

## 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.

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In the food processing industry, a food handler's role is one of the most important in ensuring the safety of food. Food handler is an essential part in the chain of preparation, cooking, packaging and delivery of food. A food handler is directly involved in, packaging or unpackaging food, food equipment and utensils, or food contact surfaces<sup>1</sup>. In order to make sure that the food is safe and free from any contamination, a food handler must fulfill the requirements to ensure the food hygiene. During the preparation, processing,

delivery and serving of food, a food handler is capable of being a potential source of bacteria that causes foodborne diseases by introducing these pathogens in to the food. It has been found that incorrect practices in the food industry by a food handler are responsible for about 97% of foodborne ailments<sup>2-3</sup>.

The contamination of food with harmful micro-organisms 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<sup>4</sup>. In addition to pathogens, toxins, and other contaminants of the food also pose a serious threat to human health, and lead to

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high morbidity and mortality. Among many micro-organisms, some of the various bacterial pathogens that have been found to be the frequent contaminants of food are *Salmonella*, *Listeria*, *S. aureus*, *Campylobacter*, *Trichinosis*, *E. coli*, *Campylobacter* and *Clostridium*. These microbes cause severe infections with high morbidity, and majority of these infections have been attributed to food borne transmission<sup>5</sup>. Recently, Australian institute of food safety reported that among many microbes, *Salmonella*, *Listeria*, *S. aureus*, *Campylobacter*, *Trichinosis*, *E. coli*, *Campylobacter* and *Clostridium* are the top 7 causes of food poisoning. The health department of Australia estimates that food poisoning affects around 5.4 million Australians each year<sup>6</sup>. Food borne or waterborne microbial pathogens are considered as leading causes of infections and deaths in developing countries, killing an estimated 1.9 million people annually at the global level. Even in developed countries, an estimated one-third of the populations are affected by microbiological food borne diseases each year<sup>7</sup>. The food borne infection usually involves the intestinal enteropathogenic bacteria and their transmission is helped directly or indirectly by objects contaminated with feces.

Food handlers capable of harboring and excreting enteropathogenic bacteria may contaminate foods from their feces through their fingers, then to food processing, and finally to healthy individuals<sup>8</sup>. It has been reported that the area of hand beneath fingernails works as a vector for transmission of harmful microorganisms through cross contamination as compared to other parts of the hand<sup>9</sup>. One of the major illness or infection due to bacterial contaminated food is diarrheal disease, and globally, diarrheal diseases are second only to respiratory diseases as a cause of adult death and are the leading cause of childhood death. In some parts of the world, they are responsible for more years of potential life lost than all other causes combined<sup>10</sup>. In addition to cause the food borne illness, the bacterial strains such as, *Salmonella* spp. and *E. coli* have tendency to evolve in order to exploit novel opportunities, for example fresh produce, and even generate new public health challenges like antimicrobial resistance<sup>11-13</sup>. The spread of foodborne disease due to pathogens which are highly resistant to antibiotics has become

a health care issue worldwide. Additionally, the toxins produced by the bacterial strains in to 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 result.

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<sup>14</sup>. 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<sup>15</sup>. 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<sup>16</sup>, 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 α-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

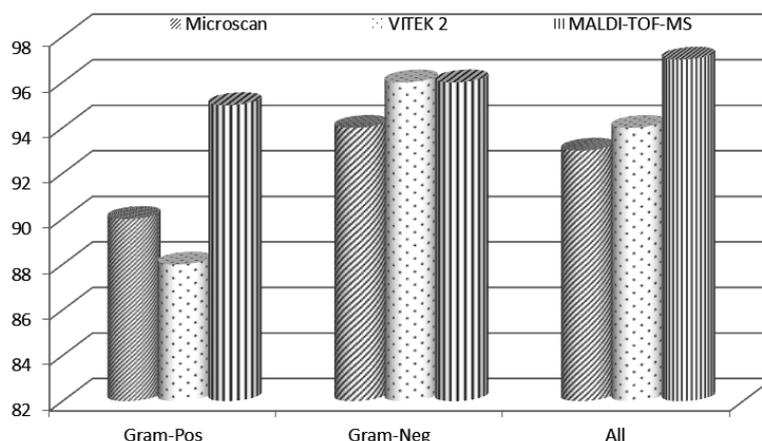
of mass spectra were performed by a Microflex LT mass spectrometer (Bruker Daltonics) using the MALDI Biotyper software package (version 3.0). The Bruker bacterial test standard (Bruker Daltonics) was used for calibration according to the instructions of the manufacturer. For each strain, two preparations of colony/sample material were analyzed. Standard Bruker interpretative criteria were applied to compare the data obtained with reference data base. Briefly, scores of  $\geq 2.0$  were accepted for species assignment and scores of  $\geq 1.7$  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	<i>Staphylococcus sciuri</i>	1	1	1	1
	<i>Staphylococcus intermedius</i>	1	1	1	1
	<i>Staphylococcus hominis</i>	1	1	1	1
	<i>Staphylococcus epidermidis</i>	4	4	3	4
	<i>Staphylococcus cohnii</i>	2	1	1	2
	<i>Staphylococcus auricularis</i>	2	2	1	1
	<i>Staphylococcus aureus</i>	11	11	11	11
	<i>Enterococcus gallinarum</i>	2	0	1	1
	<i>Enterococcus faecium</i>	6	5	6	6
	<i>Enterococcus faecalis</i>	13	13	12	13
Gram-negative	<i>Propionibacterium</i>	5	3	2	3
	<i>Proteus mirabilis</i>	19	17	19	19
	<i>Pseudomonas aeruginosa</i>	4	4	4	4
	<i>Klebsiella pneumoniae</i>	16	15	16	16
	<i>Enterobacter cloacae</i>	17	17	17	17
	<i>Enterobacter agglomerans</i>	11	10	10	10
	<i>Enterobacter aerogenes</i>	6	6	6	5
	<i>Escherichia coli</i>	17	17	17	17
	<i>Citrobacter freundii</i>	7	6	7	7
	<i>Acinetobacter lwoffii</i>	1	1	1	1
	<i>Acinetobacter baumannii</i>	6	6	6	6
	Total correct identification	152	141	143	146



**Fig. 1.** Shows the comparative identification by Microscan, VITEK 2 and MALDI-TOF-MS

Microscan walkaway machine according to the manufacturer's protocol. Results were available after 24- 48 hrs.

#### Identification and antibiotic susceptibility by VITEK 2

VITEK 2 (Biomérieux, 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*

and *K. pneumoniae* 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 Iran<sup>17</sup>. In another study from Sudan conducted by Humodi et al. *S. aureus* was found to be the most common pathogen isolated from food handlers<sup>18</sup>. 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 standard<sup>19</sup>. However, the automated systems have their own advantage and have been successfully used for identification of food borne pathogens with high sensitivity and specificity<sup>20</sup>. 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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