Detection of Colonized Pathogenic Bacteria from Food Handlers in Saudi Arabia

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Pathogenic micro-organisms from contaminated food are capable of causing serious infections and hence this issue has become a healthcare problem globally. The contamination may occur, either directly by an infected food handler, or indirectly through contact with a food contact surface that has been previously contaminated by an infected food handler. The current study was aimed to detect the pathogenic bacteria from food handlers in Ha’il region of Saudi Arabia. In this study, 152 bacterial isolates were collected from 50 food handlers. Identification of bacterial isolates was performed by conventional methods as well as by automated methods using Microscan, VITEK 2 and MALDI-TOF-MS. The results of conventional methods showed, 28.3% (43/152) bacterial isolates were Gram-positive and 71.7% (109/152) were Gram-negative. Among the Gram-positive isolates, E. faecalis, S. aureus and E. faecium were found to be 8.5% (13/152), 7.2% (11/152) and 4% (6/152) respectively. Among Gram-negative isolates, P. mirabilis, E. coli, E. cloacae and K. pneumoniae were found to be 12.5% (19/152), 11% (17/152), 11% (17/152) and 10.5% (16/152) respectively. The antibiotic susceptibility of the bacterial isolates in our study showed that 100% S. aureus were ciprofloxacin resistant. Additionally, 62% E. faecalis were resistant to gentamicin, 19% E. coli and 12% K. pneumoniae were found to be ESBL positive. The identification of bacterial isolates by 3 automated methods, showed that 93% (141/152), 94% (143/152) and 96% (146/152) bacterial isolates were correctly identified by Microscan, VITEK 2 and MALDI-TOF-MS respectively. Thus MALDI-TOF-MS proves to be the economical, fast and accurate method for identification of food borne pathogens. Incorporating this technique into food microbiology would lead to more successful and rapid identification of pathogenic bacteria from food sources.

Keywords: Food contamination, Pathogenic bacteria, MALDI-TOF-MS.
A health care issue worldwide. Additionally, the toxins produced by the bacterial strains in the food cause a substantial loss to the food industry because a large amount of money has to be spent on analyzing and identifying preventive measures.

Currently, the gold standard; traditional culture-based methods are used to identify the majority of food-associated bacteria in the daily routine of food microbiology laboratories globally. Complete identification is a time-consuming process and requires at least two days, or more for fastidious organisms. By using these phenotypic methods, sometimes, bacterial isolates with different taxonomic background and similar physiological characteristics pose a challenge and may give non-reliable results.

Thus, the development of a rapid, sensitive, specific, and cost-effective analytical method is of great importance for detection of microbial contaminants in the food. Recently, many technological improvements to methods for the identification of micro-organisms, such as MALDI–TOF-MS, have successfully been incorporated in clinical microbiology laboratories globally. MALDI–TOF-MS is a useful, fast, reliable and simple technique for the correct identification of micro-organisms and several studies have highlighted the advantages and performance of MALDI–TOF-MS including, rapidity, low sample volume requirements and low reagent costs\[14\]. MALDI-TOF-MS provides a suitable platform for quick, flexible, and reliable identification of food associated microbes because of the simple protocol and shortened analysis time\[15\]. Therefore, the aim of this study was to detect the colonized pathogenic bacteria from food handlers in Ha’il region of Saudi Arabia and to compare the results using conventional methods, MALDI-TOF-MS, Microscan and Vitek 2.

**MATERIALS AND METHODS**

**Study design**

In this study, a total of 50 food handlers (subjects working on meat shops) from the Ha’il region of Saudi Arabia were screened for the presence of pathogenic bacterial strains. A single non-repetitive, hand swab, nasal swab and swab from any wound site were collected from each individual for screening.
Bacterial identification
By conventional methods
Identification of bacterial isolates was performed by, simple staining, Gram-staining, morphology and biochemical tests.

Identification of microbes by automation methods
By MALDI-TOF-MS
The identification of the microbes by MALDI-TOF-MS was performed on Bruker Daltonics instrument\textsuperscript{16}, according to the manufacturer’s guidelines. In this method, a fresh colony material was smeared on a polished steel target plate (Bruker Daltonics) using a toothpick, overlaid with 1 µl of a saturated a-cyano-4-hydroxy-cinnamic acid (HCCA) matrix solution in 50% acetonitrile-2.5% trifluoroacetic acid (Bruker Daltonics), and air dried at room temperature. For the direct transfer-formic acid method, 1 µl of 70% formic acid was added to the bacterial spot and allowed to air dry before the matrix solution was added. The acquisition and analysis of mass spectra were performed by a Microflex LT mass spectrometer (Bruker Daltonics) using the MALDI Biotyper software package (version 3.0). The Bruker bacterial test standard (Bruker Daltonics) was used for calibration according to the instructions of the manufacturer. For each strain, two preparations of colony/sample material were analyzed. Standard Bruker interpretative criteria were applied to compare the data obtained with reference data base. Briefly, scores of ≥2.0 were accepted for species assignment and scores of ≥1.7 but <2.0 for identification to the genus level. Scores below 1.7 were considered unreliable.

Identification and antibiotic susceptibility by Microscan
Microscan walkaway (Siemens Healthcare Diagnostics, Sacramento, CA, USA) is an automated system used for bacterial identification and antibiotic susceptibility test. A small portion of a well isolated colony was taken and added to a Gram-positive or a Gram negative Microscan combo panel. The panel was loaded into the

Table 1. Identification of bacterial isolates collected from food handlers in Ha'il region of Saudi Arabia using Microscan, VITEK 2 and MALDI-TOF-MS

| Sample          | Bacterial strain                  | No. of isolates | Correctly identified by Microscan | VITEK 2 | MALDI-TOF-MS |
|-----------------|-----------------------------------|-----------------|-----------------------------------|---------|--------------|
| Gram-positive   | Staphylococcus sciuri             | 1               | 1                                 | 1       | 1            |
|                 | Staphylococcus intermedius        | 1               | 1                                 | 1       | 1            |
|                 | Staphylococcus hominis            | 1               | 1                                 | 1       | 1            |
|                 | Staphylococcus epidermidis        | 4               | 4                                 | 3       | 4            |
|                 | Staphylococcus cohnii             | 2               | 1                                 | 1       | 2            |
|                 | Staphylococcus auricularis        | 2               | 2                                 | 1       | 1            |
|                 | Staphylococcus aureus             | 11              | 11                                | 11      | 11           |
|                 | Enterococcus gallinarum           | 2               | 0                                 | 1       | 1            |
|                 | Enterococcus faecium              | 6               | 5                                 | 6       | 6            |
|                 | Enterococcus faecalis             | 13              | 13                                | 12      | 13           |
| Gram-negative   | Propionibacterium                 | 5               | 3                                 | 2       | 3            |
|                 | Proteus mirabilis                 | 19              | 17                                | 19      | 19           |
|                 | Pseudomonas aeruginosa            | 4               | 4                                 | 4       | 4            |
|                 | Klebsiella pneumoniae             | 16              | 15                                | 16      | 16           |
|                 | Enterobacter cloacae              | 17              | 17                                | 17      | 17           |
|                 | Enterobacter agglomerans          | 11              | 10                                | 10      | 10           |
|                 | Enterobacter aerogenes            | 6               | 6                                 | 6       | 5            |
|                 | Escherichia coli                  | 17              | 17                                | 17      | 17           |
|                 | Citrobacter freundii              | 7               | 6                                 | 7       | 7            |
|                 | Acinetobacter baumannii           | 6               | 6                                 | 6       | 6            |
|                 | Total correct identification      | 152             | 141                               | 143     | 146          |
Identification and antibiotic susceptibility by VITEK 2

VITEK 2 (Biomerieux, France) is an automated system used for bacterial identification and antibiotic susceptibility test. A small portion of a well isolated colony was taken and added to a Gram-positive or a Gram negative Microscan combo panel. The panel was loaded into the VITEK 2 machine according to the manufacturer’s protocol. Results were available after 24-48 hrs.

RESULTS

In this study, 152 bacterial isolates were collected from 50 food handlers in Ha’il region of Saudi Arabia as shown in Table 1. The results of the gold standard conventional methods showed, 28.3% (43/152) bacterial isolates were Gram-positive and 71.7% (109/152) were Gram-negative. Among the Gram-positive isolates, *E. faecalis*, *S. aureus* and *E. faecium* were found to be 8.5% (13/152), 7.2% (11/152) and 4% (6/152) respectively. Among Gram-negative isolates, *P. mirabilis*, *E. coli*, *E. cloacae* and *K. pneumoniae* were found to be 12.5% (19/152), 11% (17/152), 11% (17/152) and 10.5% (16/152) respectively.

The identification of bacterial isolates was also performed by 3 automated methods, namely, Microscan, VITEK 2 and MALDI-TOF-MS. The results of identification by these automated systems showed that 93% (141/152), 94% (143/152) and 96% (146/152) bacterial isolates were correctly identified by Microscan, VITEK 2 and MALDI-TOF-MS respectively as presented in Table 1.

The comparative identification analysis of Microscan, VITEK 2 and MALDI-TOF-MS are shown in Figure 1. The data revealed that among Gram-negative isolates, MALDI-TOF-MS and VITEK 2 identified 96% isolates correctly, while as, Microscan could identify 94% isolates correctly. In the case of Gram-positive isolates, MALDI-TOF-MS identified 95% isolates correctly, while as, Microscan and VITEK 2 identified 90% and 88% isolates respectively.

The antibiotic susceptibility results showed that among Gram-positive isolates, 100% (11/11) *S. aureus* isolates were resistant to ciprofloxacin and 62% *E. faecalis* isolates were resistant to gentamicin. Among Gram-negative isolates, 19% and 12% *K. pneumoniae* and *E. coli* isolates were found to be ESBL positive.

DISCUSSION

There are many factors responsible for the contamination of food. The findings of our study indicate that food handlers i.e the subjects working on meat shops may play a vital role in transmission of pathogenic bacteria to healthy people via contaminated food. In this study, 50 food handlers were screened and 152 different bacterial strains were isolated. Among these isolates, *E. faecalis*, *S. aureus*, *E. faecium*, *P. mirabilis*, *E. cloacae*, *E. coli*
S. aureus were found to be 8.5%, 7.2%, 4%, 12.5%, 11%, 11%, and 10.5% respectively. The results of our study were in agreement with a study from Iran17. In another study from Sudan conducted by Humodi et al. S. aureus was found to be the most common pathogen isolated from food handlers18. The result of current study also highlighted the significant presence of S. aureus in food handlers from Ha’il region of Saudi Arabia. The quick and reliable identification of pathogenic bacteria from the food or food handlers is essential in order to control the infections caused by these pathogens. Conventional methods of identification are time consuming and laborious, but are still considered to be the gold standard19. However, the automated systems have their own advantage and have been successfully used for identification of food borne pathogens with high sensitivity and specificity20. In our study, 3 automation methods used for identification of pathogenic bacteria from food handlers were Microscan, VITEK 2 and MALDI-TOF-MS. The results from MALDI-TOF-MS were the most accurate compared to Microscan and VITEK 2. The quick and accurate identification of pathogenic bacteria from food source is essential as many studies have shown that several antibiotic resistant bacteria have been isolated from food sources. The antibiotic susceptibility of the bacterial isolates in our study showed that all 100% S. aureus were ciprofloxacin resistant. Additionally, 62% E. faecalis were resistant to gentamicin, 19% E. coli and 12% K. pneumoniae were found to be ESBL positive.

CONCLUSIONS

This study reveals a high percentage of pathogenic bacteria with quite a few of these resistant to antibiotics isolated from food handlers in Ha’il region of Saudi Arabia. Furthermore, among the automated systems, MALDI-TOF-MS gave the maximum accuracy in identification of the pathogenic bacteria in this study. Thus in order to use a simple, accurate and reliable method for identification of food borne pathogens, MALDI-TOF-MS should be given a priority.

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