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Short Communication

Evaluation of Microbial Contamination of Chicken and Fish in Food Industries and Associated Health Effects in Saudi Arabia

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FAAA arranged the funds and designed the study. ASH carried the practical work. VK and FA analyzed the results and wrote the manuscript.

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ABSTRACT

The present study aimed determining the microbial contamination in imported fish and chicken in Saudi Arabia and impact on food industry production workers. Sufficient samples (approx. 422) from imported fish and chicken were randomly collected and analyzed for total plate count (TPC), *Staphylococcus aureus, Vibrio parahaemolyticus and Pseudomonas*. Five healthy workers directly involved with processing, handling from various departments without any prior disease were selected for blood profiling. Significant increase in *S. aureus* colonies were observed in fish samples compared to chicken in the month of September and October, 2017 and in fish and chicken meat from December, 2017 to June, 2018. In all samples TPC was more or less same for fish and chicken. *E. coli* was non traceable in any of the analyzed samples. Uniform pattern of *V. parahaemolyticus* was observed from March to June 2018 but was altered in 2017. Elevated level of C-reactive protein was observed in two of human samples, which reflected some internal inflammation without any clinical symptoms. This is fore thought to evaluate the conditions in which these livestock are grown and processed for import and the need to reset the standards for workers with periodic health checkups.

The advancement in modern technology and genetic manipulation, feed selection, and better health management practice along with disease preventive medicine the growth time is now reduced to 6-7 weeks from 10-11 weeks (Li *et al*., 2020). Antibiotics, are generally used in poultry for rapid growth and disease prevention. These antibiotic growth promoters (AGP) are always the topic of debate among food scientists (Xu *et al*., 2020a). Several intestinal commensals that include *E. coli*, *Salmonella*, and *Staphylococcus* monitored for antibiotic resistance pattern both in humans and in source of consumption. (Vasiee *et al*., 2020). There are various resistance genes of the organism that can be transmitted to humans along with the resistance pattern (Justiz Vaillant and Qurie, 2020), particularly *Staphylococcus aureus*, a Gram-positive, spherical/round bacterium, making it one of the most common antibiotic resistant both in human and animal, especially methicillin-resistant *S. aureus-MRSA* (Koizumi *et al*., 2020). They are also found in dust and on animal skin. *S. aureus* are commensals and opportunistic pathogens, present in animal skin, can cause wide range of illnesses, including skin infections, boils, including pneumonia, meningitis and osteomyelitis (Eltwisy *et al*., 2020). *S. aureus* causing food poisoning produces toxins Staphylococcal enterotoxin type B (SEB) a heat-stable and can survive normal cooking, and may not be subsequently destroyed is the most common one. Such organism should not appear in any product from any country and the best way to control is implementation of personal hygiene. *S. aureus* is found, either in raw foods from warm-blooded animals or foods that have been manually handled (Výrostková *et al*., 2020). It is not normal to detect in raw fish but it could be found in seafood products that have been heated, treated and manually handled, like crustacean products (Chen *et al*., 2020). The enterotoxins produced by *S. aureus* and *Streptococcus pyogenes*, are from pyrogenic toxin family also called super-antigens, can activate a very strong response from the host immune system (Osman *et al*., 2020). The symptoms of the diseases

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may occur with nano gram levels of enterotoxin per gram, however, in most disease outbreaks, an estimated 1 to 5 mg has been ingested (Ogonowska *et al*., 2020).

Vibrio parahaemolyticus caused deaths in 2002, an outbreak of *V. parahaemolyticus* in Hong Kong involved 56 suspect cases. The suspected foods were a collection of seafood, had been stored at ambient temperature for too long which leads to multiplication of this organism. Considering the current background, present study was designed for evaluation of the presence of microorganisms in imported fish and chicken and its correlation in the blood profile among food industry production workers as a sample study.

Materials and methods

Microbial assessment of food with respect to quality and quantity have used through total plate count method. Five chickens/fish from imported lot was selected and transferred in peptone buffer. Samples were grown at 37°C, 42°C and 24°C and were tested for *E. coli, Salmonella*, total plate count (TPC), total coliform count (TCC), *Staphylococcus. E. coli* samples were incubated in eosin methylene blue (EMB) agar and Mac-Conkey agar. *Salmonella*, in Rappaport-Vassiliadis media at 37°C for 24 h, followed by incubation at 42°C again for 24 h. The samples were tested for rest of organism at 37°C for 24 h, followed by Salmonella Shigella (SS) agar and xylose lysine deoxycholate (XLD) agar media.

S. aureus was isolated on 3 plates of baird parker agar (0.4 ml, 0.3 ml and 0.3 ml), media for 9-12 min, followed by incubation for 1 h. After absorption on agar, they were further incubated for 45-48 h at 35 to 37°C. Only plates containing 20 to 200 colonies were selected, with moist, convex, smooth, circular with 2 to 3 mm in diameter in appearance with black to gray in color and light white color in margins. Coagulase test >1 the colony was selected from each type of *S. aureus*. Colony number were determined on each plate giving positive coagulate test, with multiple of dilution factor were considered for final results.

V. parahaemolyticus was isolated from homogenized samples, from 10: 90 ml of the diluent. 1 ml of each sample was pipetted out in petri dishes with agar and incubated at 45 ± 1°C further, incubated for 48 ± 2 h or two days at 35°C. *P. pseudomonas* were isolated from homogenized samples from 10: 90 ml of the diluent. 1 ml of each sample was pipetted out in petri dishes with agar and incubated at 45 ± 1°C further, incubated for 48 ± 2 h or two days at 35°C. Meat samples from which the microorganisms were isolated were stored at 0-4°C for further use. The product was neither freeze nor chilled unless for short period not more 36 h after collection but for shellfish and shell products. Samples from shellfish products were immediately put on crushed ice at ice temperature for analysis. The ambient temperature of shellfish samples was below 10°C. The shellfish and shell were analyzed within 6 h and max 24 h. In standard petri dish for *E. coli* the count was linear over the range between 30 to 300 colony-forming unit (CFU). We counted the colonies in a part of the dish due to high number of colonies.

To ascertain the effects of these food product, the blood samples were collected in tubes containing anticoagulant, EDTA, from five healthy workers of the industry, dealing directly with incoming shipment. A routine complete blood count (CBC) including basophils, eosinophils, monocytes, CRP, interleukin-6 (IL-6) was done through respective commercial kits.

Results and discussion

Fluctuation in growth pattern with respect to months among different organism was observed. TPC was significantly high (18± 6.41) in chicken samples compared to fish (15.8 ± 2.25) irrespective of the months in which the samples were collected. However, *S. aureus* was almost double in fish samples (3.1± 1.10) compared to samples tested in chicken (1.72 ± 0.46) detailed month wise analysis gave a clear picture. In none of the samples *E. coli* was detected and uniform pattern not amounting to significant value was observed in case of *V. parahaemolyticus* along with *Pseudomonas*.

S. aureus was high in fish samples compared to chicken in September and October 2017. with significant increase in number of colonies for fish in December 2017 compared to rest of months but for chicken it remains constant in November, October, September. February 2018 both chicken and fish samples showed significant fall in *S. aureus* compared to September, October, November, December 2017 and March 2018 (Fig. 1).

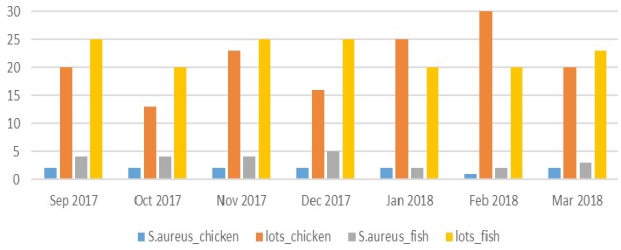

Fig. 1. *S. aureus* infection pattern in chicken and fish.

Table I shows the CBC of professional's output. WBC were in the normal range from all samples, except in one. Neutrophil percent was significantly low (45.08 ± 7.47) compared to normal mean value with a opposite trend in lymphocyte percentage. Rest of the blood picture was normal and in limits for monocytes, eosinophils,

basophils, hemoglobulin, platelets etc except RBC with a mean value slightly higher (5.342 ± 1.05 $X10^3$/ µL) compared to normal data. The mean IL-6 was less (2.402 pg/ml) than the stated limited of 5-15 pg/ml (Table I).

Table I. Blood profile of workers.

Blood parameters (Units)	Mean ± SD	Normal value
WBC ($X10^3$/µL)	6.43 ± 1.75	4.5- 10
Neutrophil (%)	45.08 ± 7.47	55 70
Lymphocytes (%)	45.08 ± 6.71	20- 40
Monocytes (%)	6.2 ± 2.64	2 to 8
Eosinophils (%)	2.06 ± 1.39	2.46±0.03
Basophils (%)	1.60 ± 0.51	1-3
RBC ($X10^3$/ µL)	5.34 ± 1.05	4.5 - 5.1
Hemoglobin (HB) (g/dl)	14.54 ± 2.42	14-17.5
Hematocrit (PCV) (%)	45.18 ± 7.21	41.5 - 50.4
MCV (fL)	85.52 ± 9.59	80 96
MCH (pg/cell)	27.6 ± 3.73	27 to 31
MCHC (g/dl)	32.26 ± 1.01	31-37
RDW (%)	14.92 ± 1.23	11.5-14.5
Platelets ($X10^3$/ µL)	270.8 ± 61.81	150- 450
Interleukin-6 (IL-6) (pg/ml)	2.40 ± 1.49	5-15
C-reactive protein (CRP) (mg/L)	2/5 positive	<6mg/l

Present study is an attempt to correlate whether the imported food stuff in form of chicken or fish is responsible for any health hazard in the handling population. It is observed that the variation in the TPC may be attributed to the climatic conditions of the source country from where the chicken has been imported. Studies have proved that the variation in the climatic condition can alter the pathogenicity and the survival rate of organism in the lie food stuff (Perrone *et al*., 2020).

Our results demonstrate that TPC was significantly high in chicken samples compared to fish irrespective of the months in which the samples were collected. However, *S. aureus* was almost double in fish samples compared to samples tested in chicken. In none of the samples *E. coli* was detected and uniform pattern not amounting to significant value was observed in case of *V. parahaemolyticus* along with *Pseudomonas* there is a significant increase in TPC. The significant variation can be attributed onset of change is temperature is also well reported by the veterinary record of England and wales (Burrows *et al*., 2019).

On an average the total TPC was 15.8 with a variation in number of average organisms, *E. coli* was nil and maximum colonies were observed for *S. aureus*. Though it is in limits but the pathogenicity of organism cannot be ruled out or it will not cause the disease. Microbiome patterns the transmission of pathogenic bacteria in hilsa fish (*Tenualosa ilisha*) marketed for human consumption in Bangladesh (Foysal, 2019). Difference in the number of colonies with respect to different organism is still a matter of concern and it may be due to change of host biological environment and its response to the environment. This change may be connected the change in temperature and the working conditions or the storage conditions of all such food foodstuff. *S. aureus* is well established as one of the most important pathogens both in animals and humans. Its prevalence resistance and virulence have been studied to established its relation with human (Dweba *et al*., 2019). The high prevalence of *S. aureus* from the livestock indicates a major food security and healthcare threat. Presence of *S. aureus* is compounded by the virulence of the pathogen, which causes numerous clinical manifestations, but in our samples the presence was nil. The reason may not be clear but use of antibiotics cannot be ruled out. . Generally, the *Pseudomonas* is not very common in the chicken sample, however a study conducted by Wu *et al*. (2020a) have shown that this organism found in the surrounding area of poultry and that have tendency to be carried in chicken. Our sample or chickens are imported from Brazil and the environment may be harboring the *Pseudomonas* there. It needs to be verified on the long-term basis to import *Pseudomonas* free live stocks. In our results *Pseudomonas* observed but with varying number of colonies.

The risk of human infections and deaths caused by highly pathogenic organism be it from viral or bacterial origin cannot be ruled out for the workers associated with food industries. The alteration in CBC along with CRP and Interleukin can predict possibility of any disease through variation in pathology or physiology of workers. CRP is an acute phase protein and its alteration is commonly seen in inflammatory or infectious disease synthesed by liver (Wu *et al*., 2020a). A known biomarker for large scale non communicable or communicable disease. Mainly concern with in chronic inflammatory and neurodegenerative diseases, such as cardiovascular disease, type 2 diabetes mellitus, age-related macular generation, hemorrhagic stroke, Alzheimer's disease, and Parkinson's disease, along with bacterial infections (Wu *et al*., 2020b).

In our study we observed that out of five two samples were with high level of CRP than the normal. The increase in level of this protein is reflecting some kind of infection or inflammation in the workers without any clinical symptoms. Our study clearly demonstrates the presence of various organisms (maybe pathogenic or may not be pathogenic) in meat samples where these workers were involved at any level. It might be possible that the

sample which they were handling might have organism that could have been transferred to these workers and could be in the latent phase for which disease symptoms were not observed. Interleukin-6 is a pro-inflammatory cytokine that may have a role in the initiation, progression, and vulnerability of many diseases (Radhakrishnan *et al*., 2020). The fluctuating level of IL-6 is observed in many infectious diseases and is one of the important diagnostic markers for the disease or any change in the pathology of the system, (Shi *et al*., 2020). In our study it is observes that in all the workers the level of IL 6 was low and much below the normal value. As on date there is no reports where in infectious disease the level of IL6 has gone down. The correlation between the CRP and IL6 is much established and as the IL6 level goes up the CRP level also shoots. These are new findings why the level of IL6 is down without decrease in the levels of CRP need further studies to provide more mechanistic approach.

Conclusions

In Saudi Arabia large portion of meat and fish exported from different countries in processed form. Presence of any pathogenic bacteria in these foods may affect health. Presence of *S. aureus, Pseudomonas* and *Vibrio* species bacteria in the food stuffs imported from different countries with varying concentration. Our study clearly demonstrates the presence of different microorganisms which may be pathogenic in meat samples were these workers were involved at any level of processing. The fluctuation of parameters of blood from normal level indicates the presence of these bacteria in foods and their regular contact with such type of foods. Further, more detailed clinical study required for establish of the claim.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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