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SHORT COMMUNICATION
ΒΡΑΧΕΙΑ ΔΗΜΟΣΙΕΥΣΗ

Cost- and time-effectiveness of application of MALDI-TOF mass spectrometry methodology in a food and water microbiology laboratory

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Εξοικονόμηση κόστους και χρόνου από την εφαρμογή της μεθοδολογίας MALDI-TOF-MS στο περιβάλλον ενός μικροβιολογικού εργαστηρίου υδάτων και τροφίμων

Περίληψη στο τέλος του άρθρου

Key words: Bacteria identification, Cost, MALDI-TOF-MS, Time

In a diagnostic laboratory not only the quality of results needs to be considered, but also the speed, cost and usefulness or clinical relevance of the test used. Laboratory tests in general are expensive and, with progress in medicine, they tend to account for an increasing proportion of the health budget. MALDI-TOF mass spectrometry (MS) has been successfully adapted to the needs of clinical microbiology laboratories throughout the world, allowing for instant identification of microorganisms by analyzing their protein fingerprint, at the same time as fulfilling all of the above prerequisites.[1–3] Taking advantage of the low cost of consumables, the extreme speed, and the labor- and specialization-free technique of MALDI-TOF-MS we used Bruker's Microflex LT MALDI Biotyper (Bruker Daltonics) in parallel with conventional biochemical and molecular techniques for the identification of 9 pathogens in a food and water microbiology laboratory in Greece. The organisms of interest according to the Greek legislation concerning food and water safety are *Listeria* sp, *Salmonella* sp, *E. coli*, *E. coli* O157:H7, *Staphylococcus* spp, *Coliforms, Pseudomonas* spp, *Cambylobacter* sp, and *Legionella* sp. The aim of the study was to evaluate the benefits, if any, in turnaround time and cost of analyzing 2,800 food and water samples that were received in a Regional Laboratory of Public Health for analysis during a 12-month period (December 2010–December 2011).

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SAMPLE ANALYSIS

In brief, the workflow of the analysis of a sample following isolation from a culture medium was the simultaneous testing of the colonies for identification by conventional and biochemical tests, API strips (where applicable), polymerase chain reaction (PCR), real-time PCR and sequencing analysis, and in parallel by MS. As regards the MS technique, the direct transfer protocol by Bruker was employed, by which a small colony was placed on the target placing one microlitre of matrix on top.

RESULTS AND DISCUSSION

Although Bruker's Biotyper obtained an IVD-CE mark with a Declaration of Conformity in accordance with the European Union *in vitro* Directive 98/79/EC in 2009, at the time of this report, the Biotyper has not yet been certified by the Greek Health Authorities for use in routine diagnosis which prompted us to focus on reproducibility, comparability and cost-effectiveness studies. Concerning turnaround time, the most characteristic example of the advantage of the MS methodology was that of *Legionella* sp testing where molecular methods were employed for the identification of the bacterium. Following bacterial growth on *Legionella* selective culture medium (BCYE) we performed DNA extraction (20 min/sample) and used real-time PCR (40 min) in order to distinguish between *L. pneumophilla* and the remaining species of the genus. When real-time-PCR indicated a non-pneumophilla species, classic PCR, gel electrophoresis, ethidium bromide staining (30 min), PCR product purification and gene sequencing

(30 min/sample) were used in order to determine the exact species of the bacterium.

As regards the rest of the pathogens (*Listeria* sp, *Salmonella* sp, *E. coli*, *E. coli* O157:H7, *Staphylococcus* sp, *Coliforms*, *Pseudomonas* sp, *Cambylobacter* sp), following isolation of the pathogen, the respective API test was applied in order to determine its identity according to the corresponding ISO methodology.

Using MS, the time required for identification was 10 min/sample irrespective of the species, without the need for implementation of the above mentioned techniques. The larger the number of the samples the more obvious was the advantage of MS compared to classical techniques concerning turnaround time.

Concerning comparison of the methodologies at the level of cost-effectiveness the results are even more dramatic. Regarding the API strips methodology, an average of 15 €/sample was spent in order to reach an identification at the species level. In contrast, identification by MS was calculated to cost 0.15 €/sample (dual runs for each sample and test standards included). Once more, the pathogen that showed the greatest difference in cost between the methods was *Legionella*. Specifically, following DNA extraction (15 €/sample) and real-time-PCR (42 €/sample), 57 €/sample was spent in total for *L. pneumophilla* to be distinguished from the other species. In the case of a non-pneumophilla species, classic PCR (35 €/sample), gel electrophoresis (7 €/sample), PCR product purification and gene sequencing (20 €/sample) added 62 €/sample, raising the total cost of identification to 119 €/sample. The cost of the MS methodology remained at 0.15 €/sample irrespective of the result.

In order to comprehend the magnitude of the difference in cost-effectiveness of the two methodologies it must be considered that 1,200 water samples that were received in the laboratory during the twelve-month period. Of the 1,200 samples cultured, 115 were finally identified as *Legionella* colonies. The identification of *L. pneumophilla* in 55 of the samples cost 3,135 €, while 6,540 € were spent for identification of non-pneumophilla species, raising the total cost to 9,675 € for the use of molecular methods. Using the MS methodology the typing of the 115 samples required just 17.25 €. These costs were calculated based only on the consumables used in each methodology. Consideration of the extra hours of work (additional personnel) required for the traditional methodology is of no small issue.

As regards the identification of the rest of the pathogens (*Listeria* sp, *Salmonella* sp, *E. coli*, *E. coli* O157:H7, *Staphylococcus* spp, *Coliforms*, *Pseudomonas* spp, *Cambylobacter* spp), in summary a total of 5,500 API tests were employed, at total cost of 63,000 €, compared with 825 € spent using the MS identification technique.

Summing up, during the one-year period of this study, the laboratory spent a total of 72,675 € in consumables in order to identify the pathogens of interest by the traditional molecular and biochemical techniques, and a total of 843 € using the MS identification technique.

It should be reported at this point that concerning the pathogens tested in this survey there was 100% concordance between the results produced by the MS methodology and the rest of the techniques. These cost- and time-related data, combined with the proven power of the MS methodology concerning accurate identification of microorganisms in clinical microbiology[1–3] are in strong support of the implementation of such state of the art instrumentation in the routine food and water microbiology laboratory.

CONCLUSIONS

Apart from the obvious huge decrease in time and costs that would be achieved by the phasing out of the traditional methodologies in favour of MALDI-TOF-MS, application of the MS methodology for the identification of microorganisms in food and water samples could practically eliminate false negative results. The reason for this is that since the MS methodology is such a rapid, cost effective and straightforward procedure, all the colonies developed on a culture plate can be analyzed, and not just those that phenotypically resemble the pathogen of interest, eliminating the possibility of biased errors.

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ΠΕΡΙΛΗΨΗ

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Η φασματομετρία μάζας έχει εισαχθεί επιτυχώς στη ρουτίνα διαγνωστικών μικροβιολογικών εργαστηρίων σε νοσοκομεία της Ευρώπης. Συγκρίθηκε η μεθοδολογία MALDI-TOF-MS με τις συμβατικές τεχνικές ταυτοποίησης μικροβίων στο περιβάλλον ενός μικροβιολογικού εργαστηρίου τροφίμων, όπου φάνηκε ότι η μεθοδολογία της φασματομετρίας μάζας είναι μια ταχεία, αποδοτική και εύκολα πραγματοποιήσιμη διαδικασία, η οποία, τηρουμένων των αναλογιών, μπορεί να εξοικονομήσει χρήματα, χρόνο και προσωπικό, εξαλείφοντας ταυτόχρονα τις πιθανότητες ανθρώπινου λάθους στη διάγνωση.

Λέξεις κλειδιά: Κόστος, Ταυτοποίηση βακτηρίων, Φασματομετρία μάζας, Χρόνος

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