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Microbiological Quality of Meat at the Abattoir and Butchery Levels in Kampala City, Uganda

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Abstract

Contaminated meat has been implicated in many cases of foodborne illnesses. The study assessed microbiological quality of meat at abattoir and butchery levels. Forty eight meat samples were collected and microbiologically examined in the laboratory. Samples were 100%, 97.9%, 93.8%, 83.3% and 4.2% positive for APCs, S. aureus, coliforms, E. coli and Salmonella respectively. Mean APCs were 1.64x109cfu/g for abattoir and 1.92x109cfu/g for butcheries. Coliform, E. coli and S. aureus counts were 5.0x102cfu/g, 8.4x104cfu/g and 2.7x103cfu/g at the abattoir level. Coliform, E. coli and S. aureus counts were 4.98x106cfu/g, 1.06x105cfu/g and 5.4x105cfu/g at the butchery level. Mean APCs were 3.95x109cfu/g, 1.10x109cfu/g and 7.02x109cfu/g for Wandegeya, Nakulabye and Kasubi butcheries respectively, and 1.90x109cfu/g for morning and 1.77x109cfu/g for evening. S. aureus counts were 1.86x105cfu/g for morning and 7.71x105cfu/g for evening. Overall, E.coli, S. aureus, coliforms and APCs were higher than conventionally accepted levels indicating that meat was contaminated. More stringent inspection, regular supervision and monitoring besides good hygiene practices are recommended for meat processing facilities.

Key words: meat samples, bacterial counts, abattoir, butchery, contamination

Introduction

Consumption of meat contaminated with pathogenic bacteria precedes many food-borne illnesses (Nouich and Hamdi, 2009, Food Standards Agency (FSA), 2004), with human health consequences ranging from illness to death (Iroha et al. 2011; Hassan Ali et al. 2010).

Pathogenic bacteria such as Salmonella spp., Staphylococcus aureus (S. aureus), Listeria monocytogens, Campylobacter spp. and Escherichia coli (E. coli) 0157:H7, have been implicated in a number of food borne illnesses (Nouichi and Hamdi 2009).

These bacteria arise from contamination in the slaughter house during processing of live animals into meat where the routine veterinary inspection procedures cannot detect presence of bacteria on meat (Nouichi and Hamdi 2009).

The main sources of contamination include the slaughtered animals themselves, personnel and slaughterhouse. If microbial contamination exceeds certain levels, it adversely affects shelf-life and renders the meat unfit for human consumption (Fasanmi et al. 2010), as per the guidelines stipulated to ensure safe meat handling by World Health Organization, Food and Agricultural Organization and Codex Alimentrarius Commission such as hazard critical control points (HACCP) and Good Manufacturing Practices (Hassan Ali et al. 2010). The study assessed bacterial counts of meat at the abattoir and butchery levels as an indicator for microbial quality of meat.

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Materials and Methods

Study sites, sample collection and transportation. The study was carried out at Kampala City Abattoir (KCA) on Old Portbell road in the industrial area of Kampala and three butcheries in Wandegeya, Kasubi and Nakulabye. The butcheries were selected purposively by selecting the operators at the time of buying meat from KCA and were traced to their butcheries. The study also investigated whether time of day (morning or evening) influenced the bacterial counts of meat sold at the butcheries. Meat samples from the abattoir were aseptically cut from the brisket, rump and posterior side of the fore leg of the selected carcasses to make approximately 250g and those from the butcheries were bought by the researcher just like any other customer. Meat samples were put in sterile polythene bags and placed under ice in a cool box before transportation to the Laboratory in the College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University. In the laboratory, meat samples were processed within 6 hours after collection to avoid contamination and multiplication of bacteria.

Sample preparation and inoculation. Representative samples were prepared aseptically using sterile surgical blades to cut 25grams from each food sample. The 25grams were weighed and then put into sterile stomacher bags to which 225mls of sterile buffered peptone water were added. The contents of the stomacher bags were homogenized using a pulsefier to give 10-fold dilutions in the 1:9 ratios. Food homogenates obtained were shaken to mix thoroughly and then 1ml from each stomach was pipetted into a glass bottle containing 9mls of peptone water and mixed further to give a 10^{-2} dilution. This procedure was repeated through serial dilutions of 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7} 10^{-8} 10^{-9} according to Harrigan, (1998). The universal bottles were labeled respectively. Selected dilutions of the food samples were appropriately mixed by vortexing. Inoculations were made within 25 minutes of processing. The inoculation methods used were adopted from the Compendium of Methods for microbiological examination of foods (Downes and Ito, 2001). Samples were analyzed in duplicates.

Samples were cultured onto Nutrient agar, Salted nutrient agar, Xyline Lysine Deoxycholate (XLD) agar and Coliform Chromocult agar, and incubated at 37^{o}C for 18-24 hours (Harrigan, 1998). Colony morphology on the plates was observed and colonies were counted. Sub-culturing was done to obtain pure colonies for biochemical tests. Gram staining was done to differentiate Gram positive from Gram negative bacteria. Suspected E.coli colonies when Gram stained revealed Gram negative short rods that were biochemically confirmed as *E.coli*. The biochemical tests included Tripple Sugar Iron (TSI), Urease, Indole, IMVIC and Citrate (Harrigan 1998). Using IMVIC reactions, Indole production test was considered positive if a distinct red color appeared in the upper layer, Voges-Proskauer test was considered positive if the Eosin pink color developed and methyl red test was considered positive if Simmon's citrate media remained green (Downes and Ito 2001).

Determination of total aerobic plate counts. The total aerobic plate counts (APCs) were determined by taking 0.1ml of the processed food samples of specified dilutions and inoculating them onto sterile plate count agar (PCA) using the surface spread method. The inoculated plates were incubated for 24 hours at 37^{o}C (Refai 1979, Harrigan 1998). After incubation, plates containing 25-250 colonies were selected for counting. The counts obtained were characterized by the reciprocal of the dilution factors used and additionally by 10^{1}. The bacteria population were expressed as a number of colony forming units per units per gram (cfu/g). APC is a useful indicator of the level of contamination of food with bacteria and reflects the quality, shelf-life and post-heat processing contamination.

Enumeration of total coliforms and *Escherichia coli*. Chromocult coliform agar (Merck, Germany) was used as a selective indicator media for the enumeration of *E. coli* and other coliforms. Inoculation of the processed food samples from selected dilutions were done using a procedure similar to that of APCs above. After inoculation, dark blue colonies were classified as *E. coli* colonies while pink colonies were classified as other coliforms (Petrick and Parsch 1996). Positive controls were included by using *E. coli* culti-loops, ATCC 25922. Gram staining was carried out on the suspected *E. coli* colonies. Cultures giving gram negative short rods were biochemically confirmed as *E. coli.* using the IMVIC reactions, indole production test was regarded positive if there was an appearance of a distinct red color in the upper layer (Downes and Ito 2001). The Voges-Proskauer (VP) test was considered positive if the Eosin pink color developed. The methyl red test was considered positive if a distinct red color developed. Using the citrate utilization test, *E. coli* was considered negative if Simmon's citrate media remained green.

Isolation of *Staphylococcus aureus*. *Staphylococcus aureus* (*S. aureus)* were isolated using Salted Nutrient Agar (SNA) as the selective media. The SNA was prepared using a procedure similar to that of Nutrient Agar (NA), as per the manufacturer's instructions. Sodium chloride was added to nutrient agar in the range of 8-10% weight by volume. 0.1ml of each of the selected dilutions were inoculated onto the SNA plates and spread evenly over the surface of the media using a sterile surface spreader. The inoculated plates were then inverted and incubated for 24hours at 37^{o}C. gram staining revealed gram positive cocci which were violet in color when viewed under the light microscope. *S. aureus* was confirmed biochemically using the coagulase test. Suspected *S. aureus* colonies were transferred into tubes containing 5mls of brain heart infusion broth and incubated for 20-24 hours at 37^{o}C and 0.1mls of the resulting broth was added to 0.3mls of rehydrated rabbit plasma in small tubes and incubated at 37^{o}C. The tubes were examined for clotting after 6 hours.

Detection of Salmonella in the food sample. The ten-fold dilution of food homogenates was pre-enriched for 24 hours at 37^0C (Refai 1979). To enhance the recovery of Salmonella, pre-enrichments were followed by enrichment on selective media (Tetrathionate Brilliant Green Broth (Merck, Germany), at a ratio of 1:9 for 24 hours at 37^0C (Downes and Ito, 2001). Detection of Salmonella was aided by the use of the enriched cultures streaked onto Xylose Lysine Desoxycholate agar. The inoculated selective agar was incubated at 37^0C for 24 hours and examined for typical Salmonella colonies. Large sized and completely black colonies were suspected for Salmonella (Andrews 1997). The suspected Salmonella colonies were then sub-cultured to produce a pure culture that was used in the confirmatory tests namely Urease, TSI and Citrate tests (Harrigan 1998).

Statistical analysis. The data for aerobic plate counts (APCs), Coliform, *E. coli* and *S. aureus* were statistically analyzed using R-software and plots of means were applied to the data.

Results

During sample collection, observation of the operations of the study butcheries revealed that study butcheries had similar operating conditions and hygiene practices. All the butcheries displayed meat (beef) mixed with offals openly on tables and wooden logs, had no screens which let flies into the butcheries, floors were not clean, knives and other cutting tools were handled carelessly, weighing scales were unclean and all the butcheries lacked handwashing facilities. The butcher men did not wear protective gear such as white coats, caps and gumboots and the same people handled meat and received money. The butcheries were located along the road side probably for display and marketing purposes but this exposed the meat to dust raised by automobiles. Several butcheries were located next to each other and the butcher men shared weighing scales and stones.

Table 1 presents bacterial frequencies on meat samples and it indicates that very high frequencies (above 80%) for APCs, Coliforms, *E. coli* and *S. aureus* were obtained in this study.

The mean counts for APCs were 1.64x109cfu/g and 1.92x109cfu/g for the abattoir and butcheries respectively (Table 2). The mean bacterial load was slightly higher at the butchery level than at the abattoir level (Table 2). All the meat samples (100%) showed growths of aerobic bacteria and mean APCs were 1.64x109cfu/g for the abattoir and 1.92x109cfu/g for the butcheries. Mean APCs for the individual butcheries were 3.95x109cfu/g for Wandegeya, 7.02x108cfu/g for Kasubi and 1.10x109cfu/g for Nakulabye.

The bacterial load of meat samples from the study butcheries did not vary significantly though Wandegeya butchery registered the highest bacterial load (Table 3).

Contamination of meat with Coliforms and E.coli was higher at the butchery level than at the abattoir level (Figures 1 and 2). The mean Coliform and E.coli counts were 4.98x106cfu/g and 1.06x105cfu/g at the butchery while respective mean counts for the abattoir were 5.0x102cfu/g and 8.4x104cfu/g. Nakulabye butchery had the highest counts for both Coliforms and E.coli (Figures 3 and 4). Mean S. aureus counts were 2.7x103cfu/g for the abattoir and 5.40x105cfu/g for the butcheries (Figures 5 and 6).

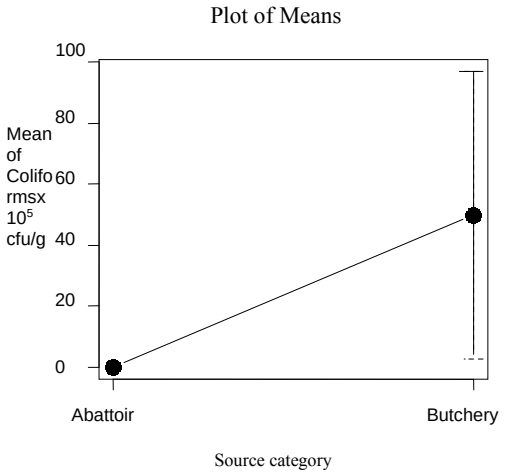

Figure 1. Mean Coliform counts at the abattoir and butchery levels

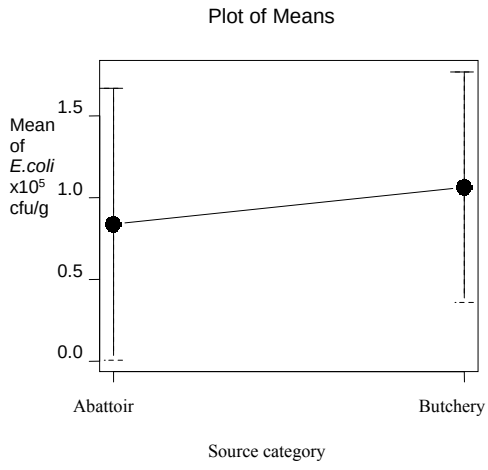

Figure 2. Mean *E.coli* counts at the abattoir and butchery levels

Table 1. bacterial frequencies on the meat samples

Bacteria	APC	Coliform	*E.coli*	*S. aureus*	*Salmonella*
Positive	48 (100%)	45 (93.8%)	40 (83.3%)	47 (97.9%)	2 (4.2%)
Negative	0 (0%)	3 (6.2%)	8 (16.7%)	1 (2.1%)	46 (95.8%)
Total	48	48	48	48	48

Bacterial load or counts of meat at the abattoir and butchery levels

Table 2. Mean bacterial load at abattoir and butchery levels

Bacterial load (APCs)	Mean $x10^9$cfu/g	p value	Confidence interval, 95%	
Abattoir	1.64	0.812	-26.25[#]	20.70[*]
Butcheries	1.92			

[#]lower limit of confidence interval, [*]upper limit of confidence interval

Table 3. Mean bacterial load at the three butcheries in peri urban Kampala

Bacterial load	Mean cfu/g	Standard deviation	p-value
Wandegeya	$3.95x10^9$	83.9	0.311
Nakulabye	$1.10x10^9$	18.3	
Kasubi	$7.02x10^8$	9.3	

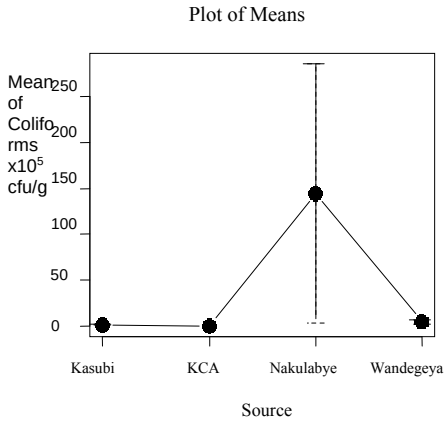

Figure 3. Mean Coliforms at different sampling points

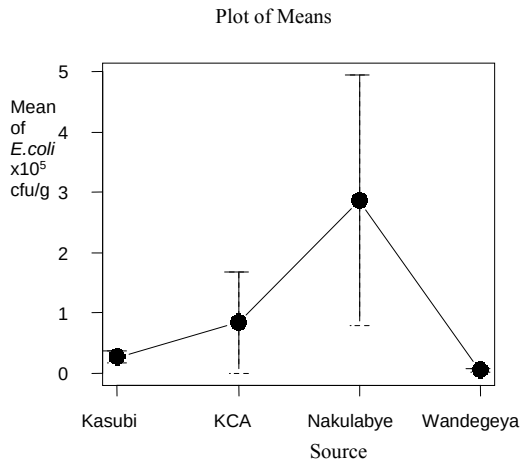

Figure 4. Mean *E.coli* counts at different sampling points

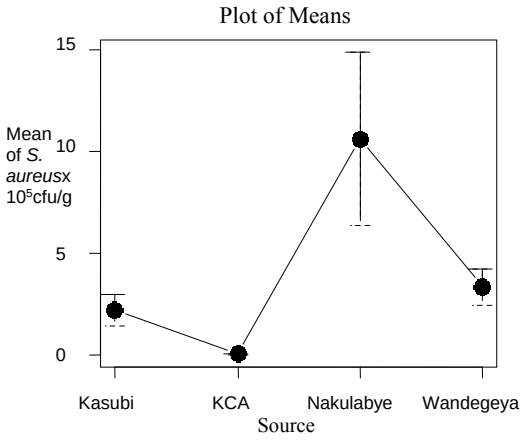

Figure 5. Mean *S. aureus* counts at the different sampling points

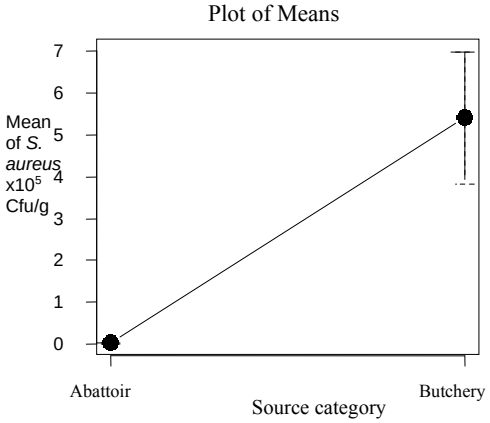

Figure 6. Mean *S. aureus* counts at the abattoir and butchery levels

The mean bacterial counts for the butcheries in the morning and evening samples at the 5% test level (CI = 95%, p-value = 0.92) were not significantly different (Table 4). Analysis was done to compare the effect of time of day on the counts of E. coli and S. aureus on the meat at the butcher level. S. aureus counts were higher in the evening than in the morning. On the contrary, E. coli counts were lower in the evening than in the in the morning (Figures 7 and 8).

Table 4. Mean bacterial load of meat as influenced by time of day

Bacterial load	Meanx10^9cfu/g	p-value	Confidence interval	
Morning	1.90	0.92	- 28.07[#]	25.54[*]
Evening	1.77			

[#]lower limit of confidence interval, [*]upper limit of confidence interval

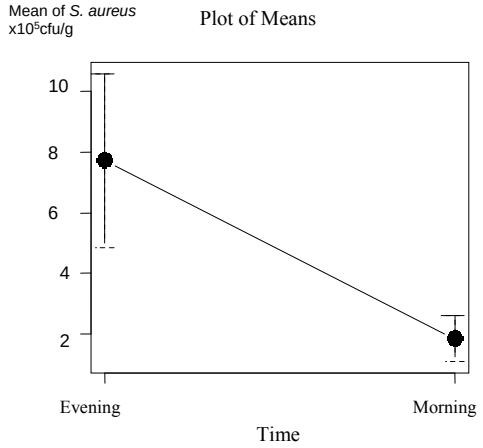

Figure 7. Variation of *S. aureus* with time

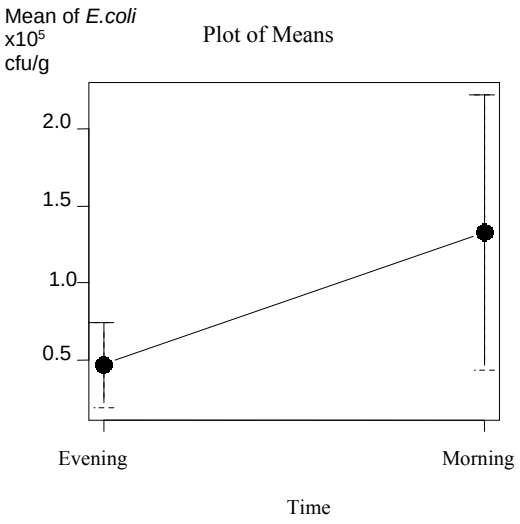

Figure . Variation of *E.coli* with time

Discussion

The practices of meat handlers at the abattoir and butchery levels seem to favor cross contamination of meat thus the high microbial load found by this study irrespective of the time of day. Salmonella had the lowest frequency i.e. only two samples tested positive for Salmonella (Table 1), which casts a low prevalence compared to an earlier study (Aftab et al. 2012). However, presence of Salmonella in any ready to eat food compromises food safety and signals poor hygiene practices such as failure to practice regular and effective handwashing after visiting the toilet or mixing meat with intestinal contents during dressing of carcasses since Salmonella is capable of direct and indirect transmission (Filimon et al. 2010).

Though presence of bacteria on meat has been reported in many parts of the world (Iroha et al. 2011), all the bacterial counts obtained in this study were higher than the internationally accepted level i.e. 105cfu/g (The Prevention of Food Adulteration Act and Rules 2004).

The high APCs on meat at the abattoir and butchery levels (Tables 2 and 3) indicates that meat at both levels is heavily contaminated with various bacteria and highlights poor hygiene practices by the meat handlers. Additionally, poor storage conditions besides the high nutritive value of meat might be favoring growth and rapid multiplication of bacteria hence the high counts (Ukut et al. 2010). The mean APCs for morning and evening samples were similar hence whether a consumer bought meat in the morning or evening, chances of purchasing contaminated meat were similar (Table 4).

Meat from the butcheries was more contaminated with S. aureus than that from the abattoir which agrees with (Centre for Health Protection 2011) that Staphylococcal contamination of foods increases with handling (Figures 5 and 6). Meat contamination levels with S. aureus at the abattoir and butcheries were different i.e. Kasubi, Nakaulabye and Wandegeya (Figure 5). Among butcheries, meat from Nakulabye had the highest mean counts for S. aureus (1.06x106cfu/g) followed by Wandegeya (3.35x105cfu/g) and Kasubi (2.22x105cfu/g) as shown in Figure 6.

The present study revealed a slight but not statistically significant difference (p-value = 0.812) between the bacterial load at the abattoir and butcheries. Mean APCs at the butcheries were slightly higher than at the abattoir. Contamination of meat during transportation from the abattoir to the butcheries and poor hygiene practices at the butchery level e.g. lack of handwashing and cold chain facilities may be contributory factors besides bacterial growth on the meat considering the bacteria growth curve.

Coliforms were higher at the butcheries (4.98x106cfu/g) than at the abattoir (5.0x102cfu/g), E. coli counts were 8.4x104cfu/g at the abattoir compared to 1.06x105cfu/g at the butcheries. These findings agree with (Fazlina et al. 2012) who found lower contamination of meat with

Coliforms at the abattoir level. Presence of E. coli and other Coliforms on meat signals fecal contamination. Good evisceration techniques at the abattoir could explain the low levels of E. coli since these organisms are abundant in the viscera of cattle. Iroha et al. 2011) also found out that E. coli was the most prevalent micro-organism in meat sold at retail markets in Abakaliki, Ebonyi state in Nigeria.

Fazlina et al. (2012) found very low levels of S. aureus contamination of meat at the government abattoirs. Similarly this study found low contamination of meat at the abattoir with S. aureus count of 2.7x103cfu/g compared to the contamination at the butcheries (5.40x105cfu/g), though both values were higher than the standard limit for S. aureus (PFA rules 2004) which is 102cfu/g. The higher staphylococcal contamination of meat at the butcheries than at the abattoir is probably due to continuous touching of meat with bare hands by the butchers unlike at the abattoir since S. aureus is easily carried by the hands of food handlers.

The study showed no significant difference between the bacterial load in the morning and evening which differs from (Lawan et al. 2011) which showed a statistically significant difference in APCs between meat samples collected in the morning and evening. The difference could be explained by a common practice in Kampala whereby if butcheries do not sell all the daily meat stock obtained from the abattoir, they mix it with the next day's meat stock so as convince customers that the meat is fresh in order to sell the previous day's meat stock which otherwise be rejected by many customers. Hence bacteria from the previous evening may be carried onto the next morning meat thus the lack of a significant difference in the bacterial counts.

The most prevalent bacteria isolated in this study were S. aureus (97.9%) and Coliforms (93.8%) in comparison with (Mekonnen et al. 2013) where the major bacterial pathogens isolated were S. aureus, E. coli and B. cereus. Presence of high counts for Coliforms and E.coli in meat (Figures 3 and 4) is probably associated with the poor hygiene practices involved in meat processing in developing countries (Abdalla et al. 2009), Abdalla et al. 2010) that result into higher chances of fecal contamination (Siragusa et al. 1995; Siragusa et al. 2000; Lawan et al. 2011).

The study showed that the meat sold at the study abattoir and butcheries was heavily contaminated with bacteria including, S. aureus, E. coli and other aerobic bacteria that can cause foodborne illnesses to the consumers unless the cooking processes are effective enough to destroy these bacteria. The study butcheries had similar working conditions with poor hygiene practices as reflected in the high mean bacterial counts. The study recommends more stringent inspection and regular supervision and or monitoring of hygiene practices in the study abattoir and butcheries as well as in other meat processing facilities in Uganda by both the veterinary and public health inspectors. Further research should be done to assess, the meat safety and hygiene knowledge levels of meat handlers, bacterial load on meat cutting equipments including knives, blades, and machetes, and hand microbiology of meat handlers at the abattoir and butchery levels.

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