Research Article

Trial Based Method for Detection of *Shigella spp* and *E.coli* by MALDI – TOF Mass Spectrometry

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ABSTRACT

Shigella spp was found to have very close relations with *E.coli*. Traditionally confirmation of both Organisms can be carried out by biochemical and serological tests. Most of the *Shigella spp* and *E.coli* had ended up with positively in both biochemical and serological testing. After the confirmation both the Organisms were processed for MALDI – TOF (Matrix – Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry). The total of 50 samples was processed, out of that 5 were positive for *Shigella spp* and the other 45 were identified as mixed cultures of *E.coli* and mixed cultures. The routine test result was indicative of the identified strains belongs to *E. coli* and other *Coliforms.* None of test organism was identified as *Shigella spp.*This technique could not help in the detection of *E.coli* to mostly as 95%. This study further urges that streamline in research to be done in order to fasten the diagnosis of *Shigella spp* in the near future. Other molecular diagnostic testing like PFGE, MLST, MVLA and WGS can be tested for pathological strains with better results.

Keywords: *Shigella spp, E.coli,* MALDI – TOF, Mass spectrometry.

INTRODUCTION

The Genus *Shigella* belongs to the Family *Enterobacteriaceae* and consists of four species, as *Shigella dysenteriae, Shigella flexneri, Shigella boydii* and *Shigella sonnei*[2]. *Shigella spp* appears as Gram negative rods, 0.3 to 1 micrometer in diameter in length. It shall also appear as lengthy, pairs and even as single too. They are facultative anaerobes, non-lactose fermenting and non-spore formers. The *Shigella spp* may be further differentiated by following the series of biochemical and serological tests.

Differentiation of *Shigella spp* from *E. coil* is one of the commonly faced issues in the diagnostic microbiological laboratory. This may reflect the fact, as the DNA relatedness study *E. coli* and *Shigella* should be considered as a single genetic species[5,7].

Thus, it is not surprising that it was practically impossible to distinguish on the basis of their DNA sequences. Both of them are very closely related and falls in the category of the Family named as *Enterobacteriaceae*.

The species of *Shigella* is found very closely related in the aspect of phenotype, whereas genotypically both of them differ. Due to its close relatedness the differentiation being most difficult, both of them fall under the same category as non-lactose fermenting, non-gas producing, non – motile organisms. Unfortunately 16S rRNA sequencing and MALDI-TOF is not tending to give reliable results.

Hence the researcher who spends his time to confirm the presence of *Shigella spp* by this technique will end him up with the waste of time spent, man power and expenses incurred for this purpose.

Shigella spp is one among the leading etiological agent, further known to the cause of diarrheal disease in humans exclusively in developing countries. It is estimated at more than 163 million cases, inclusive of 1 million fatalities; occur annually[4]. Even in areas relatively free from shigellosis, massive destruction due to war or natural disasters, such as the earthquake of 1999 in Kocaeli, Turkey, can suddenly give rise to multiple numbers of species, multi – focal increase in dysentery[10]. For example, in operation Iraqi freedom the incidence of *S. flexneri* and *S. sonnei* was about equal during the early phase of war[9].

The processes of horizontal gene transfer; most of the bacteria get the mutated plasmids from other bacteria which face and overcomes all the hurdles. Like antibiotic resistance, gaining vast characters for its survival.

The plasmid profiling study shall further state *Shigella spp* has increased the number of plasmids. This is a trail based approach based on MALDI – TOF MS to distinguish between both the *Enterobacteriaceae* members.

MATERIALS AND METHODS

Totally 50 bacterial strains were studied during the research and this clinical isolates were further screened for biochemical, serological and by MALDI – TOF MS. The study area as Edayarpalayam falls under Sultanpet block

has a population density as 234.19 persons per square kilometer as the least in the district whereas the other block considered for this study was Madukkarai block which have the population density as 1023.98 persons per square kilometer. It was the highest dense region in the entire district. Hence, in this study one block with the highest population density and the other block with the lowest population density were studied. An attempt was made in this study to identify any positive incidence in three different villages for *Shigella spp* in villages namely Edayarpalayam, Ettimadai and Malumichampatti located within Coimbatore district, Tamilnadu, India.

Primary Isolation Media

The diarrheal samples obtained from children of 0-5 were cultured in XLD, DCA and SS agar. It was further incubated at 35-37 ^{o}C for 18 -24 hours. The results were noted after the incubation period[8].

Biochemical Screening

Diarrheal isolates were tested for routine diagnostic as phenotypic and biochemical tests such as Gram's staining, Lactose fermentation test and TSI. Serotyping was done for the characterization of the species further. The sera by which the isolates get agglutinated will help in finding it in species level.

MALDI – TOF Analysis

The data of 7 clinical isolates of BrukerDaltonik MALDI Biotyper Classification results were considered as the proof for detecting the presence of *Shigella spp*. It was an automated approach as the sample was processed by laser and made to fly within the given time. The data obtained was considered for concluding the findings during the course of study.

RESULTS

In XLD agar plate the colonies of pink red with no black in center, ranges from 1-2 nm in diameter. Some strains of *Shigella spp* will have pink or yellow in color (**Fig 1**). At the DCA agar plate the colonies may be colorless (**Fig 1**). In SS agar translucent colorless colonies were identified (**Fig 1**). White translucent colonies were observed in Nutrient agar (**Fig 1**).

Figure 1: Culture plates showing the growth

On an EMB agar plate *E. coli* growth was observed as green metallic sheen (**Fig 2**). TSI agar slant shows the alkaline slant (k) and acid butt (A).

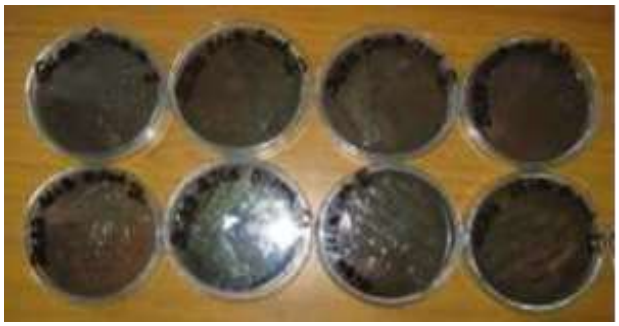


Figure 2: Culture plates showing the growth of *E.coli* on EMB agar

The automated approach of MALDI – TOF was given with the result overview, matching hints and the peaks (**Fig 4 & 5**). The findings of the study show that out of 7 clinical isolates one is showing as *E. coil* and the other may belong to *Enterobacteriaceae* (**Fig 3**). The last isolate was known as *E.coli* and the matching hint suggests that it shall closely relate to *Shigella spp*. Meanwhile the Biochemical tests and serotyping had confirmed that the seventh isolate was *Shigella spp*. These results gave a great surprise and I arrive into conclusion as the peaks of *Shigella spp* were not completely stored in the instrument.

SS agar, XLD agar, MacConkey agar & Nutrient Agar

***E.coli* on EMB agar**

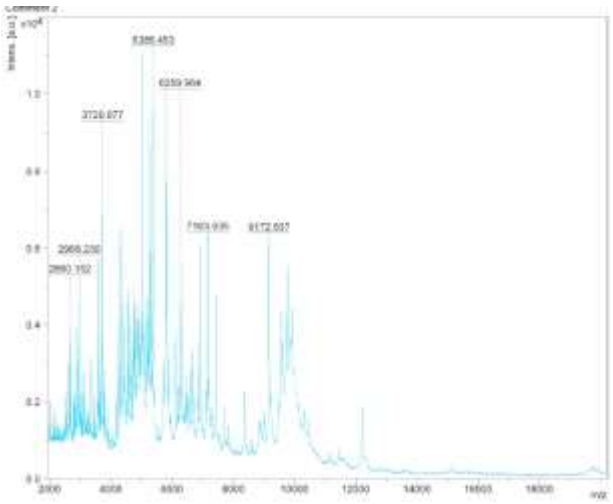
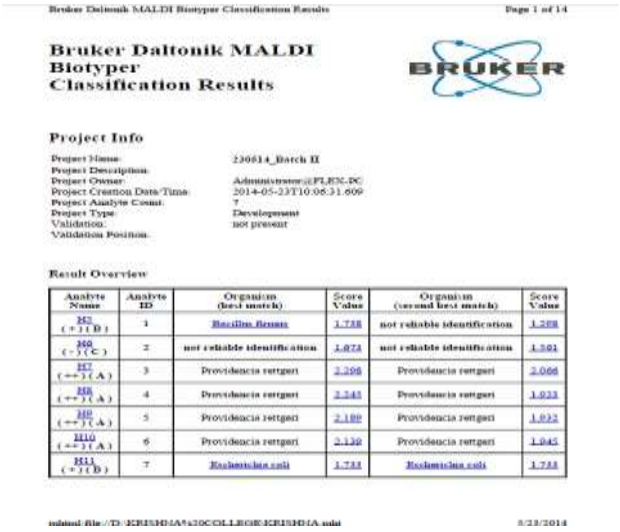


Figure 3: Acquisition parameter obtained from Bruker

DISCUSSION

The colony morphology as its growth in selective and differential agar hints that the strains may be a member of the Genus *Shigella* and *E.coli*. The Biochemical tests as TSI agar slant also support the strain may belong to the same as by showing acid slant and alkaline butt. Serotyping also confirmed that the strains obtained were *Shigella dysentriae* and *Shigella flexneri*.

One of the limitations of MALDI – TOF MS is its current inability of discrimination of the pathogenic *E.coli* from *Shigella spp* because of its close relatedness of organisms makes it as challenging[1]. The inability of routine MALDI – TOF MS to differentiate *Shigella spp* and *E.coli* is well recognized[3,6]. Most of the researchers state their finding with MLADI is not giving the exact result as it is as *Shigella spp*. Hence, to conclude the time spent by the researcher, money spent and manpower will not end up in a fruitful manner. This study further suggests that more peaks of *Shigella* have to be stored in the data bank of Bruker in order to make the researcher and the diagnostic process as simple and fast and reliable.



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Bruker Daltonik MALDI Biotyper Classification Results

BRUKER

Project Info

Project Name:	230814_Batch II
Project Description:	
Project Owner:	Administrator@FLEX-PC
Project Creation Date/Time:	2014-05-23T10:06:31.609
Project Analyte Count:	7
Project Type:	Development
Validation:	not present
Validation Position:	

Result Overview

Analyte Name	Analyte ID	Organism (best match)	Score Value	Organism (second best match)	Score Value
H7 (*1(D))	1	Bacillus flexus	1.738	not reliable identification	1.298
H9 (*1(C))	2	not reliable identification	1.074	not reliable identification	1.301
H7 (**1(A))	3	Providencia rettgeri	2.206	Providencia rettgeri	2.066
H8 (**1(A))	4	Providencia rettgeri	2.345	Providencia rettgeri	1.933
H9 (**1(A))	5	Providencia rettgeri	2.189	Providencia rettgeri	1.932
H10 (**1(A))	6	Providencia rettgeri	2.139	Providencia rettgeri	1.945
H11 (*1(D))	7	Escherichia coli	1.733	Escherichia coli	1.733

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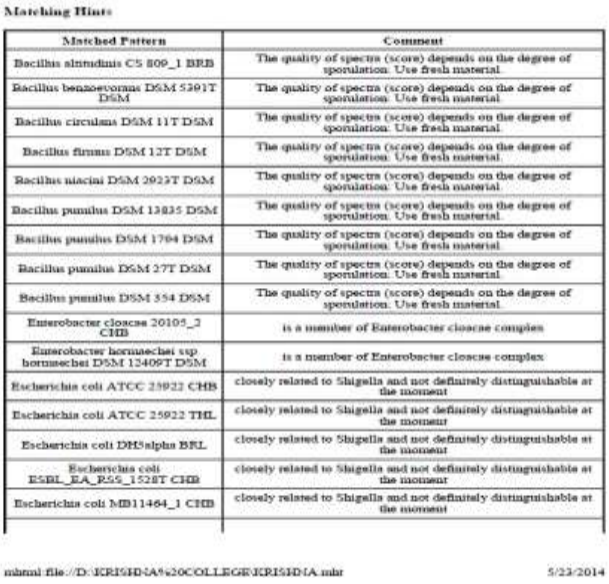
Figure 4: Results obtained for Maldi from Bruker



Matching Hints

Matched Pattern	Comment
Bacillus altitudinis C5 809_1 BRB	The quality of spectra (score) depends on the degree of sporulation. Use fresh material.
Bacillus benzoevorans DSM 5391T DSM	The quality of spectra (score) depends on the degree of sporulation. Use fresh material.
Bacillus circulans DSM 11T DSM	The quality of spectra (score) depends on the degree of sporulation. Use fresh material.
Bacillus firmus DSM 12T DSM	The quality of spectra (score) depends on the degree of sporulation. Use fresh material.
Bacillus niacini DSM 2023T DSM	The quality of spectra (score) depends on the degree of sporulation. Use fresh material.
Bacillus pumilus DSM 13835 DSM	The quality of spectra (score) depends on the degree of sporulation. Use fresh material.
Bacillus pumilus DSM 1704 DSM	The quality of spectra (score) depends on the degree of sporulation. Use fresh material.
Bacillus pumilus DSM 27T DSM	The quality of spectra (score) depends on the degree of sporulation. Use fresh material.
Bacillus pumilus DSM 354 DSM	The quality of spectra (score) depends on the degree of sporulation. Use fresh material.
Enterobacter cloacae 20105_2 CHB	is a member of Enterobacter cloacae complex
Enterobacter hormaechei ssp hormaechei DSM 12409T DSM	is a member of Enterobacter cloacae complex
Escherichia coli ATCC 25922 CHB	closely related to Shigella and not definitely distinguishable at the moment
Escherichia coli ATCC 25922 THL	closely related to Shigella and not definitely distinguishable at the moment
Escherichia coli DH5alpha BRL	closely related to Shigella and not definitely distinguishable at the moment
Escherichia coli ESBL_EA_RSS_1528T CHB	closely related to Shigella and not definitely distinguishable at the moment
Escherichia coli MB11464_1 CHB	closely related to Shigella and not definitely distinguishable at the moment

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Figure 5: Matching hints suggestive of the presence of *Shigella spp*

CONCLUSION

A wide range of rapid detection, typing has been developed for the detection of clinical samples. Molecular techniques PFGE, MLST, MVLA, WGS and the other new techniques shall tested for getting better result in the research. The researcher may consider the traditional detection/diagnosis procedure.

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