Norwegian Institute of Public Health

Salmonella identification by MALDI-TOF MS

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Introduction

Biochemical and serological methods are traditionally used to identify *Salmonella* from clinical samples. The National Reference Laboratory for Enteropathogenic Bacteria; Norwegian Institute of Public Health wanted to examine if MALDI-TOF MS (Bruker Daltonik GmbH) could replace biochemical tests in the identification of *Salmonella* to genus level prior to serological testing for final identification. At the same time, we wanted to examine whether various growth media would influence the results obtained using this method.

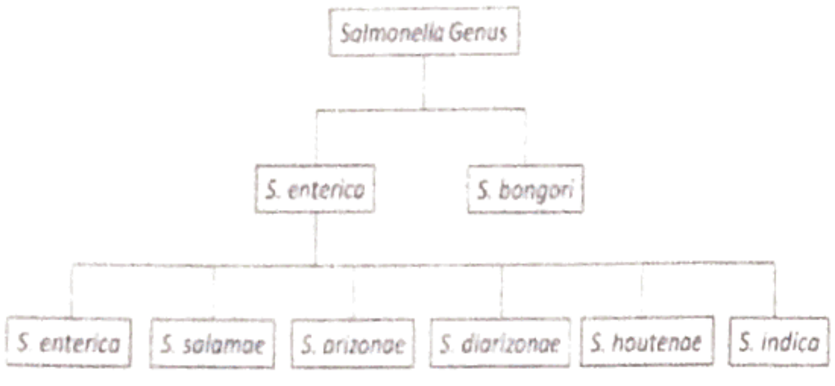

Fig. 1. Overview of taxonomic classification of *Salmonella*.

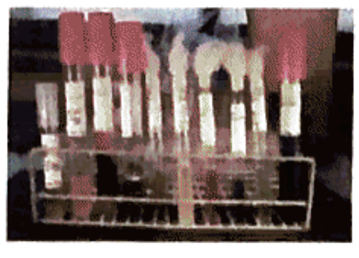

Fig. 2. Example of biochemical tests.

Material and Methods

A total of 101 *Salmonella* isolates from our national strain collection were analysed:

- Species *S. enterica* (including all six subspecies:
  - *S. enterica* (50)
  - *S. salamae* (11)
  - *S. arizonae* (6)
  - *S. diarizonae* (13)
  - *S. houtenae* (10)
  - *S. indica* (1)
- Species *S. bongori* (10)

Fig. 3. *Salmonella* grown on Columbia blood- ,lactose- and nutrient agar.

Morphological and phenotypical deviant strains from *S. enterica* ssp. *enterica* were among the included isolates. Each isolate had previously been identified using standard biochemical and serological methods (Fig. 2 and Table 1).

Bacteria were grown on lactose-, nutrient- and Columbia blood-agar, which are traditionally used in our laboratory (Fig. 3), and subsequently analysed using Microflex LT MALDI-TOF MS with MALDI biotyper 3.1 software (Fig. 4). Each isolate was prepared in duplicate using the smear method described by the manufacturer. The parallell with the lowest score was excluded.

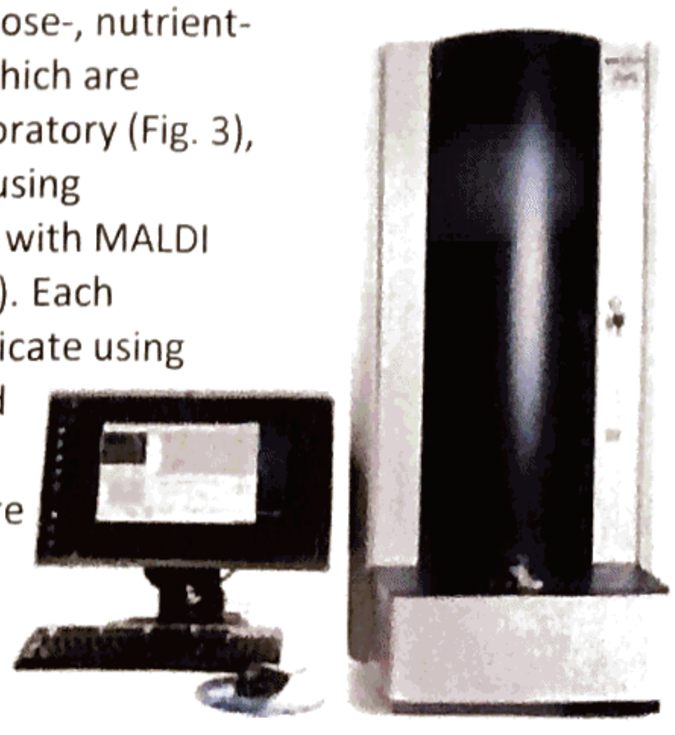

Fig. 4. Microflex LT MALDI-TOF MS (Bruker Daltonik GmbH).

Species	*S. enterica*						*S. bongori*
Subspecies	*enterica*	*salamae*	*arizonae*	*diarizonae*	*houtenae*	*indica*	
Characters							
Dulcitol	+	+	–	–	–	d	+
ONPG (2 h)	–	–	+	+	–	d	+
Malonate		+	+	+	–	–	–
Gelatinase	–	+	+	+	+	+	–
Sorbitol	+	+	+	+	+	–	+
Growth with KCN	–	–	–	–	+	–	+
L(+)-tartrate[a]	+	–	–	–	–	–	–
Galacturonate	–	+	–	+	+	+	+
γ-glutamyltransferase	+[(R)]	+	–	+	+	+	+
ß-glucuronidase	d	d	–	+	–	d	–
Mucate	+	+	+	(70%)	–	+	+
Salicine	–	–	–	–	+	–	–
Lactose	–	–	– (75%)	+ (75%)	–	d	–
Lysed by phage O1	+	+	–	+	–	+	d
Usual habitat	Warm-blooded animals		Cold-blooded animals and environment				

(a) = *d*-tartrate.
(R) = Typhimurium d, Dublin –.
\+ = 90 % or more positive reactions.
– = 90 % or more negative reactions.
d = different reactions given by different serovars

Table 1. Differential characters of *Salmonella* species and subspecies (Grimont and Weill, 2007).

Results

The results showed acceptable scores (≥2.0) for all *Salmonella* strains to genus level except for *S. enterica* ssp. *Houtenae* (Fig. 5). This subspecies was incorrectly identified in some duplicate tests and generally showed lower scores (<2.0). The score was ≥2.0 for 295 out of 303 analyses independent of growth media.

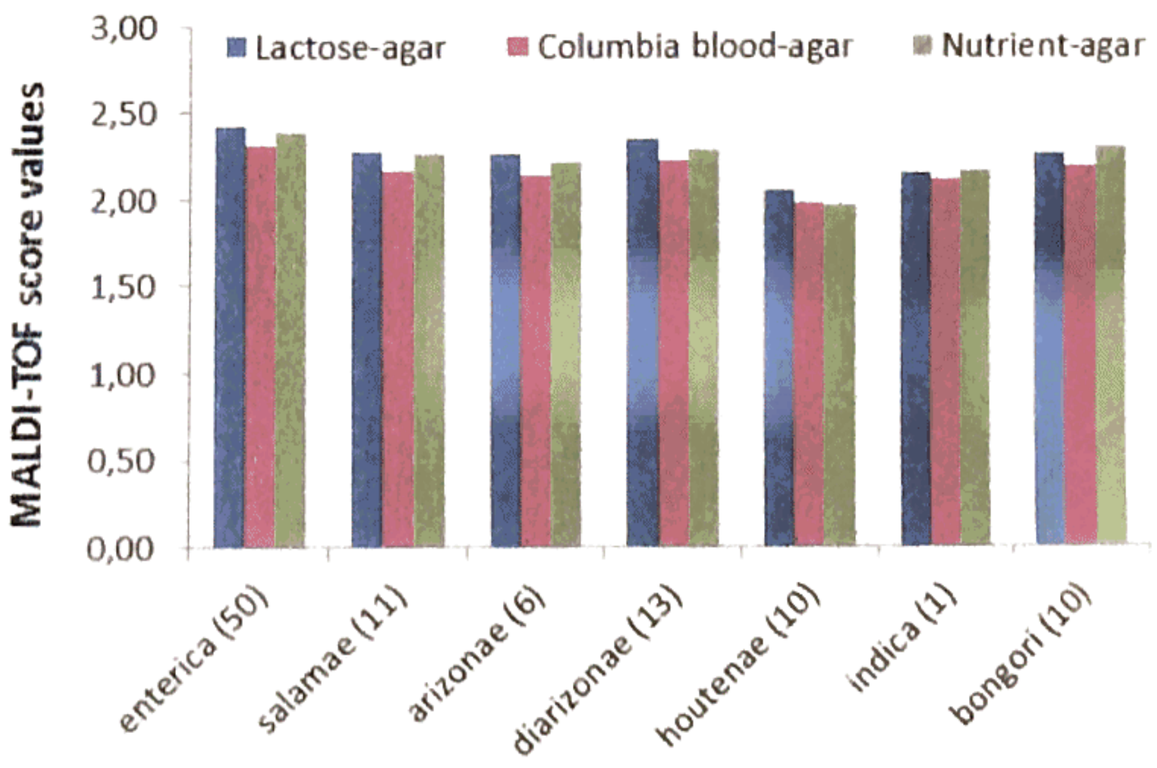

Fig. 5. Score values of *Salmonella* subspecies on different growth media.

Conclusions

- MALDI-TOF MS can replace biochemical methods for detection of *Salmonella* to genus level. The method did, however, have difficulties in identifying *S.houtenae,* but infections caused by this microbe are very rare in humans.
- Serological tests are still necessary to identify serotypes. Additional biochemical testing must be performed if different subspecies have equal antigenic formulae.
- The three growth media utilized are all equally suitable for use in the identification of *Salmonella* with MALDI-TOF MS.