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# Quantitative Exposure Assessment of Enterotoxin-Producing *Staphylococcus aureus* in Chicken Briyani

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**A B S T R A C T**

A Quantitative Risk Assessment Model was constructed to estimate the probability that a serving of Chicken Briyani prepared in the catering units at Tamil nadu, India is contaminated with *S. aureus*. Existing data, predictive models and user input were used to define the input settings. The retail to table pathway was modeled as a series of nodes and associated pathogen events that included initial contamination of raw chicken at retail, growth during caterer transport, storage at catering establishments, thermal inactivation during cooking, post process contamination during serving (on the day consumption) and storage of leftover (consumption after overnight storage). The extent of *S. aureus* growth in each node (with exceptions) were assessed using Combase predictive models whereas thermal inactivation during cooking was quantified using simple mathematical calculations as there was no suitable inactivation model for *S. aureus*. The data, models and user inputs were entered into an Excel (Microsoft, Redmond, WA) spreadsheet. Modeling @RISK software (Palisade Corporation) was used to perform Monte Carlo simulation of ~10000 iterations. Results of exposure assessment and probability of illness revealed that 36.67% of the population consuming chicken briyani is at risk of staphylococcal food intoxication (at both node 5&6). In 95% of the cases, the probability of getting illness due to the consumption of *S. aureus* contaminated Chicken Briyani to at least one recipient is equal to or less than 0.99 or 99%.

## Introduction

Urbanization, consequent changes in pattern of human consumption, globalization of food market and climate change lead to new challenges against foodborne pathogens. Convenient, ready to eat foods provides a favourable atmosphere for toxin producing bacteria such as *Staphylococcus aureus* mainly due to inhibition of competitive microbial flora and post-process contamination. The European Food Safety Authority (EFSA) reported in 2009 that cheese followed by mixed or buffet meals were two main food vehicles involved in outbreaks of food poisoning caused by staphylococcal toxins. Scallan *et al.,* (2011) also stated that *S. aureus* is a significant cause of foodborne diseases, causing an estimated 241,000 illnesses per year in United States and Shale *et al.,* (2005) too observed that in developing countries *Staphylococcus aureus*

has been consistently shown to be one of the most important micro-organisms responsible for food poisoning outbreaks. In India, there are no systematic studies to understand the types of foods involved and the etiological agent causing the foodborne diseases (Sudershan *et al.,* 2014) which is mainly due to neglected surveillance, under reporting, poor consumer awareness about food safety issues. Consequently, the information on incidence of Staphylococcal Food Poisoning (SFP) and implicated foods are very limited. However, several studies have indicated the prevalence *Staphylococcus aureus* in foods and environment in general (Ghosh *et al.,* 2007; Mahale *et al.,* 2008; Tambekar *et al.,* 2011; Agarwal *et al.,* 2012) and chicken meat and its products in particular (Ruban *et al.,* 2012; Arul Kumar and Saravanan, 2011; Badhe *et al.,* 2013; Sudershan *et al.,* 2014). Biryani is a rice-based mixed meal containing rice, spices, condiments and chicken pieces. One of the most popular, often consumed Prepare, Cook and Serve (PCS) meat preparation in the Indian subcontinent and however, the customary handling and storage practices in catering environments as well as households including eating habits of consumers pose a threat of staphylococcal intoxication.

In this context, Predictive Modeling (PM) and Quantitative Microbiological Risk Assessment (QMRA) are the promising tools where the former operate to fill the knowledge gaps by predicting the possible growth and multiplication of bacterial pathogens in different steps/modules of food supply chain and the latter to assess the consequent risk posed to the public health upon exposure (i.e., consumption), provided risk factors i.e., adequate information on food supply chain, especially, food processing and handling environment, pathogen behavior and incidences and consumption pattern of the food concerned are known. Hence, the present

study was carried out to determine the possibility of Chicken Briyani serving as a source of risk to contract Staphylococcal Food Poisoning to the consumers, using predictive modeling and Quantitative Microbiological Risk Assessment, in the given context.

## Materials and Methods

### Overview of modeling contamination of S. aureus in chicken briyani

A QRAM for *S. aureus* in Chicken Briyani (Fig. 1) was constructed in an Excel spreadsheet and was simulated using @ risk (version 4.1, Palisade, Newfield, NY). The retail-to table pathway was modeled as a series of unit operations and associated pathogen events that included initial contamination of raw chicken at retail (node 1), growth during caterer transport (node 2), storage at catering establishments (node 3), thermal inactivation during cooking (node 4), post process contamination during serving (node 5; on the day consumption) and storage of leftover (node 6; consumption after overnight storage). This risk model explicitly assumes that all the Coagulase Positive Staphylococci in raw retail chicken (results from the previous study; 3.79 log CFU/g) are *S. aureus* and 95% of them carrying enterotoxigenic gene sequences. Further, this model assumes that all retail raw chickens were individually packaged and thus, cross contamination did not occur during caterer transport (node 2) and subsequent storage (node 3) and hence, the prevalence was same in node 1, 2 and 3.

This model also presumes that other ingredients added while cooking chicken /preparing chicken briyani (node 4) did not contain any *S. aureus* whereas post-process contamination occurred immediately after cooking (node 4) but before serving (node 5).

The level of contamination and incidence of *S. aureus* in the latter node was defined by the data from an unpublished work on chicken briyani. An overview of the simulation variables and distributions that were used in this risk assessment model are discussed in more detail in input settings (2.1.2). In general, pathogen events were modeled using PERT, Triangular and Discrete distributions as well as predictive modeling software 'Combase' either singly or by linking these distributions to model rare pathogen events.

**Input settings**

### Node 1 - Initial contamination of raw chicken at retail

The first node in the QRAM simulated the initial contamination of raw chicken with *S.aureus* at retail. The data from case study 1 on CPS count in retail chicken meat and prevalence were used to define input settings with necessary modifications as per our assumptions made earlier. The data on *S. aureus* count in retail raw chicken (initial contamination) were subjected to distribution fitting in @risk. The best fitting distribution was chosen according to chi-square test, probability-probability plot, quantile-quantile plot and also by comparing the descriptive statistical parameters.

### Node 2 - Caterer transport

The second node simulated growth of *S.aureus* on retail raw chicken. To establish input settings for this node, personal opinion was made on time (30 min, 120 min) and temperature (min. 23.5°C, max 35.3°C) data for caterers transport of raw chicken meat from retail stores to catering units and the same were used in the growth model. Uniform distribution was used to define time and temperature data.

### Node 3 - Storage at catering unit

The third node simulated growth of *S. aureus* on retail raw chicken while the caterers store them for a while in the catering unit before cooking. To establish input setting for this period, again personal opinion was made on temperature (min 23.5°C, max 35.3°C) and duration of storage (min 1h, max 3h) as there were no available data and the same were utilized in the growth model. As in the previous node, uniform distribution was used to define data (Fig. 2).

### Node 4 - Cooking of chicken briyani

The fourth node simulated the thermal inactivation of *S. aureus* during preparation of Chicken Briyani (cooking of chicken). In this node, it was assumed that chicken briyani was cooked in the catering unit at a minimum temperature of 68°C, maximum of 72°C and median of 70°C for a minimum duration of 45 min, maximum of 90 min and most likely of 60 min. These time and temperature values were used to define the input settings for the PERT distributions in constructing the thermal inactivation model. Result of the cooking model simulation indicated that the log cycle reduction of *S. aureus* ranged from -266.17 to -137.02 with a median value of -189.39. These values were used to define the PERT distribution for cooking.

### Node 5 - Serving - on the day consumption

The fifth node simulated post process-contamination of Chicken Briyani with *S. aureus* and its growth during serving. Here it was assumed that the contamination of chicken briyani was occurred immediately after cooking when left at ambient temperature for considerable period of time that would promote growth of *S. aureus*. To establish input setting for this period, again personal opinion was made on temperature

(min 27°C and max. 32°C) and duration of storage (min 1h, most likely 2½ h and max. 8h) as there were no available data and the same were utilized in the growth model and thereby subjected to uniform and PERT distributions, respectively. With respect to level (initial) of post process-contamination and incidence of *S. aureus,* as mentioned earlier, the data from an unpublished work were used to establish input setting and discrete distribution was applied on incidence data (Fig. 3).

## Node 6 - storage of leftover (Consumption after overnight storage)

The sixth node simulated growth of *S. aureus* in chicken briyani that has been kept overnight at refrigerated temperature in the households. To establish input setting for this period, again personal opinion was made on temperature (min 6°C; most likely 7.5°C and max 9°C) and duration of storage (min 8h, most likely 10h and max 11h) as there were no available data and the same were utilized in the growth model. Pert distribution was used to define data (Fig. 4).

## Exposure assessment

*S. aureus* causes food borne diseases by the enterotoxins it produces. Several studies have shown that the concentration of *S. aureus* needed for detectable toxin production in a variety of foods varies between $10^6$ and $4 \times 10^7$ CFU/g (Gockler *et al.,* 1988; Anunciacao *et al.,* 1995; Walls and Scott, 1997; Lee *et al.,* 2004; Fujikawa and Morozumi, 2006). According to the U.S. Food and Drug Administration, a toxin dose of less than 1.0 μg in contaminated food will produce symptoms of staphylococcal intoxication and this toxin level is reached when *S. aureus* populations exceed $10^5$ CFU/g (Food and Drug Administration, 1992). Based on all this information, $10^5$ CFU/g was used as the minimum concentration of *S. aureus* needed to cause foodborne disease, although further research might refine this value to a greater degree. In the present study, exposure assessment has been carried out in two nodes (Node 5 and 6).

## Probability of illness

The probability of illness due to consumption of chicken briyani has been calculated by the exponential model proposed by the Rose and Haas (1999). The model proposed is as follows.

P (response) = 1-(-k x dose) where 'k' is optimized parameter

## Simulation modeling

The data, models and user inputs were entered into an Excel (Microsoft, Redmond, WA) spreadsheet. Modeling @ RISK software (Palisade Corporation) was used to perform Monte Carlo simulation of ~10000 iterations.

## Results and Discussion

## Estimated level of contamination at the point of consumption (using Combase predictive models and @ risk software)

The data on concentration of *S.aureus,* as per assumption made earlier, in retail raw chicken meat from previous study was utilized to demonstrate the distribution of the initial contamination level of *S.aureus*. The triangular distribution was chosen to define the extent of pathogen events (initial contamination) in node 1. In 90% of the chicken meat samples the *S. aureus* count varied between 3.28 and 4.26 log CFU/g of chicken and discrete distribution was used to define the incidence of *S. aureus* contamination. From these probabilistic distributions on extent of initial contamination

and incidence, the output distribution at retail has been obtained. The maximum number of colonies obtained in output distribution at retail were approximately $2.87 \times 10^4$ CFU/g and the same was used to determine the logarithmic growth per hour (0.541 log CFU/h) using Combase growth model and extent of potential growth during the period of transport to catering unit (Node 2) was estimated to be about 0.68 log CFU/g under given time and temperature conditions as well as incidence rate. Similarly, the maximum number of colonies obtained in the output distribution for node 2 (Transport to catering unit) was used to determine the output for node 3 (storage at catering unit).

As there was no suitable thermal inactivation model for *S.aureus,* both in Combase and Pathogen Modeling Program, the logarithmic reduction in the colonies during preparation of Chicken Briyani/cooking of chicken (node 4) was determined by mathematical calculation using D-value (D70=0.33 min) under the given risk factors such as temperature and time. The results revealed that there would be approximately 193.45 log CFU/g reduction in *S. aureus* under given cooking time and temperature combination and consequently, no colonies could be identified during the end of cooking process (node 4). As per the assumption mentioned in *2.1,* post-process contamination of Chicken Briyani occurred immediately after cooking (node 4) but before serving (node 5) with 3.35 log CFU/g of *S. aureus* (unpublished data). Based on the prevailing practices followed in the catering units, it was presumed that there would be growth of contaminating (post-process) *S.aureus* during the time delay between cooking and serving and hence the same was predicted using Combase growth model. The output distribution for serving based on cross contaminating microorganisms and its growth was calculated. Similarly, output distribution was calculated for node 6

under given temperature - time conditions to determine the pathogen event.

## Exposure per serving

As mentioned in section 2.3 exposure assessment has been carried out in two nodes (Node 5 and 6). Further, $10^5$ CFU/g was used as the minimum concentration of *S. aureus* needed to cause foodborne disease. At node 5 (on the day consumption), 63.33% of the population consumes less than or equal to $10^5$ cfu/serve. Conversely, 36.67% of the population is at risk of staphylococcal food intoxication. From this exposure assessment study, it could be inferred from the detailed statistics that 50%, 90%, 95% and 99% of the population had the exposure level of equal to or less than 0.00, 8.54, 8.82 and 12 log cfu/serve, respectively when exposure assessment was made at Node 5. Surprisingly, similar percentile of the population consumes less than or equal to $10^5$ cfu/serve at Node 6. However, the detailed statistics revealed that 50%, 90%, 95% and 99% of the population had the exposure level of equal to or less than 0.00, 12, 12 and 13 log cfu, respectively when exposure assessment was made at Node 6. This finding is concordance with Sudershan *et al.,* 2014 who reported that chicken briyani with *Staphylococcus aureus* count of 8.5- $9.5 \times 10^8$ cfu/g involved in a food poisoning outbreak in Hyderabad city, Andhra Pradesh.

## Estimation of probability of illness

From the cumulative distribution and descriptive statistics, it could be inferred that in 50% of the cases, the probability of risk in getting ill to at least one recipient is equal to or less than 0.00 or 0%. In 95% of the cases, the probability of getting illness due to the consumption of *S. aureus* contaminated Chicken Briyani to at least one recipient is equal to or less than 0.99 or 99%. In 99% of the cases, the probability of getting illness to

at least one recipient is equal to or less than 1 or 100% at node 5. This study reveals the higher probability of illness due to Staphylococcal Food Poisoning in consumers of Chicken Briyani. The time delay between preparation and consumption as well as temperature of storage are the important risk factors (Fig. 5).

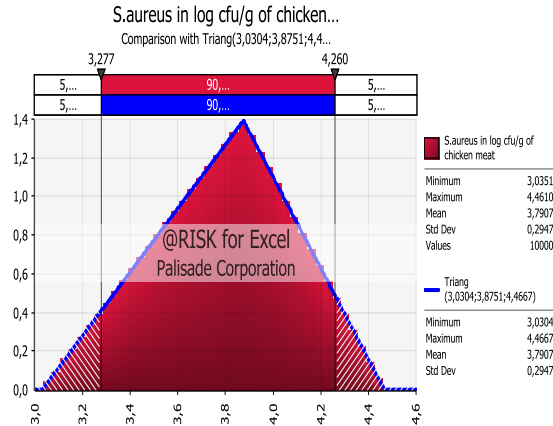**Fig.1** Distribution of initial contamination (extent) of retail raw chicken with *S. aureus*
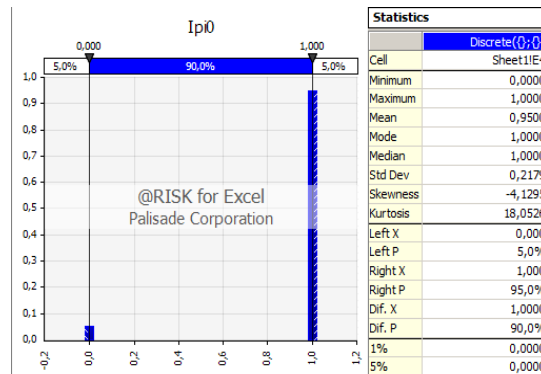
**Fig.2** Prevalence distribution for nodes 1,2 & 3



**Fig.3** Exposure/serve or week at Node 5

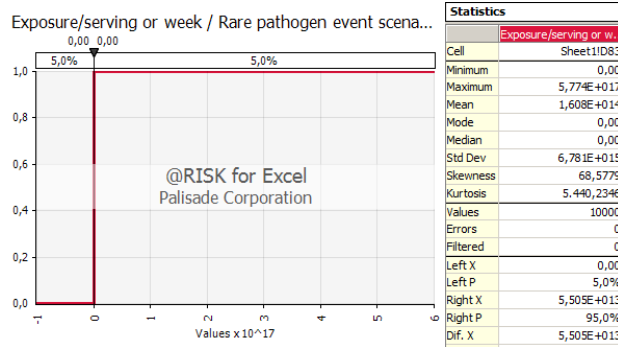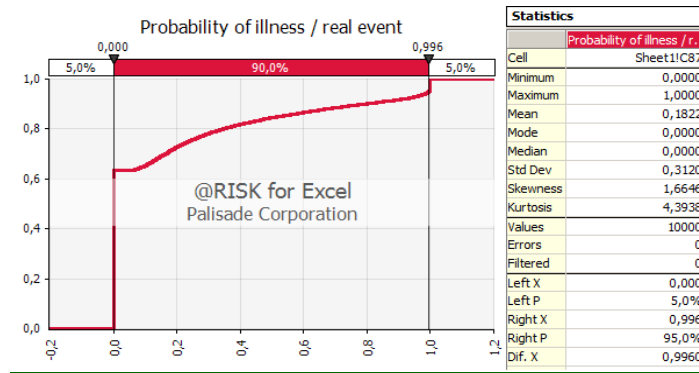**Fig.4** Exposure/serve or week at Node 6



**Fig.5** Estimation of probability of illness



In conclusion, initial microbial load in raw chicken, transport and storage at ambient temperature significantly influence the microbial load of the chicken before cooking.

Cooking is a crucial event which should be monitored to avoid happening of untoward events.

Post-process contamination and eating habits appears to important factors makes the population vulnerable to SFP.

## Acknowledgement

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