Mechanism of inactivation of Pseudomonas aeruginosa by cold gas plasma

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phenotypic tests have mostly remained unchanged. However, where PCR methods can only detect the targets for which they are designed, phenotypical testing will detect strains with that characteristic, irrespective of the underlying genetic mechanism. Recently, a new test was published to detect the capability of an isolate to hydrolyze carbapenems, the Carba NP Test. Up until now, the ability to hydrolyze carbapenems was tested using the Modified Hodge Test. However, interpretation of this test is highly subjective and both specificity and sensitivity leaves much to be desired. The Carba NP test claims to be 100% specific and sensitive and to be faster.

Methods: The Carba NP test relies on the creation of an enzymatic bacterial suspension from each isolate. This suspension is then added to mixture of imipenem monohydrate and phenol red. Upon hydrolysis of the imipenem by carbapenemases within the enzymatic bacterial suspension, pH levels will decline and the phenol red solution will turn from reddish purple to orange / yellow. Forty isolates of various species of Enterobacteriaceae and non-fermenters were tested with this method, both with and without known carbapenemase genes, with both low and high meropenem MIC’s and also including reference strains.

Results: The Carba NP test was positive for isolates containing a carbapenemase gene and negative for those lacking these genes, regardless of their MIC’s. One false-negative isolate was found to be carrying a carbapenemase gene not routinely tested for. One false-negative isolate is believed to carry a non-functional NDM gene, which shall be further investigated.

Conclusion: The phenotypic test in order to detect the ability of isolates to hydrolyze carbapenems was already successful in identifying an additional carbapenemase positive isolate. The Carba NP test offers a fast (< 3 h) and reliable method to do so. However, this method requires a large amount of imipenem (0.3 mg) for each isolate, making it a costly test ($68/40 isolate) for general screening purposes. Efforts to modify the test to reduce cost are being made at this time.

P042 Characterization of extended spectrum beta-lactamase-producing, multiluqdrug-resistant Enterobacteriaceae in culinary herbs imported from South-East Asia

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Objectives: As part of a national study on the occurrence of ESBL-producing Enterobacteriaceae in food samples, imported herbs from different South-East Asian countries were included.

Methods: The Netherlands Food and Consumer Product Safety Authority (NVWA) in Wageningen collected 32 fresh herb samples imported from South-East Asia. Ten gram of each sample was selectively enriched in 50 ml Luria Bertani broth with 1 mg/L cefotaxime. After 16 - 20h incubation at 37 °C all samples were inoculated on MacConkey with 1 mg/L cefotaxime as well as on Brilliance ESBL agar. After 16 - 20h incubation at 37 °C isolates with growth typical for Enterobacteriaceae were pure cultured on blood agar plates and sent to the Central Veterinary Institute (CVI) in Lelystad for further analysis. The analysis included susceptibility testing by broth microdilution according to ISO 20776-1:2006, screening for resistance genes with micro-array (Affymetrix GeneChip Technology), PCR and sequencing of specific beta-lactamase and plasmid mediated quinolone resistance (PMQR) genes, transformation of plasmids in DH10B cells followed by PCR bases replication typin (PBR) of plasmids. To determine the location of the ESBL and/or PMQR genes S1-PFGE was performed followed by SacIoh resonance hybridization. Finally, the bacterial isolates were identified to species level by MALDI-TOF mass spectrometry at the GD Animal Health Service in Deventer.

Results: The study resulted in 24 cefotaxime resistant Enterobacteriaceae obtained in 32 imported herb samples, which comprised of 18 samples from Thailand (Morning Glory, Acai and Betel Leaf), 5 samples from Vietnam (Palely, Rais M, Huoutamya leaf and Peppermint) and 1 sample from India (Holy Basil). A total of 17 isolates (70.8%) were identified as Klebsiella pneumoniae (n = 9), Enterichia coli (n = 6), Enterobacter cloacae (n = 5) and Enterobacter spp. (n = 1). All isolates tested were remarkably multidrug-resistant showing resistance against 4 to 10 different antibiotic classes. Variants of CTX-M enzymes (CTX-M-9, -14, -15 and -49) were predominantly found (n = 15) followed by SHV-12 (n = 4). Furthermore, a high number of isolates with PMQR genes were detected (n = 18). PCR and sequencing revealed 2 isolates with qnrB, 4 isolates with both aac(6’)-1b-cr and qnrB, 2 isolates with both qnrB and qnrS and 10 isolates with qnrS. In almost all cases the ESBL gene and the quinolone resistance genes were located on the same plasmid. These plasmids were replicon typed as IncF, IncN, IncH1 or IncHI2. In 7 isolates the ESBL genes were located on a non-typeable plasmid. Finally, in one E. coli isolate hybridization experiments revealed both blaCTX-M-9 and qnrS were chromosomally located.

Conclusion: Imported culinary herbs from South-East Asia are a potential source for contamination of food with ESBL-producing, multiluqdrug-resistant bacteria. The extent of bacterial inactivation was further related to the distance, duty cycle and treatment time. Plasmas operated at low duty cycles (10-20%) reduced the viability of fibroblasts. Higher settings did not damage viability of fibroblasts. Plasma treatments of keratinocytes did not result in loss of activity. The effects of heat, ion- and UV- fluxes, electric fields, and gas flow were estimated not to have a prominent direct effect on bacterial inactivation. Rather, plasma exerts its effect through liquid phase chemistry, most likely via liquid ion chromatography and hydrogen peroxide concentration.

P043 Mechanism of inactivation of Pseudomonas aeruginosa by cold gas plasma

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Introduction: Bacterial contamination is a constant threat in burn wound care and needs to be treated. It is however important to keep a balance between inactivating the bacteria and maintaining the wound healing potential. Cold atmospheric pressure plasmas might provide additional means to reduce the bacterial load in a burn wound. Atmospheric pressure plasmas have been used for many years for different applications. Atmospheric pressure plasmas deliver electrons, ions, UV radiation and an electric field, which together are effective in killing bacteria. We studied the effects and mechanism of cold argon plasma treatment on in vitro inactivation of Pseudomonas aeruginosa, which is commonly isolated from burn wounds.

Methods: For the treatment, a pulsed cold atmospheric plasma jet (35-36 MHz micro-jet) was used. Bacteria were heat killed in physiological salt with plasma at different settings for different times. Surviving bacteria were counted by plating dilutions. Because reactive radicals in plasma can interfere with the healing process, cell cultures of keratinocytes and fibroblasts in physiological salt were treated with cold plasma after which culture medium was added. To estimate cell damage due to the treatment, activity of the cells was quantified with a tetrazolium based assay.

To elucidate the mechanism of plasma mediated inactivation, we studied gas temperature, absolute UV irradiance, emitted ions (mass spectrometry) and induced liquid chemistry (liquid ion chromatography and hydrogen peroxide concentration).

Results: Bacterial inactivation reached up to 7-log reduction after 1 min. Plasma treatment leads to a pH decrease in non-buffered solutions which is critical for plasma activity. Inactivation was hardly observed in phosphate buffered saline or culture broth. The extent of bacterial inactivation was further related to the distance, duty cycle and treatment time. Plasmas operated at low duty cycles (10-20%) reduced the viability of fibroblasts. Higher settings did not damage fibroblasts. Plasma treatments of keratinocytes did not result in loss of activity.

The effects of heat, ion- and UV-fluxes, electric fields, and gas flow were estimated not to have a prominent direct effect on bacterial inactivation. Rather, plasma exerts its effect through liquid phase chemistry, most likely via liquid ion chromatography and hydrogen peroxide concentration.

Conclusion: Non-thermal argon plasma can be used to kill bacteria and yet preserve the viability of skin cells. The bactericidal effect can be ascribed to plasma induced liquid chemistry, leading to the production of stable and transient chemical species.

P044 Detection of Streptococcus pneumoniae in saliva samples from Dutch primary school children

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With the introduction of pneumococcal conjugate vaccines, more in-depth carriage studies are required to monitor the effects of vaccination. Here, we investigated saliva as a means of detecting Streptococcus pneumoniae colonization for potential use in surveillance on pneumococcal carriage. We were inspired by Gundel and Okura (Zerst Lrg, 1993) describing rates of up to 80% pneumococcal carriage in healthy schoolchildren, detected in saliva using a mouse inoculation method. We applied conventional culture and molecular methods to detect pneumococci in uncultured saliva samples from 49 schoolchildren, aged 5 to 12 years, from a rural school near Utrecht, on a single morning in June 2012. Each child spat into a disposable container. Samples were placed on wet ice and transported to the lab within 2 hours. Blood agar plates supplemented with 5 mg/l gentamicin were inoculated with 100 ul of sample and the remaining 50 ul was streaked on blood agar and culture broth. Samples were overnight incubated at 37 °C and 5% CO2 cultures were inspected for the presence of S. pneumoniae. Next, all bacterial growth was harvested in 1 ml of 10% glycerol in BHI and stored at -80 °C. DNA was extracted from 100 ul of thawed raw culture enriched samples, using either a modified Qiagen DNAeasy kit protocol or bead-beating of the samples followed by magnetic separation of DNA with Agena reagents. Puriﬁed DNA was tested by quantitative-PCR (qPCR), targeting S. pneumoniae speciﬁc genes lytA and piaA. Samples were considered positive with CT values for both genes > 40. Saliva cultures show abundant polymicrobial growth, which allowed detection of S. pneumoniae in 81% of S. pneumoniae positive saliva samples. We compared efﬁciency of DNA isolation methods, DNA templates from raw and culture-enriched samples from 15 randomly selected children were tested by qPCR. The lytA-speciﬁc signal was stronger for templates puriﬁed with 15 mg of material compared to Qiagen protocol (average ΔCt 24 CT for raw and D6.94 CT for culture-enriched sample). Thus, DNA was isolated from all samples using the Agowa protocol.