PREVALENCE OF SEAFOOD BORNE PATHOGENS IN SHELLFISH AT RETAIL LEVEL

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ABSTRACT

Seafood borne diseases associated with consumption of shellfish are the major challenge to the food hygienists in the 21st century, especially in the costal cites-Egypt. Therefore, a total of 740 shellfish samples such as crab (Neptunus plegicus), shrimp (Penaeus spp.), clam (Tapes decussata) and wedge shell (Donax drunculus) were randomly collected from the retail markets of Ismailia city over one year. Salmonella species, Staphylococcus aureus and Vibrio species were detected in 10 (1.4%), 443 (59.9%) and 93 (12.6%) of the shellfish respectively. Mollusca (clam and W. shell) had a high positive sample for S. spp., S. aureus and V. spp. when compared with crustacea (crab and shrimp) samples. Twenty-five S. strains represented by S. senftenberg (81.8%), S. typhi (9.1%) and S. typhimurium (9.1%) were isolated from W. shell samples. Only 10 (1.4%) W. shell samples were positive for S. spp. On the other hand, S. aureus was considered as the most prevalence seafood borne pathogens in both molluscs and crustaceae samples. W. shell samples were ranked the top (10%) for contamination with S. aureus coagulase positive, while shrimp samples revealed the lowest positive number of this pathogen (3.3%). According to the standard limit of the S. aureus (more than 104 CFU/Gram) in the fresh shellfish, 107 (14.5%) samples were unfit for human consumption. Sixty-five strains of V. spp. were isolated from the 740 shellfish samples, the most recorded strains were V. parahaemolyticus (25) followed by V. furnissii (22) and V. alginolyticus (18). Only 27 (3.5%) of shellfish samples were unfit for human consumption due to high counts of V. spp. more than 10^4 CFU/g. Good hygienic conditions, prevent the cross-contamination from raw food to other food, clean disposable gloves were used during shellfish handling and thorough cooking of shellfish must be adopted to control the hazard of seafood-borne pathogens.

Abbreviations: CFU=colony forming unit ;W.shellfish=wedge shellfish; S.aureus=Staphylococcus aureus;V=vibrio;S=salmonella.
INTRODUCTION

Food safety is one of the major challenges for the 21st century implying a significant redirection of food microbiologist efforts in many parts of the world toward the prevention of foodborne diseases (44). Nowadays, there is a global increase in the consumption of seafood leading to a significant global problem concerning hazards of seafood borne pathogens (43).

Shellfish, including Crustacea and Mollusca are marine water seafood, which harbor the pathogens already mentioned as constituting the flora of fish. Since shellfish is rich in free amino acids, they are considered as a good medium for bacterial growth (22). Mollusca: oysters, mussels and clams are filter feeding bivalve mollusks and can accumulate human pathogens at levels higher than those in their surrounding waters (10,45).

Seafoods act as a vehicle for all important species of foodborne pathogens (25,36). Environmental conditions play an important role on the pathogens count in fresh fish (39). Temperature abuse during raw seafood harvesting and storage may help in microbial pathogens multiplication, thus posing a potential health threat to susceptible consumers (24).

Shellfish may be contaminated with food-borne pathogens which are naturally present in aquatic environments, such as V. spp. (9); or derived from sewage contaminate water such as Salmonella (13) and/or associated with workers, equipments, processing or retail establishment, such as S. aureus (49), when the seafood harvested, the above mentioned pathogens invade the flesh through the gills, along blood vessels and directly through the skin and belly cavity lining (35).

Regarding to the potential public health threat that the shellfish may constitute, Legnani et al. (21) emphasized the importance of the systematic and periodically monitoring of the microbiological quality of shellfish. In addition, Schlundt (38) confirmed the urgent need for better foodborne disease data in most countries to improve food safety allover the world. Therefore, the aim of this study was to detect some seafood born pathogens in the flesh of shellfish that were randomly collected from the retail markets in Ismailia city as well as discusses their public health importance.

MATERIALS AND METHODS

1. Collection of the samples: A total of 740 fresh shellfish samples were randomly collected from different retail markets at Ismailia city over a period of one year. Two kinds represent the Crustacea (each 4 pieces considered as one sample): crab, Neptunus plegicus (120) and shrimp, Penaeus spp. (120); other two kinds
represent the Mollusca (each 4 pieces considered as one samples): clam, Tapes decussata (250) and w. shell, Donax drunculus (250). Each shellfish group was almost similar in shape and weight. Shellfish samples were packed in sterile polyethylene bags, stored in ice-box, and sent to the laboratory without delay.

2. Preparation of the Samples: in case of Crustacea samples, the shells were scrubbed with a sterile stiff brush under running potable water with special attention to crevices at the junction of the shells, and left for draining on a sterile towel. The dried samples were transferred to a sterile plate where the shell was removed by a sterile forceps and scissors. The internal content was transferred under complete aseptic conditions into a sterile plate. On the other hand, for the Mollusca samples, it was shucked by using a sterile thin bladed knife, the clams were held in the hand, the knife edge placed at the junction of the bills and forced between the shells with squeezing motion then cut the two adductor muscles. The soft body of the animal and the shell liquor transferred into sterile plate.

3. Detection of Seafood Borne Pathogens:
3.1. Detection of Salmonellae: Salmonella was detected according to the using procedure described by APHA (4).
3.2. Detection of S. aureus: S. aureus count was done according to the technique recommended by AOAC (3). Coagulase positive S. aureus was detected by using technique of Sperber and Tatini (42).
3.3. Detection of Vibrio: According to the technique recommended by APHA (4).
4. Identification of Isolates: Identification of the isolated strains were done according to MacFadyean (27) and API 20 E system BioMerieux (6).

RESULTS AND DISCUSSION
Edible shellfish may be contaminated with pathogens that naturally occur in marine ecosystems that are potential human pathogens (11). In this study, S. spp., S. aureus and V. spp. were detected in 10 (1.4%), 443 (59.9%) and 93 (12.6%) of all shellfish samples respectively (Table, 1). Generally, S. spp., S. aureus and V. spp., were isolated from all kinds of seafood, they were recovered from fresh seafood (28, 29, 32), chilled seafood (31); frozen seafood (35, 40); salted fish (1,17) and smoked fish (16,41). In the present study, wedge shell samples were the only shellfish species positive for S. spp. (4%) and have the high percent in S. aureus (72%), while shrimp samples were reported to have the highest positive percent for V. spp. (18.3%). On the other hand, 100% of crab, shrimp and clam samples were free from salmonella and they also constitutes the lowest positive percent for S. aureus. V. spp. were isolated
from all shellfish samples in 14 (11.7%), 22 (18.3%), 32 (12.8%) and 25 (10%) for crab, shrimp, clam and w. shell respectively. FDA (12) listed the standard limit of the seafood borne pathogens of fresh shellfish, and mentioned that shellfish must be free from S. spp. and S. aureus producing enterotoxins. However, S. aureus count is equal to or greater than $10^4$/g and V. spp. level equal to or greater than $1 \times 10^4$/g. According to FDA standard, 10 (1.4), 107 (14.5%) and 27 (3.6%) of shellfish samples have exceeded the standard limit for S. spp., S. aureus and V. spp. respectively and considered unfit for human consumption. It is worthy to mention that out of 740 shellfish samples, 730 (98.6%), 633 (85.5%) and 713 (96.4%) samples were considered free from the hazard of S. spp., S. aureus and V. spp. respectively and safe for human consumption.

The obtained results in figure 1 clearly revealed that Mollusca (clam and w. shell) had a high percent of S. spp., S. aureus and V. spp. when compared to Crustacea (clam and shrimp). It is obvious that, S. aureus is considered as the most recorded seafood-borne pathogens in both Mollusca and Crustacea.

At the retail level, S. aureus was the most prevalent pathogens in fresh crustaceans especially under mishandling condition (32). The expected higher positive samples of seafood pathogens in the Mollusca rather than Crustacea is possibly because of the distinct feeding habitat of Mollusca. Shellfish do not usually harbor pathogens, but they acquire them by filtering water through their systems and concentrating them in their flesh (20).

Contamination of shellfish with Salmonella due to their growth in polluted water has been a problem in many parts of the world (34). Eleven Salmonella strains represented by S. senftenberg (81.8%) which considered as the most predominate strain followed by S. typhi (9.1%) and S. typhimurium (9.1%) that isolated from wedge shell samples only (Table, 2). Wilson (50) detected Salmonella serotypes in 8% out of 433 Mollusca, while Nora (32) recorded that Salmonella was detected in fresh Crustacea at retail level with a percentage of 15.8%. Recent study by Martinez_Urtaza et al. (29) declared that the incidence of Salmonella was 1.8% out of 2,980 samples of shellfish, nine serovars out of 54 Salmonella were isolated. S. senftenberg was the most frequent type (50%), followed by S. typhimurium (18%) and S. agona (17%). Heinitz and Johnson (16) isolated S. spp. from 5 (3.2%) out of 156 inspected shellfish samples. Mohamed et. al. (30) isolated Salmonella in only one sample from 846 samples of raw shrimp ( Penaeus monodon ), serotyping of this strain revealed that it was S. typhimurium.

Salmonella is a pathogenic microorganism even when present in low percentage, so it is important to be systematically monitoring for their presence in
shellfish (21). It causes a severe public health hazard for shellfish consumers, S. typhi and S. typhimurium produce typhoid and typhoid-like fever in human being. The fatality rate of typhoid fever is 10% compared to less than 1% for other forms of salmonellosis (7).

The mean values of S. aureus were 9x10^{4}, 3.4x10^{3}, 3.2x10^{6} and 13x10^{5} CFU/g in crab, shrimp, clam and wedge shell samples respectively (Table 3). S. aureus was the most prevalent seafood borne pathogens detected in the seafood (26,41,47). The presence of S. aureus in the shellfish is generally considered as an indication of poor sanitation which is due to bad handling or contaminated food contact surfaces. Food handlers are the main source of this contamination through poor personal hygiene standards. Wilhelmsson et. al. (47) reported that the count of S. aureus was ranged between 2.3x10^{2} to 4.6x10^{2} in the shellfish samples and 25% of the isolated S. aureus were coagulase positive, they also confirmed that handling of the shellfish by the seafood handler either in growth areas or during the collecting and selling operation is responsible for increasing in the S. aureus number.

The obtained results in table 4 revealed the frequency distribution of coagulase positive S. aureus in shellfish samples, 49 (6.6%) out of 740 shellfish samples were positive for S. aureus coagulase positive. W shell samples were ranked the top (10%) for contamination with S. aureus coagulase positive, while shrimp was the lowest positive number for this pathogen (3.3%). The difference in the number of positive samples in between the shellfish samples, may be due to the ability of mollusca to concentrate the pathogens than the crustacea as well as the difference in handling technique in the randomly collected samples. Sixty three strain of S. aureus coagulase positive were isolated from shellfish samples; the frequency rate were 12 (19.1%), 5 (7.9%), 14 (22.2%) and 32 (50.8%) for crab, shrimp, clam and W. shell samples respectively. Most of the isolated S. aureus from the seafood were enterotoxogenic (37).

When these pathogens are given time to grow on seafood, they can produce a heat resistant toxin that causes food poisoning. Keeping seafood not cold enough will allow the pathogens to produce toxins that cause food poisoning. Once the toxins have formed they are difficult to destroy even by boiling (32).

Staphylococcus food poisoning is a major form of seafood borne disease, there are six antigenically different enterotoxin types A, B, C1, C2, D and E. Enterotoxin A is considered to be the most toxic and is the most commonly detected in staphylococcal food poisoning outbreaks along with enterotoxin type D (19). S. aureus intoxication symptoms usually appear within 0.5 to 7 or 8 hours after consumption of food contaminated with enterotoxin (5). Commonly reported
symptoms include nausea, vomiting and less frequently diarrhoea. Headache, dizziness and weakness are reported in a minority of cases. There were few deaths recorded especially in elderly or very young (46).

The mean values of V. spp. were 2.2x10^3, 17x10^4, 10x10^5 and 5.7x10^4 CFU/g for crab, shrimp, clam and W. shell respectively (Table 5). Sixty-five strain of V. spp. were isolated from 740 shellfish samples (table 6), the most prevalent strain was V. parahaemolyticus (25) followed by V. furnissii (22) and V. alginolyticus (18). Shellfish at the retail stage of distribution generally contain greater densities of V. parahaemolyticus than do shellfish at harvest, V. parahaemolyticus can grow rapidly in unrefrigerated shellfish (15).

More recently, DePaola et al., (8) strongly confirmed the predomination of V. parahaemolyticus in the shellfish, where 34 (21.8%) of oysters samples were positive for V. parahaemolyticus. Moreover, Kaufman et. al. (18) found that nearly 90% of the shellfish prior to storage contained V. parahaemolyticus at levels of 200 to 2,000 CFU/g. On the other hand regarding shellfish samples, clam had the most frequency of Vibrio strains 21 (32.3%) followed by shrimp, wedge shell and crab in a percent 27.7, 26.2 and 13.8% respectively.

V. spp. leads the list of the fish-borne pathogens (33). It is a natural inhabitants of estuarine environments and may be transmitted to human by ingestion of raw or inadequate cooked shellfish causing a sever public health hazard (2). V. parahaemolyticus was first described as the cause of gastroenteritis in Japan (14), it is an important pathogen for humans being and aquaculture animals (51). Foodborne disease caused by V. parahaemol-lyticus is almost associated with consumption of fish and shellfish (23).

It could be concluded that the presence of such pathogens even in a low frequency in the shellfish at the retail level are in need of more governmental attention to ensure safety of these foods. Clean food, rapid chilling, proper heating and cold storage are the principles for quality control of food to ensure food safety. To achieve this goal, shellfish consumers should be only purchase fresh shellfish handled in good hygienic conditions, prevent cross-contamination to other raw food, wear clean disposable gloves when handling the shellfish and thoroughly cooking the shellfish.
Table 1. Numbers of negative, positive and exceeded the standard limit for S. spp., S. aureus and V. spp. in the shellfish samples

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>S. spp.</th>
<th>S. aureus</th>
<th>V. spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>`-ve %</td>
<td>`+ve %</td>
<td>No. &lt; *S.L. %</td>
</tr>
<tr>
<td>Crab</td>
<td>120</td>
<td>0</td>
<td>120 (100)</td>
</tr>
<tr>
<td>Shrimp</td>
<td>120</td>
<td>0</td>
<td>120 (100)</td>
</tr>
<tr>
<td>Clam</td>
<td>250</td>
<td>0</td>
<td>250 (100)</td>
</tr>
<tr>
<td>W. shell</td>
<td>250</td>
<td>10</td>
<td>240 (96)</td>
</tr>
<tr>
<td>Total</td>
<td>740</td>
<td>10</td>
<td>730 (98.6)</td>
</tr>
</tbody>
</table>

`-ve Means Negative Samples for the Presence of Pathogens
>+ve Means Positive Samples for the Presence of Pathogens
*S.L. Standard Limit according to FDA (12)
Figure 1. Incidence of positive samples for S. spp., S. aureus and V. spp. in the crustacea and mollusca samples

Table 2. Frequency distribution of S. spp. in the shellfish samples

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Crab</th>
<th>Shrimp</th>
<th>Clam</th>
<th>W. shell</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. senftenberg (%)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>9 (81.8)</td>
</tr>
<tr>
<td>S. typhi (%)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>S. typhimurium (%)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11 (100)</td>
</tr>
</tbody>
</table>

ND Means Not Detected
Table 3. Mean values of S. aureus in shellfish samples

<table>
<thead>
<tr>
<th>Shellfish</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crab</td>
<td>$9 \times 10^4$</td>
<td>$2.1 \times 10^2$</td>
<td>$4.3 \times 10^6$</td>
<td>$\pm 5.8 \times 10^6$</td>
</tr>
<tr>
<td>Shrimp</td>
<td>$3.4 \times 10^3$</td>
<td>$&lt; 10^2$</td>
<td>$3.3 \times 10^3$</td>
<td>$\pm 8.6 \times 10^3$</td>
</tr>
<tr>
<td>Clam</td>
<td>$3.2 \times 10^6$</td>
<td>$5 \times 10^2$</td>
<td>$5.2 \times 10^5$</td>
<td>$\pm 4 \times 10^6$</td>
</tr>
<tr>
<td>W. shell</td>
<td>$13 \times 10^3$</td>
<td>$7 \times 10^2$</td>
<td>$2.2 \times 10^8$</td>
<td>$\pm 1.6 \times 10^7$</td>
</tr>
</tbody>
</table>

*S.D.* = standard deviation
Counts expressed as CFU/g

Table 4. Frequency distribution of S. aureus in the shellfish samples

<table>
<thead>
<tr>
<th>Shellfish</th>
<th>Sample No.</th>
<th>Positive No S. aureus (%)</th>
<th>S. aureus coagulase positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crab</td>
<td>120</td>
<td>55 (45.8)</td>
<td>9 (7.5)</td>
</tr>
<tr>
<td>Shrimp</td>
<td>120</td>
<td>43 (35.8)</td>
<td>4 (3.3)</td>
</tr>
<tr>
<td>Calm</td>
<td>250</td>
<td>165 (66)</td>
<td>11 (4.4)</td>
</tr>
<tr>
<td>W. shell</td>
<td>250</td>
<td>180 (72)</td>
<td>25 (10)</td>
</tr>
<tr>
<td>Total</td>
<td>740</td>
<td>443 (59.9)</td>
<td>49 (6.6)</td>
</tr>
</tbody>
</table>

F. means the frequency

Table 5. Mean values of V. spp. in the Shellfish Samples

<table>
<thead>
<tr>
<th>Shellfish</th>
<th>Sample No.</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crab</td>
<td>120</td>
<td>$2.2 \times 10^4$</td>
<td>$2 \times 10^4$</td>
<td>$3.4 \times 10^6$</td>
<td>$\pm 6.1 \times 10^9$</td>
</tr>
<tr>
<td>Shrimp</td>
<td>120</td>
<td>$17 \times 10^4$</td>
<td>$&lt; 10^4$</td>
<td>$2 \times 10^6$</td>
<td>$\pm 4.2 \times 10^9$</td>
</tr>
<tr>
<td>Clam</td>
<td>250</td>
<td>$10 \times 10^4$</td>
<td>$10^2$</td>
<td>$3.5 \times 10^7$</td>
<td>$\pm 6.2 \times 10^7$</td>
</tr>
<tr>
<td>W. shell</td>
<td>250</td>
<td>$5.7 \times 10^4$</td>
<td>$2 \times 10^2$</td>
<td>$3 \times 10^7$</td>
<td>$\pm 10 \times 10^7$</td>
</tr>
</tbody>
</table>

*S.D.* = Standard Deviation
Counts Expressed as CFU/g
Table 6. Frequency distribution of V. spp. in the shellfish samples

<table>
<thead>
<tr>
<th>Shellfish</th>
<th>V. parahaemolyticus (%)</th>
<th>V. furnissii (%)</th>
<th>V. alginolyticus (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crab</td>
<td>2 (8)</td>
<td>3 (13.6)</td>
<td>4 (22.2)</td>
<td>9 (13.8)</td>
</tr>
<tr>
<td>Shrimp</td>
<td>7 (28)</td>
<td>-</td>
<td>11 (61.1)</td>
<td>18 (27.7)</td>
</tr>
<tr>
<td>Clam</td>
<td>11 (44)</td>
<td>7 (31.8)</td>
<td>3 (16.7)</td>
<td>21 (32.3)</td>
</tr>
<tr>
<td>W. shell</td>
<td>5 (20)</td>
<td>12 (54.6)</td>
<td>-</td>
<td>17 (26.2)</td>
</tr>
<tr>
<td>Total</td>
<td>25 (100)</td>
<td>22 (100)</td>
<td>18 (100)</td>
<td>65 (100)</td>
</tr>
</tbody>
</table>

ACKNOWLEDGEMENT

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REFERENCES

10.


مدي أنتشار جراثيم الأغذية في المحاريات المصدرة في متجار الأسماك

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يشهد القرن الحادي والعشرين تحديات من قبل الباحثين في علم وصحة الأغذية بخصوص أمراض التسمم الغذائي الناتجة عن استهلاك الأطعمة البحرية التي قد تكون ملوثة بالجراثيم وخصوصا في المدن الساحلية بجمهورية مصر العربية ولذلك فقد تم تجميع 740 عينة شعاعية لهذه الدراسة على مدار عام كامل من عينات الكاوبوريا (سرطان البحر) والروبيان (المجامعي) والبطليوس (الجدانوفي) وأم الخول من مختلف متاجر الأسماك بمدينة الإسماعيلية. وقد تبين من الدراسة أن عدد 10 (4.1%) و 443 (59.9%) و 93 (6.6%) من عينات المحاريات لела نتيجة أجابة تواجه جراثيم السالمونيلا والمكور العنقودي الذهبي والفيبرو على التوالي، وكان أكثر تأرجح تلك العينات الإيجابية في عينات الروبيان (بطليوس وأم الخول) عنها في عينات القشريات (الكابوريا والروبيان). هذا وقد تمكّن من عزل احدي عشر شبه عذر من عينات جراثومة السالمونيلا من عينات المحاريات وكان نسبة تواجه جراثومة السالمونيلا سبتيصرح 81.8% وسالمونيلا تيفاي 9.1% وسالمونيلا تيفيموريم 9.1%. وقد اتضح أن عدد عشرة (1.1%) عينة من إجمالي 740 عينة من المحاريات الإيجابية لجراثومة السالمونيلا يجب إعادتها لأنها قد تشكل خطورة على صحة المستهلكين. وعلى الجانب الآخر كانت جراثومة المكور العنقودي الذهبي هو الأكثر تكرارا عن الجراثيم الأخرى في عينات الروبيان والقشريات وكانت عينات أم الخول هي الأكثر نتيجة إيجابية (10%) بينما عينات الروبيان هي الأقل نتيجة إيجابية (3.3%) لهذه الجراثوم. وأفاد بعض من الدراسة أن عدد 107 (14.5%) من عينات المحاريات غير صالحة للاستهلاك الغذائي ويلزم إعادتها لزيادة عدد جراثيم المكور العنقودي الذهبي أكثر من 4جراثومة لكل جرام وذلك تواجه جراثومة المكور العنقودي الذهبي الإيجابي لأنزيم الجلوتاجولين. وكذلك تمكّن من عزل 65 عذر من عينات جراثومة الفيبرو وكان جراثومة الفيبرو بارا هيلونتينكس هو الأكثر تكرارا بعدد 25 عذر ثم جراثومة الفيبرو فرينسي بعدد 22 عذر وجراثومة الفيبرو الجنونليتيكس بعدد 18 عذر وبناء علي الحد الإقليمي المسموح به لتواجد هذا الجراثوم في
المحاريات فقط تبين ان عدد 27 (3.5%) عينة من عينات المحاريات غير صالحة للاستهلاك لتواءد جراثيم الفيروبا بنسبة أكبر من 10% جراثيم لكل جرام. ويمكن لمستهلكي المحاريات الوقاية من خطرة تلك الجراثيم المسببة للأمراض وذلك بالاعتماد على شراء تلك المحاريات اذا أمكن طازجة ومن معارض الأسماك التي تتوفر فيها الاستردادات الصحية الجيدة وكذلك تنبج التلالس بين هذه المحاريات والأطعمة الطازجة الأخرى كالخضروات والفواكه لتجنب انتقال الجراثيم وأيضا التعود على ارتداة قفازات واقي عند ملائمة تلك المحاريات وأخير الطهي الجيد لضمان التخلص من كل الجراثيم إذا تواجدت.