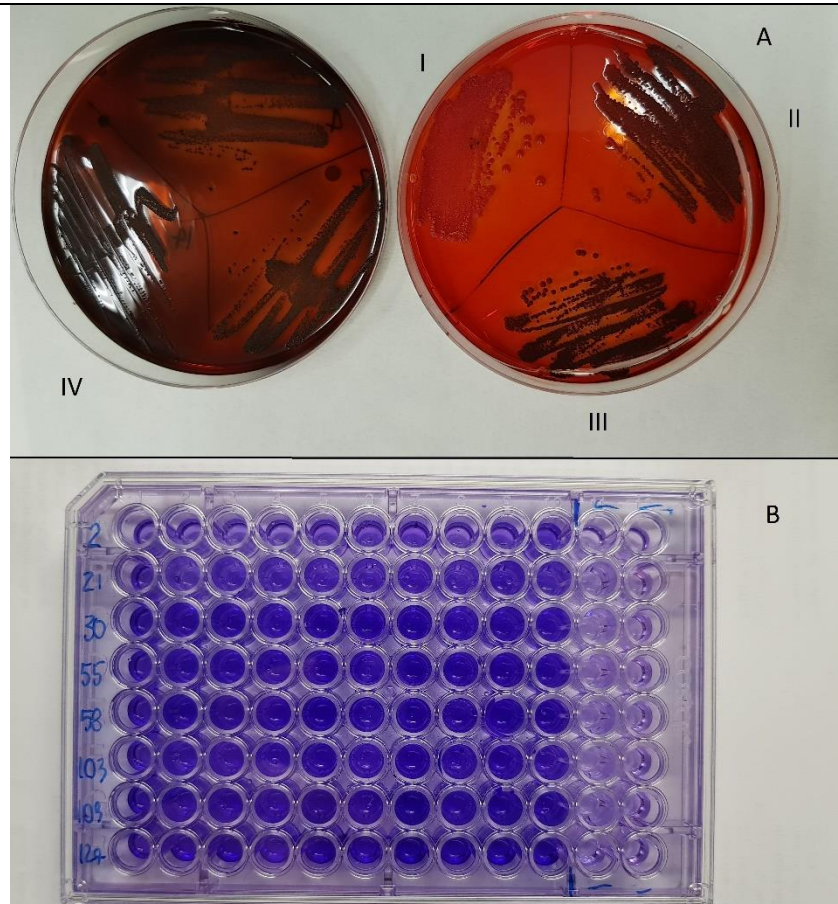
Forty-two SP strains, previously determined as multi drug resistant (MDR) by the disk diffusion method using a panel of 17 antimicrobial agents, were selected from our collection and tested phenotypically for the minimum inhibitory concentration (MIC) of methicillin and genotypically for the presence of *mecA* and *blaZ* genes. The ability to produce biofilm was assessed phenotypically by two different assays: the Congo Red Agar plates (CRA) and the Microtiter Plate test (MtP) and genetically by the amplification of *icaA* and *icaD* genes. Three virulence factors genes coding for bicomponent leukocidin and enterotoxins (*luk-I*, *seC*, *se-int*) were searched.

Thirty-six strains revealed a value of MIC major than 16 µg/mL (threshold for resistance); the remaining six isolates were sensible to this antibiotic. The 73% (31/42) were *mecA*-positive and 86% (36/42) resulted *blaZ*-positive; all the strains positive for *mecA* were also positive for *blaZ*. All SP strains resulted biofilm-producers by MtP assay (Figure 1) and classified as weakly producers (4.7%), moderate producers (47.6%) and strongly producers (47.6%). In contrast, only 35.7% of all strains were considered biofilm-producers by CRA method. The amplification of *icaA* and *icaD* gene occurred respectively in 66.6% and 97.6%; only one strain was negative for both genes. Almost all strains were positive for *luk-I* (95%), *seC* (74%) and *se-int* (84%).

Our data reveal the pathogenicity potential of SP strains from dogs, suggesting that they could be considered zoonotic potential agents and confirming other previous studies (Osland et al., 2010; Singh et al., 2013; Stefanetti et al., 2017). In particular in this study we focused only on the dissemination of *mecA* and *blaZ* genes both coding for methicillin-resistance (*mecA* is present in

chromosome while *blaZ* in plasmid), but nowadays other authors reported the presence of methicillin-resistant SP strains mediated by *mecC* gene instead of *mecA*. Moreover could be observed a clear linkage between antibiotic-resistance and ability to produce biofilm.

Figure 1: A) Example of colourimetric appearance of colonies onto CRA: very red (I), bordeaux (II), almost black (III), black (IV); (B) MtP assay for detection of biofilm production in some strains (column 11 and 12: negative controls).



References

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