Detection of Mollicute species (Mycoplasma) in House Dust Mites

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BACKGROUND

House dust mites are associated with asthma, rhinitis and many allergies. The products of house dust mites are used by pharmaceutical and diagnostic companies to gain a better understanding of diseases and making new medicines and cures. Ideally, all mite products have to be made by Good Manufacturing Practice and should be free of contaminants. Since HDM products are made from animal material, different kind of bacteria could be present in HDM products and HDM its self. Sterilization of mite extract products cannot take place by autoclaving and filtering over 0,22µm filter would be the solution diminish bacterial and fungal contamination, but this does not apply to one bacterial specie called Mollicutes. **Mollicutes** are the smallest known bacteria that lack a cell wall and infect hosts of humans, animals and plants. With their size of approximately 0.1 µm, they can easily pass through a filter of 0,22µm. Different studies have already revealed that HDMs have distinct species-specific bacterial communities. They conclude that "other Gram-negative bacteria" and "bartonella-like organisms" are present in DNA of HDMs, but none of them mention Mollicutes species specifically[1]. This is the very first study where Mollicutes are detected in house dust mites.

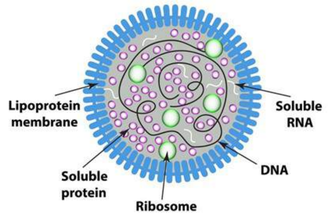

FIGURE 1: *Structure of a Mollicute cell*

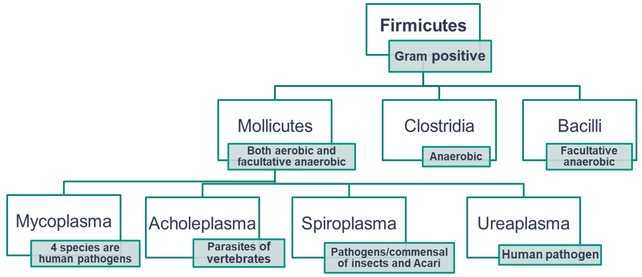

FIGURE 3: *phylogenetic diagram of different Mollicute species*

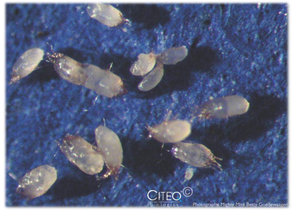

FIGURE 2: *House dust mites close-up*

MATERIAL & METHODS

SAMPLES USED FOR THIS STUDY

The table below shows the different products from 5 different suppliers (A to E). These products contain the whole culture (**WC**), mite bodies (**MB**) from two different HDMs. The products include the European house dust mite Dermatophagoidus Pteronyssinus (**DP**) and the American house dust mite Dermatophagoidus Farinae (**DF**).

TABLE 1: *Different HDM products used for this study from supplier A to E*

SUPPLIER	Medium	WC DP	WC DF	MB DP	MB DF	Living mites
A				X	X	
B		X	X	X		
C	X	X	X	X	X	
D		X	X			
E		X	X			

1ST METHOD; AGAR CULITVATION

The mycoplasma agar base was used with enrichment of Mycoplasma supplement-G for the isolation of Mycoplasmas in HDMs. After homogenizing the mites, the sample was spread on the plate and were then incubated at 35 °C up to 6 weeks. After incubation, the plates were selected for bacterial colonies and used for PCR to confirm the presence of mycoplasma.

2ND METHOD; PCR

For PCR-based method, HDMs was extracted using a DNA extraction kit. After extraction, the DNA was used for detection of Mollicutes with PCR, with an universal generic-specific primers as well as a total of 11 Mollicute species-specific primers. The 11 species-specific primers are based on the most common pathogenic mycoplasma species in humans, including Mycoplasma, Acholeplasma and Ureaplasma.

TABLE 2: *11 Mollicute species specific primers used for PCR method*

	Primers used for PCR					
Mycoplasma	Arginini	Orale	Hyorhinis	Fermentans	Genitalium	Laidlawii
	Pirum	Pneumoniae	Salivarium	Synoviae	Bovis	Hominis
Ureaplasma	U. urealyticum					
Acholeplasma	A. laidlawii					

RESULTS

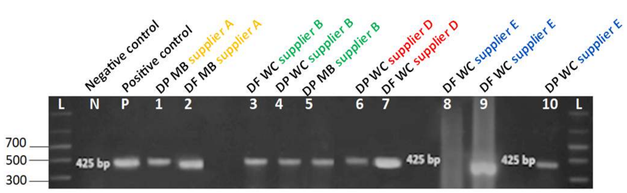

FIGURE 4 *Agarose gel (1.2% agarose) of PCR amplified products using a general Mycoplasma PCR primer set. Lane 1 to 10 are different product samples from only supplier A, B, D and E. The description above the lane number indicates which sample is loaded. Lane L is a 200 bp DNA size ladder. Lane N is a negative control and Lane P is a positive control of A.laidlawii.*

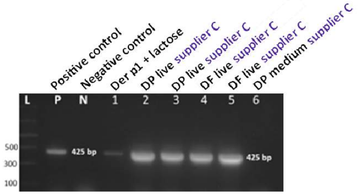

FIGURE 5 *Agarose gel (1.2% agarose) of PCR amplified products using a general Mycoplasma PCR primer set. Lane 1 to 6 are different products from only supplier C. The description above the lane number indicates which sample is loaded. Lane L is a 200 bp DNA size ladder. Lane N is a negative control and Lane P is a positive control of A.laidlawii.*

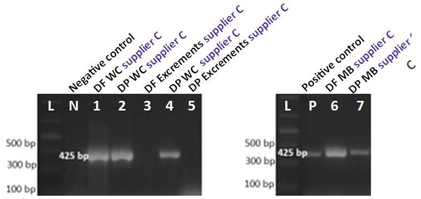

FIGURE 6 *Agarose gel (1.2% agarose) of PCR amplified products using a general Mycoplasma PCR primer set. Lane 1 to 7 are different products from only supplier C. The description above the lane number indicates which sample is loaded. Lane L is a 200 bp DNA size ladder. Lane N is a negative control and Lane P is a positive control of A.laidlawii.*

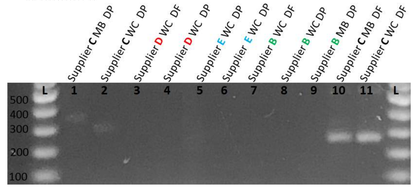

FIGURE 7 *Agarose gel (1.2% agarose) of PCR amplified products using M.Pneumoniae primers. Lane 1 to 11are different products from supplier B to E. The description above the lane number indicates which sample is loaded. Lane L is a 100 bp DNA size ladder.*

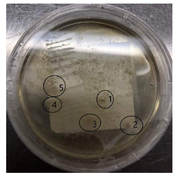

FIGURE 8 *Microorganism growth on Mycoplasma-G agar plate of sample (Mite bodies DP, supplier C). Number 1, 2 and 3 are formed colonies after 5 days of growth at 35 °C. The black circle is one formed colony that is used for control with PCR.*

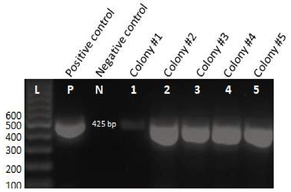

FIGURE 9 *Agarose gel (1.2% agarose) of PCR amplified products using a universal Mycoplasma PCR primer set. Lane 1-5 are different PCR products from colonies of MB from supplier C. Lane L is a 200 bp DNA size ladder. Lane N is a negative control and Lane P is a positive control of A.laidlawii.*

CONCLUSION

Results show that it is very likely that HDM source material from supplier A to E contain Mycoplasma after detection with PCR with the universal primer. Only MB from supplier C showed a mycoplasma positive result with the agar cultivation method and colony PCR with the universal primer. Only HDM extracts do not contain Mycoplasma. PCR of the HMD mite bodies, whole culture, medium with 11 species specific Mollicute primers (see table 2) showed only presence of Mycoplasma Pneumoniae in DF. Based on our results, we recommended that HDM product suppliers should test all their products on Mollicutes. Exclusion of Mollicutes in HDM products ensures that they are free of contamination and made by GMP. Both methods must be optimised and extended for detecting more Mollicutes since only 11 different specific species were tested.

1 Alejandra Perotti & Braig, 2011; Hubert, Kopecky, Nesvorna, Alejadra Perotti, & Erban, 2016; Hubert et al., 2012; Valerio et al., 2005