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Quantitative Microbiological Risk Assessment of Two Street Foods Sold in a Kenyan Town with Regard to *Salmonella* Contamination

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Abstract

Street sold foods have gained popularity in recent times, particularly in developing countries where their sale is often unregulated, hence, has a potential to transmitting pathogenic microorganisms which are of public health concern. A survey of two street foods, *kachumbari* (a type of a vegetable salad) and *mandazi* (a type of a wheat-based flour snack) was carried out in a Kenyan town to determine the prevalence and conduct a quantitative microbiological risk assessment to estimate the risks of infection due to consumption of the two products contaminated with *Salmonella*. A prevalence of 19% (16 out of 86 samples) and 7% (6 out of 86 samples) was observed for *kachumbari* and *mandazi* respectively. A risk assessment model composed of three different steps (nodes) comprising finished product (processed ready-to-eat), waiting (storage) period and consumption was used for the microbiological risk assessment. Models built in excel spreadsheets using @Risk software package, version 6 (Palisade USA) was used to obtain the inputs, outputs, and run the Monte Carlo simulations at 5000 iterations. The model estimated that in 95% of the cases, the consumers of *kachumbari* would be exposed to a maximum dose of 8.3×10^4 *Salmonella* cells per single serving. On the other hand, in 95% of the cases, consumers of *mandazi* would be exposed to a maximum dose of 4.0×10^4 *Salmonella* cells per single serving. The model also predicted that 64.3% and 69% of the population was at risk of developing salmonellosis upon consumption of contaminated *kachumbari* and *mandazi* respectively. The results indicate that these two products can contribute to high levels of salmonellosis morbidity. Nevertheless, a significant reduction in the level of ingested *Salmonella* cells in *kachumbari* and *mandazi* could be attained through a reduction of the prevalence of the pathogen contamination at or before the point of sale by the employment of good hygienic practices during their preparation and subsequent handling, in addition to enforcement of food hygiene regulations regarding street foods to ensure microbiologically safe foods are sold to the consumers. In order to improve the accuracy of this risk assessment model, more data, whenever available should be used in such studies.

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Introduction

The business of street food selling is an age-old occupation which is common worldwide[1]. These foods, which usually do not require further processing before consumption, are widely accepted by many people in developing countries because they are viewed as nutritious, inexpensive, convenient and attractive[2]. The World Health Organization (WHO) defines street foods as 'foods and beverages prepared and/or sold by vendors in streets and other public places for immediate consumption or consumption at a later time without further processing or preparation[3]. Since street foods are considered among the sources that can transmit pathogenic microorganisms that can cause foodborne illnesses worldwide[4], their microbiological quality is of great food safety concern as the consumers are continuously exposed to the risk of contracting these diseases[5]. A recent review publication about street foods[2] talks about the pros and cons of these products, including the significance of microbiological contamination.

The World Trade Organization (WTO) recommends the use of risk analysis as a great approach towards production and provision of safe foods in an effort to assure good health to human population[6]. Risk analysis comprises risk assessment, risk management and risk communication[7].The aim of risk analysis is 'to provide a global standard for the interpretation of the acceptability of risks associated to foods to which consumers might be exposed'[8]. Quantitative microbiological risk assessment (QMRA), which is one of the three components of risk analysis process, is a somewhat new scientific discipline capable of linking information from food production to consumption (farm-to-fork) and information on foodborne diseases to give an approximation of the effect of contaminated food on consumer health[9]. This QMRA concept was developed by the Codex Alimentarius Commission (CAC), an executive body of WHO and FAO[8]. QMRA highlights risks associated with pathogens along the food chain whose outcome is to give an approximation of the possibility of disease development from a foodborne microbe in a given population. QMRA is a scientifically-based process made up of four concise steps according to CAC[10] namely; hazard identification, hazard characterization (dose-response), exposure assessment and risk characterization.

The QMRA concept is progressively gaining a lot of interest and attention especially in the developed countries. In fact there currently exist a sizeable number of scientific publications and reports on microbiological risk assessment of various types of foods. Unfortunately, this is not the case with developing countries where research and publication, as well as reporting in this aspect are largely lacking. Most of the microbial food safety research in developing countries seems to focus more on incidence and prevalence of pathogenic microorganisms in foods, with the microbial risk assessment taking a back seat. QMRA, just like HACCP, is an important tool that can be used to increase safety of foods and food products through assessment of their safety as well as predicting the effects of intervention measures in food production processes[11,12,13]. QMRA can be used to obtain important microbiological information for risk managers to mitigate, prevent or control a microbiological problem[11]. QMRA is currently applied in numerous developed countries as a useful instrument to enable realistic resolutions to be made to minimize the effect of disease-causing organisms on human health[15]. It is for this reason that the approach should be encouraged for adoption especially in developing countries, particularly in the African context because it has been shown that the number of QMRAs that are performed is low, and the discipline calls for awareness with regard to resource allocation[15]. The objective of this investigation was to conduct a QMRA of two common street-vended foods, *kachumbari* and *mandazi* sold in one of Kenyan towns with regard to *Salmonella* contamination.

Materials and Methods

Kachumbari and *Mandazi* Products Descriptions

Kachumbari is a fresh salad dish that is popular in East Africa. Variations of this product can be found in Kenya, Tanzania, Rwanda, Uganda and Burundi. It is prepared using fresh chopped tomatoes, onions and chili peppers. There is extensive handling of

this product during preparation. There is no cooking (heat treatment) step involved in its preparation and consumption.

Mandazi (singular, *andazi*), also known as *swahili* bun is a form of deep fried bread made from wheat. The snack is popular in East Africa as it is convenient to make, can be eaten with almost any food or just as a snack by itself. It is made of wheat, sugar, salt, cooking oil and baking powder.

Determination of Prevalence of *Salmonella* in *Kachumbari* and *Mandazi*

Salmonella prevalence data used in this QMRA is derived from a separate unpublished study which sought to determine the prevalence of *Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Vibrio cholerae* in *kachumbari* and *mandazi* in the said town. Data for prevalence of many foodborne pathogens and consequences of the diseases they cause in Kenya is generally not readily available, and is largely lacking in scientific literature. The choice for *Salmonella* for the investigation of risk assessment in these two products was therefore based on the fact that salmonellosis is one of the principal causes of gastroenteritis traceable to most foods globally. This pathogen is generally estimated to cause the greatest health impact in financial terms and results in loss of Quality Adjusted Life Years (QALYs), a measure of health-related quality of life in developed countries like the USA[16]. There have also been sporadic but undocumented media reports in Kenya on salmonellosis incidences, particularly in institutions of higher learning where students are known to buy street foods as convenience foods due to their affordability and busy studies lives meaning that they rarely prepare their own meals. *Kachumbari* and *mandazi* are some of the most common street vended foods in Kenya and media reporting seem to attribute them, alongside other foods to sporadic salmonellosis outbreaks in many urban centers. Table 1 shows the prevalence of *Salmonella* in the two products alongside other pathogens.

Table 1: Prevalence of *Samonella, Staphylococcus aureus, Listeria monocytogenes* and *Vibrio cholerae* in *kachumbari* and *mandazi* sold in Thika town. Values in parentheses represent prevalence.

	Food type						
	Kachumbari				***Mandazi***		
Microbial test	**Total samples**	**+ve (%)**	**CFU/g range**	**CFU/g Mean**	**+ve (%)**	**CFU/g range**	**CFU/g Mean**
Salmonella	**86**	**16 (18.6)**	**-**	**-**	**6(7)**	-	-
Staph. aureus	86	63 (73)	0-1.3x10^7	1.1x 10^7	46(53.5)	0-2.2x 10^5	1.2 x 10^4
Lis. mono.	86	56(65)	0- 1.6x10^7	1.3x10^6	11(12.8)	0-1.2x 10^5	1.1 x 10^3
Vib. cholerae	86	0	0	0	0	0	0

+ve = number of positive samples

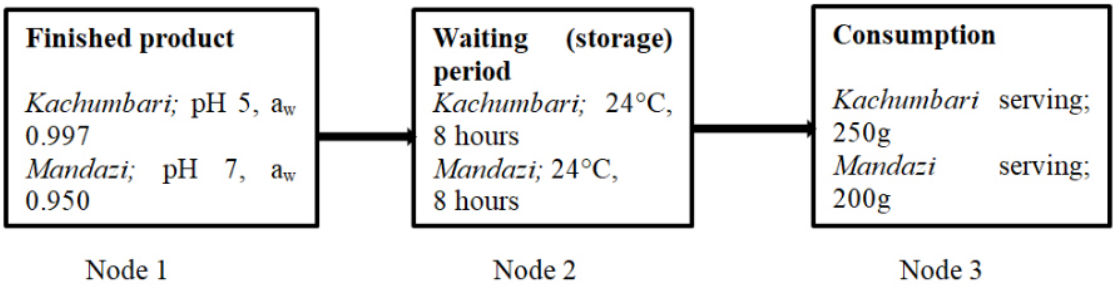

Figure 1: Flowchart showing the exposure assessment pathway for *Salmonella* in *kachumbari* and *mandazi.*

Exposure Pathways

Three nodes (steps) were considered in the exposure pathway for this study as shown in Figure 1. The pathway was modeled as a series of three unit operations and associated pathogen events that included initial contamination of the finished products (Node 1), growth during the waiting period (storage) before sale (Node 2) and dose-response after consumption (Node 3) (Figure 1).

The first node, finished product, starts with the evaluation of the initial prevalence, which is the chance of occurrence and the concentration of the pathogen in the ready-to-eat *kachumbari* and *mandazi* (Table 1). Changes were then assessed throughout the remaining nodes as a result of *Salmonella* growth dynamics. The exposure assessment concludes in an estimation of the probability and level of exposure to the pathogen following the ingestion of a single serving of either *kachumbari* or *mandazi* food. The cell addresses, formulas and input settings used in the assessment using the @Risk software are shown in Tables 2a and 2b for *kachumbari* and *mandazi* respectively.

Use of @Risk Software Package to Carry out the Risk Assessment

A risk assessment model composed of the three different nodes as highlighted in Figure1 (finished product (processed ready-to-eat), waiting (storage) period and consumption) was used for the microbiological risk assessment. Models built in excel spreadsheets using @Risk software package, version 6 (Palisade USA) was used to obtain the inputs, outputs, and run the Monte Carlo simulations at 5000 iterations.

Steps in the Exposure Pathway

Node 1: Prevalence Estimate (Initial Contamination)

The proportion of contaminated ready-to-eat *kachumbari* and *mandazi* was modeled as a random variable with a discrete distribution; Discrete ({xi},{pi}) (Table 2a and 2b) as this distribution is normally used for modeling activities and phenomena with a small number of outcomes[17]. The assumption made here was that the initial concentration of *Salmonella* in finished product ranged from 1 CFU/g (minimum), 10 CFU/g (median) and 100 CFU/g (maximum)[17].

Node 2: Waiting (Storage) Period-Microbial Growth

Waiting period is that time duration between production and consumption (Figure 1). During this time, the food products are maintained at ambient temperature (approximately 24°C) and this happens throughout until they are purchased and consumed. There is a possibility of *Salmonella* growth and multiplication during this time depending on both intrinsic and extrinsic properties of the two food categories. After production, the two foods are maintained at conditions shown in Figure 1. ComBase, an online tool for quantitative food microbiology (https://www.combase.cc) used to study and predict how microorganisms survive and grow under a variety of food-related conditions was used for modeling the level of growth of *Salmonella* at this step. Zero, 1 and 2 log CFU/g for minimum, median and maximum *Salmonella* concentration in the product was assumed at this step. A continuous distribution, PERT distribution (PERT(minimum, most likely, maximum)), was used to model the concentration of *Salmonella* (Table 2a and 2b)[17]. The PERT distribution was selected as the continuous distribution for extent of *Salmonella* pathogen event.

Node 3: Consumption (Dose-Response)

The third step modeled consumers' response upon consumption of *Salmonella* contaminated products. A PERT distribution was used to simulate illness dose of the consumption event (Table 2a and 2b). The dose-response data for salmonellosis-causing *Salmonella* in consumers is scarce and the little that is available shows that the degree of virulence is strain dependent, with the least dose causing the disease in healthy individuals ranging from 10^5 to 10^{10} for the 13 strains tested[18]. However, estimated doses of *Salmonella* ingested in outbreaks that may have involved less resistant consumers, more virulent strains of *Salmonella* and/or more permissive meals ranges from 10^1 to 10^{11} with a dose of $< 10^3$, usually causing illness[19]. The input settings for PERT distribution for illness dose were adopted from previous studies[17] where a minimum of 1 log MPN, a median of 3 log MPN, and a maximum of 7 log MPN were used[17].

Table 2a: Cell addresses and formulas used in the quantitative risk assessment model for *Salmonella* in *kachumbari* using the @Risk software.

Cell	Variable	Description	Unit	Distribution/value	Reference
Node 1 Finished product (*Kachumbari*)					
A2	N	Number of samples		86	Own data
A3	X	Number of positive samples		16	Own data
A4	P	Prevalence of *Salmonella* in *kachumbari*		=RiskDiscrete({0\1}; {0,814\0,186})	Calculated
A5	IC	Initial concentration of *Salmonella*	Log CFU/g	=RiskPert(0;1;2)	Assumed
A6	IC	Initial concentration of *Salmonella* with prevalence	Log CFU/g	=IF(A4=0,0,A5)	Calculated
Node 2 (Storage)					
A8	T	Storage temperature	°C	26	Own data
A9	pH	Product pH		6	Assumed
A10	SG	Growth at storage	Log CFU/g	=RiskPert(0,19;1, 19;2,19)	ComBase prediction
A11	SC	Concentration of *Salmonella* during storage	Log CFU/g	=A6+A10	Calculated
A12	SC	Concentration of *Salmonella* during storage	CFU/g	=POWER(10;A11)	Calculated
Node 3 (Consumption)					
A14	Ps	Portion size	g	250	Own data
A15	C/S	Concentration of *Salmonella* in a serving	CFU/serving	=A12*A14	Calculated
A16	CI	Concentration of *Salmonella* causing illness	CFU	=POWER(10; Risk Pert(1;3;7))	Oscar, 2004
A17	R	Risk of illness as a result of consuming *Salmonella* contaminated *kachumbari*		=A15/A16	Calculated

Table 2b: Cell addresses and formulas used in the quantitative risk assessment model for *Salmonella* in *mandazi* using the @Risk software.

Cell	Variable	Description	Unit	Distribution/value	Reference
Node 1 Finished product (*Mandazi*)					
A22	N	Number of samples		86	Own data
A23	X	Number of positive samples		6	Own data
A24	P	Prevalence of *Salmonella* in *mandazi*		=RiskDiscrete({0\1}; {0,93\0,0698})	Calculated
A25	IC	Initial concentration of *Salmonella*	Log CFU/g	=RiskPert(0;1;2)	Assumed
A26	IC	Initial concentration *Salmonella* with prevalence	Log CFU/g	=IF(24=0,0,A25)	Calculated
Node 2 (Storage)					
A28	T	Storage temperature	°C	26	Known data
A29	pH	Product pH	pH	6	Assumed
A30	SG	Growth at storage	Log CFU/g	=RiskPert(0,71; 1,71;1,71)	ComBase prediction
A31	SC	Concentration of *Salmonella* at storage	Log CFU/g	=A26+A30	Calculated
A32	SC	Concentration of *Salmonella* at storage	CFU/g	=POWER(10;A31)	Calculated
Node 3 (Consumption)					
A34	Ps	Portion size	g	200	Own data
A35	C/S	Concentration of *Salmonella* in a serving	CFU/serving	=A32*A34	Calculated
A36	CI	Concentration of *Salmonella* causing illness	CFU	=POWER(10; Risk Pert(1;3;7))	Calculated
A37	R	Risk of illness as a result of consuming *Salmonella* contaminated *mandazi*		=A35/A36	Calculated

Results and Discussion

Table 3 shows the summary of model predictions of the minimum, maximum and mean of *Salmonella* concentration per servings of *kachumbari* and *mandazi* at the time of consumption. It also presents the predicted level of contamination at P50, P75, P90, P95 and P99. The model estimated that in 95% of the cases, the consumers of *kachumbari* would be exposed to a maximum dose of 8.3 x 10^4 or less of *Salmonella* cells per single serving. Only 5% of the servings would contain greater than this number, and less than 5% of the same would contain a level of contamination less than 1.0 x 10^3 *Salmonella* cells per serving. On the other hand, in 95% of the cases, consumers of *mandazi* would be exposed to a maximum dose of 4.0 x 10^4 *Salmonella* cells per single serving (Table 3).

Table 3: Concentration of *Salmonella* in *kachumbari* and *mandazi* per serving at the time of consumption. Values are exposure of the pathogen in CFU/serving.

	Exposure per serving								
Food type	**Minimum**	**Maximum**	**Mean**	**Std Dev**	**P50**	**P75**	**P90**	**P95**	**P99**
Kachumbari	$4.5x10^2$	$1.7x10^6$	$2.0x10^4$	$6.6x10^4$	$5.0x10^3$	$1.1x10^4$	$3.3x10^4$	$8.3x10^4$	$2.9x10^5$
Mandazi	$1.2x10^3$	$6.2x10^5$	$1.4x10^4$	$3.9x10^4$	$7.8x10^3$	$9.3x10^3$	$1.0x10^4$	$4.0x10^4$	$2.1x10^5$

The model estimated that only 5% of the servings would contain a level of contamination of greater than this number, while less than 5% of the servings would contain less than 3.7 x 10^3 *Salmonella* cells. On average, exposure by *kachumbari* is higher than that of *mandazi.* One of the possible reasons is probably because the prevalence of *Salmonella* in the former product was higher than in the later product at the start. It is also worth noting that since there is no heat processing step involved in the production of *kachumbari*, there is no chance of reducing the numbers of the pathogen already present. It is of paramount importance to also note that, in addition to *kachumbari* being a fresh product, it is also extensively handled by hand during production, an action that has been greatly associated with food contamination by microorganisms[2]. On the other hand, although handling is also common for *mandazi*, the product is deep fried and therefore, the only problem could result from post-heat contamination. The storage conditions for the two products are basically the same, but their intrinsic factors differ significantly (Figure 1). It is widely known that higher water activity encourages multiplication of microorganisms in foods, which is the case with *kachumbari,* compared to mandazi. Data on the two products is non-existent in the literature and it is therefore difficult to do any comparison with previous work. For any food to be safe for consumption in relation to *Salmonella*, this pathogen is expected to be absent in every 25 g of food tested[20] which is clearly not the case with these findings.

Figures 2a and 2b shows the models estimation of the population's risk of illness upon consumption of *Salmonella*-contaminated *kachumbari* and *mandazi.*

The likelihood of risk of illness per exposure per serving (due to single exposure) was determined by calculating the ratio of exposure to dose-response of *Salmonella* i.e. risk of illness per serving = Exposure (CFU per serving)/Dose-Response. The value equal to one meant that there would be illness. Figures 2a and 2b, shows the proportion of the population at risk of contracting salmonellosis as a result of consuming contaminated servings of *kachumbari* and *mandazi* respectively. For *kachumbari* (Figure 2a), 64.3% of the population would be at risk of developing salmonellosis, while only 35.7% would be protected from the illness. In case of *mandazi* (Figure 2b), 69% of the population would be at risk of developing salmonellosis while 31% of the population would be protected. With estimated daily consumers (population) of about 200000, then, approximately 128600 and 138000 would be at risk of developing salmonellosis upon consumption of contaminated *kachumbari* and *mandazi* respectively. Although both

products seem to be high-risk products based on this result, it seems that mandazi would contribute to more morbidity compared to *kachumbari*. The reason for this finding is not well understood and thus requires further investigation, particularly by use of larger data sets and taking note of every single detail involved in the entire production line, up to the point where the consumers are served, clearly noting the general hygiene and the personal hygiene of the food handlers.

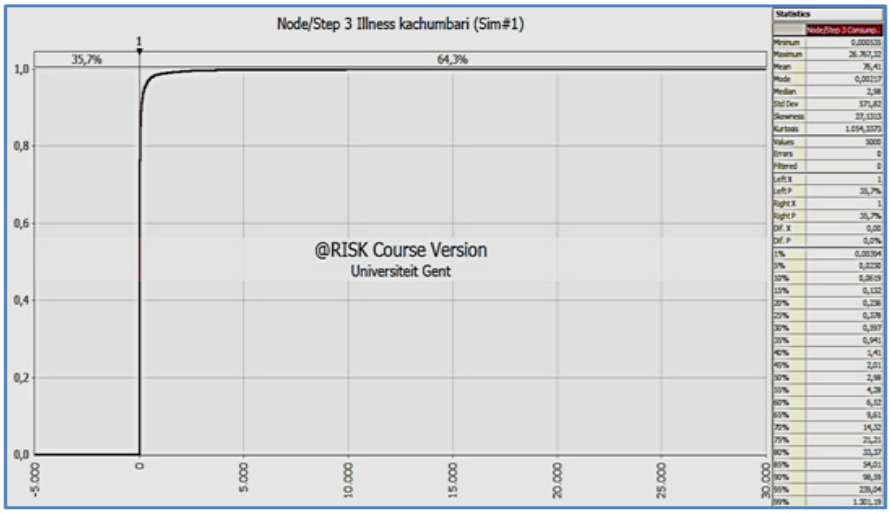

Figure 2a: Model estimation of the percentage of the population at risk of developing salmonellosis as a result of consuming *Salmonella*-contaminated *kachumbari* servings.

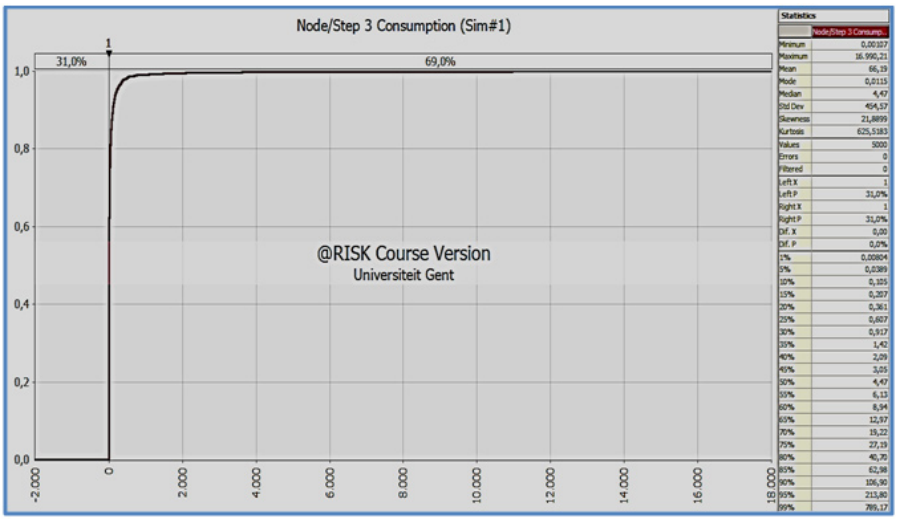

Figure 2b: Model estimation of the percentage of the population at risk of developing salmonellosis as a result of consuming *Salmonella*-contaminated *mandazi* servings.

Conclusion

Quantitative microbiological risk assessment is an important method that helps in approximation of the effect of microbiologically unsafe food on the consumers, even in cases where there is limited data. This method can provide scientifically sound information with regard to the presence and growth of pathogenic microorganisms in foods. This valuable scientifically-sound information derived from such a method can be used by food safety managers and in deed any other individual involved in food safety to draw inferences and make necessary decisions to assure food safety. It has clearly been seen that QMRA due to its ability to quantify microbiological risks presented by foods, can very well supplement the popular HACCP approach which is qualitative in nature. From this study, both *kachumbari* and *mandazi* are potentially hazardous food products which is not a surprise given the conditions in which they are produced and/or handled. High exposures lead to a high estimated risk of developing salmonellosis. A significant decrease in the number of ingested *Salmonella* cells in *kachumbari* and *mandazi* could be achieved through a reduction of the prevalence of the pathogen contamination at or before the point of sale by the use of simple hygienic measures during their preparation and subsequent handling. These findings have demonstrated that none of the two foods can be deemed risk-free, they both have potential to cause an illness, and therefore, each and every step, from farm-to-fork needs to be looked at as a potential point of intervention to mitigate *Salmonella* contamination and the possible development of salmonellosis in consumers. In addition to embracing QMRA to guide in food safety decision making anchored on scientific evidence, it is also important to have policies that reinforce hygiene education of food handlers in the street food subsector to help limit risks associated with *Salmonella* and other foodborne pathogens.

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