

# Performance of four different diagnostic tests for *C. difficile* infection in piglets

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## Abstract

*Clostridium difficile* is emerging as pathogen in man as well as in animals. In 2000 it was described as a cause of neonatal enteritis in piglets and it is now the most common cause of neonatal diarrhoea in the USA. In Europe, *C. difficile* infection (CDI) in neonatal piglets has also been reported. Diagnosis of this infection is based on detection of the bacterium or its toxins A and B. Most detection methods, however, are only validated for diagnosing human infections. In this study three commercially available Enzyme Immuno Assays and a commercial RT-PCR were evaluated by testing 172 pig faecal specimens. The results of each test were compared with cytotoxicity assays (CTA) and toxigenic culture as gold standards. Compared with CTA, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were for RT-PCR respectively 91.6%, 37.1%, 57.6%, and 82.5%, and for Premier Toxin A+B (Meridian): 83.1%, 31.5%, 53.1% and 66.7%, and for Immunocard tox A/B (Meridian) 86.6%, 56.8%, 66.9%, and 80.7%, and for VIDAS (BioMérieux): 54.8%, 92.6%, 85.0%, and 72.8%.

Compared with toxigenic culture sensitivity, specificity, PPV and NPV were; for RT-PCR 93.0%, 34.7%, 50.0%, and 87.5%, and for Premier Toxin A+B: 80.3%, 27.7%, 43.8%, and 66.7%, and for Immunocard tox A/B: 80.0%, 46.2%, 52.8% and 75.4%, and for VIDAS: 56.4%, 89.8%, 77.5%, and 76.7%.

We conclude that all tests had an unacceptable low performance as a single test for detection of *C. difficile* in pig herds and that a two step algorithm is necessary. Of all assays, the RT-PCR had the highest NPV compared to both reference methods and is therefore the most appropriate test to screen for absence of *C. difficile* in pigs as a first step in the algorithm. The second step would be a confirmation of the positive results by toxigenic culture.

# Risk assessment of MRSA isolates from swine using a diagnostic DNA microarray

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## Abstract

The aim of this study was to support a risk assessment of MRSA-isolates from swine using a diagnostic DNA-microarray to detect virulence-, toxin- and resistance related genes. In comparison with other species like poultry, cattle and humans there were only few isolates with virulence genes.

## Introduction

Methicillin- resistant *Staphylococcus aureus* (MRSA) is an important pathogenic agent causing nosocomial infections. The clonal lineage ST398-MRSA-V dominates in swine, in humans with occupational exposure to swine and in regions with intensive swine production (Tenhagen, B.-A. et al. 2009). Investigation of their antimicrobial resistance and SCCmec typing produced multiple analogue results. The aim of this study was to detect virulence-, toxin- and resistance related genes of swine isolates to support a risk assessment, in particular with respect to the transmission to humans.

## Material and Methods

We used DNA-chip-technology based on the STAPHY TYPE Kit (Alere Technologies GmbH, Jena) which identifies 333 clinically relevant markers for resistance and virulence in a single test.

After a linear PCR amplification the resulting single stranded and biotin labelled amplicons are stringently hybridised to a set of highly discriminative probes that are covalently bound onto the microarrays. Additionally, the phenotypic resistance of 71 isolates was investigated by broth microdilution to compare the genotypic and phenotypic resistance profiles. 83 isolates from swine and 48 isolates from other species were included in this genotype characterisation.

## Results and Discussion

The tests showed a good correlation between genotype and phenotype resistance. The array provides information about genes for PVL, toxic shock syndrome, exfoliativ toxins and enterotoxins and in addition resistance genes for example pleuromutiline and streptothricin. 10 of 131 investigated isolates contained virulence genes; only 2 of them were isolated from pigs. Within one spa-type we can discriminate a lot of different resistance gene patterns. For example in 39 isolates of the Spa-type t011 we investigated 16 different resistance gene patterns.

This microarray-technique is useful for risk assessment in veterinary diagnostic like it is in human medicine (Monecke et al, 2008) and it also provides information for epidemiological studies.

## Conclusion

Only 2,4% of the investigated isolates from swine showed virulence genes for enterotoxin, and none was positive for PVL, toxic shock syndrome or exfoliativ toxins. The isolates showed good correlation between phenotypic and genotypic resistance. The DNA-Chip-technology provides essential information for epidemiological studies in a one step analysis.

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## References

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