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Prevalence and Predictors of *Salmonella* spp. in Retail Meat Shops in Kathmandu

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Abstract: A cross-sectional study was conducted from November 2008 to May 2009 to estimate the prevalence of *Salmonella* in retail meat shops in Kathmandu. The methods followed were ISO 18593:2004 for swab sample collection, ISO 6579:2002 for *Salmonella* isolation and manufacturer's instructions (SIFIN®, Germany) for serotype identification. A questionnaire was used to collect information on some of the risk factors of shops likely to be associated with *Salmonella* identification. A total of 492 environmental swab samples (164 chopping board samples, 164 knife samples and 164 table samples) from 82 retail meat shops were analyzed. The prevalence of *Salmonella* positive shops was 40.2% (95% CI: 29-51). The isolation rates of *Salmonella* from chopping boards (36.0%), knives (32.9%) and tables (25.0%) were not significantly different ($P > 0.05$). Retail meat shops were 1.9 times more likely to yield *Salmonella* in the evening (38.2%) as compared to the morning (24.4%) ($P = 0.001$). *S. Typhimurium* (54.5%) was the most common serotype found in retail meat shops followed by *S. Enteritidis* (16.9%), *S. Haifa* (13.6%), *S. Virchow* (10.4%), *S. Agona* (3.9%) and *S. enterica* (0.6%). Among the risk factors examined, "hygiene status of shop", "type of shops", "number of person handling meats", "number of knives used", "number of kinds of meat sold" and "number of kinds of meat sold using different numbers of knives" were individually significantly ($P < 0.05$) associated with *Salmonella* contamination in the retail meat shops. After univariate analysis of these risk factors, a final logistic regression model with *Salmonella* yes or no category of shops as outcome variable identified four significant predictors. Odds ratios, indicating the likelihood increase of a shop to achieve *Salmonella* positivity status were 10.17 for multiple persons rather than a single person involved, 7.66 for open rather than closed shops, 9.44 for use of several knives rather than one knife and 5.18 for single kind of meat using several knives. The results of this investigation revealed that retail meat shops to a noticeable extent are *Salmonella* contaminated, with a considerable degree of cross-contamination between meats and personnel and equipment used during a day in processing of meats.

Key words: *Salmonella*, retail meat shops, prevalence, swab samples, risk factors, Kathmandu.

1. Introduction

Salmonella is one of the most widespread food borne pathogen and a growing public health problem both in developed and developing countries including Nepal. It was reported that *Salmonella* causes an estimated 1.4 million cases of food borne illness and more than 500 deaths per year in the US [1]. Each year, approximately 40,000 *Salmonella* infections are culture-confirmed, serotyped, and reported by the United States Centers for Disease Control and Prevention. Of the total cases, 96% are estimated to be caused by foods [2]. In Europe, *Salmonella* was the second most reported cause of food-borne diseases in humans with 160,649 people suffering from *Salmonella* infections in 2006, approximately 35 people in every 100,000 [3].

Meat comprises a substantial source of high-quality protein in Nepal. The major retail outlets of meat in Nepal are the butcher's shops. Butchers slaughter goats and poultry in their premises with poor hygienic

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conditions [4]. Kathmandu receives about 20% of meat animals from its own sources while 80% of meat animals are received from the neighboring districts. There is one buffalo slaughterhouse and few poultry slaughterhouses and processing plant in Kathmandu.

Kathmandu, the capital city, has an estimated population of 1.4 million (GeoNames geographical database) which is ever increasing due to tourists and immigrants. As a result of this, Kathmandu is continuously facing high demand for food, quantitatively and qualitatively. This has led to increases of food establishments, for example, food vendors, small shops, cold stores and butchers shops. A great majority of consumers buy meat from butcher's shops at which food hygiene and safety conditions are not assured. There is no information on the prevalence of *Salmonella* in retail meat shops in Nepal. Therefore, this study was conducted to determine: (1) the prevalence of *Salmonella* spp., (2) the serotypes and (3) to know some of the risk factors associated with cross contamination of the retail meat in Kathmandu.

2. Materials and Methods

This study was carried out from November 2008 to May 2009 in retail meat shops in Kathmandu, Nepal. A total of 492 environmental swab samples (knives 164, chopping boards 164 and tables 164 each) were taken from randomly selected 82 retail meat shops located in five different divisions of Kathmandu (Fig. 1, Table 1). Three environmental samples in the morning and three environmental samples in the evening were collected from the same selected sites from each shop.

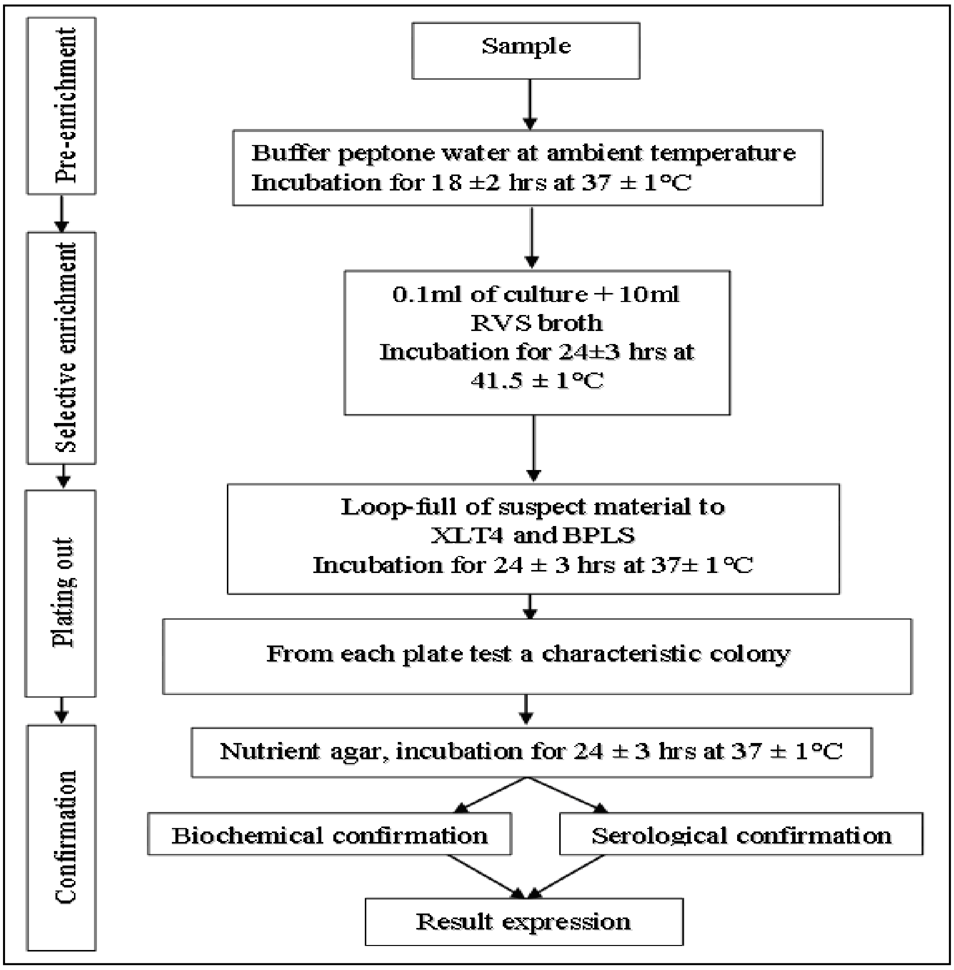

Fig. 1 Procedure for isolation of *Salmonella* spp. (ISO 6579).

The swab samples were collected following ISO 18593 2004 [5] and kept in an icebox (4-5 °C). These samples were sent for analysis as soon as possible, but not more than 24 hours later, to the Central Veterinary Laboratory, Kathmandu. The samples in test tubes were incubated at 37 ± 1 °C for 18 ± 2 h. Before starting isolation, the test tubes were shaken vigorously. The microbial analysis was done using the methods for the detection of *Salmonella* following standard procedures from ISO 6579:2002 with slight modifications [6] (Fig. 2).

After incubation on nutrient agar, pure colonies were picked up and inoculated into Triple Sugar Iron (TSI; Merck KGaA, Germany) slant, Voges-Proskauer (VP; Merck KGaA, Germany) broth, Motile-indole-lysine (Difco™ MIL Medium, Germany) broth and Urea (Urea; Merck KGaA, Germany) slant. All inoculated biochemical media were incubated at 37 °C for 18-24 h and checked for confirmation.

The serological confirmation of *Salmonella* antigens was performed by slide agglutination test according to the instructions of the manufacturer (SIFIN®, Germany).

The data collected from the field, laboratory investigation and the questionnaire were managed using Excel® version Microsoft Office® Excel 2003. The STATA version 10 (STATA Corp., College Station, Texas, USA) and the Statistical Package for Social Sciences (SPSS) version 16 were used for analysis of data. The significance level and confidence interval were considered to be 0.05 and 95% respectively. The prevalence of *Salmonella* was expressed by dividing the number of positive shops with

Table 1 Retail meat shops sampling frame.

N	Division	No. of wards	wards selected	No. of shops selected	Samples per shop	No. of samples
1	Center	6	4	18	6	105
2	East	7	4	24	6	144
3	North	5	2	16	6	96
4	City Core	14	3	16	6	96
5	West	3	1	8	6	48
Grand Total		35	14	82	6	489

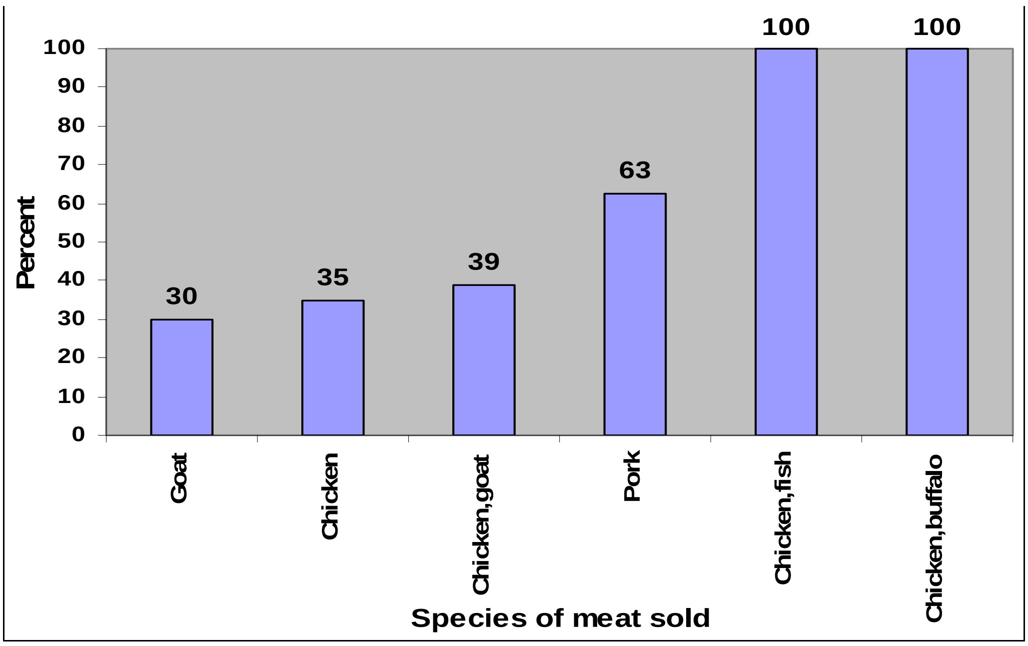

Fig. 2 ***Salmonella*** **in retail meat shops selling meats of different species.**

the number of total shops tested [7]. A Chi-square Fisher exact test was used to compare the prevalence of *Salmonella* according to retailer shops, time, months and administrative divisions. McNemar's Chi-square test was used to determine significant differences between morning and evening isolation of *Salmonella* from different sample types. Data from the questionnaire were used to evaluate the association of the risk factors with *Salmonella* identification. A univariate analysis (Chi-square Fisher exact test) was conducted using the *Salmonella* status of the meat shops as the outcome variable. A multivariable analysis was performed to relate the potential risk factors to *Salmonella* outcomes (present or not present) in samples and shops. All variables with a significant value $P \leq 0.05$ were selected for further analysis in a multivariable logistic model. A backward stepwise elimination process was used with a *P*-value for retention of a variable equal to 0.15 [8]. Interactions between variables were tested and retained with similar retention *P*-values. The Hosmer and Lemeshow goodness-of-fit test was carried out to assess the fitness of the model [8]. Finally, a receiver operating characteristic (ROC) curves was plotted to look the specificity and sensitivity of *Salmonella* predictions by risk factors of retail meat shops.

3. Results

3.1 Salmonella in Shops and Samples (Overall level)

Out of the total of 82 shops sampled, 33 were positive for *Salmonella,* giving an overall shop prevalence of 40.2% (95% CI: 29.4-51.1).

Out of a total of 489 samples collected from the retail meat shops, 154 samples were found positive giving an overall sample prevalence of 31.3% (95% CI: 27.2-35.6) (Table 2).

3.2 Salmonella in Morning and Evening Samples

Morning and evening prevalences of *Salmonella* in the samples of retail meat shops were statistically significant ($P = 0.001$); prevalences were higher in the evening (38.21%) compared to the morning (24.39%). The proportions of *Salmonella* on chopping boards, knives and tables in the morning were 31.71%, 26.83% and 14.63%, while in the evening the proportions were 40.24%, 39.02% and 35.37%, respectively (Table 3).

The prevalence of *Salmonella* on chopping boards, knives and tables was compared between two time points (morning and evening) by McNemar's Chi-square test. The proportions of *Salmonella* in the morning and evening samples from chopping boards were statistically significantly different (McNemar X^2, $P = 0.016$). Likewise, the proportions of *Salmonella* on the knives in the morning and evening samples were different (McNemar X^2, $P = 0.002$), as were the *Salmonella* proportions of morning and evening samples from tables (McNemar X^2, $P = 0.000$).

3.3 Serotypes of Salmonella

Of the total 154 isolated samples, five serotypes were identified. The most frequent serotype identified in retail meat shop was *S. Typhimurium* (54.5%) followed by *S. Enteritidis* (16.9%), *S. Haifa* (13.6%), *S. Virchow* (10.4%), *S. Agona* (3.9%) and *S. enterica* (0.6%) (Table 4).

3.4 Salmonella in Different Hygienic Status of Shop

The prevalence of *Salmonella* in shops with subjectively assessed poor hygiene was 55.81% (24 out of 43) in comparison to 23.08% (9 out of 39) in shops with good hygiene. The chance of getting *Salmonella* in shops with poor hygiene was five times

Table 2 *Salmonella* positive meat shops in different divisions in Kathmandu.

Division	Total	Positive	Percent	95% CI
Center	18	5	27.78	9.7-53.5
City Core	16	7	43.75	19.8-70.1
West	8	3	37.50	8.5-75.5
East	24	11	45.83	25.6-67.2
North	16	7	43.75	19.8-70.1

CI = Confidence Interval, **P*-value = Fisher exact test.

higher than in shops with good hygiene (OR = 4.21, *P* = 0.003 (Tables 5 and 6).

3.5 Salmonella in Shops Selling Meats Using Different Number of Knives

The retail meat shops sold multiple species of meat using different number of knives. The prevalence of *Salmonella* in the shops that sold two species of meat using two knives (one for each species of meat) (29.17%) was lower than when shops only sold a single species of meat using two or more knives (44.44%). This prevalence did even increase when the number of knives was increased, e.g., in shops that sold two meats (*P* = 0.023, Fisher exact test). The decisive factor therefore was the number of knives used, not the number of (different) meats sold (Fig. 3). There was an increasing trend of the likelihood of *Salmonella* detections in the shops when the number of knives used in the shops increased (*P* = 0.002, Test for trend) (Table 7).

3.6 Univariate and Multivariate Analysis of Risk Factors

The distributions of proportions of *Salmonella* contaminations per level of each risk factor and number

Table 3 *Salmonella* in samples in mornings and evenings in meat shops.

Time	Sample types	Total samples	Positive samples	Percent	95% CI
Morning	Knives	82	22	26.83	17.6-37.8
	Chopping boards	82	26	31.71	21.9-42.9
	Tables	82	12	14.63	7.8-24.2
	Total [a]	246	60	24.39	19.2-30.3
Evening	Knives	82	32	39.02	28.4-50.4
	Chopping boards	82	33	40.24	29.6-51.7
	Tables	82	29	35.37	25.1-46.7
	Total [b]	246	94	38.21	32.1-44.6
Grand total		492	154	31.3	27.2-35.6

[a,b] = Statistically significantly different, CI = Confidence Interval, **P*-value= Chi^2 test.

Table 4 *Salmonella* serotypes in each type of samples in meat shops.

Serotpyes	No. of isolates (%) in different sample types			Total
	Chopping boards	Knives	Tables	
S. Typhimurium	32 (54.2%)	30 (55.6%)	22 (53.7%)	84 (54.5%)
S. Enteritidis	8 (13.6%)	10 (18.5%)	8 (19.5%)	26 (16.9%)
S. Haifa	10 (16.9%)	7 (13.0%)	4 (9.8%)	21 (13.6%)
S. Virchow	6 (10.2%)	4 (7.4%)	6 (14.6%)	16 (10.4%)
S. Agona	2 (3.4%)	3 (5.6%)	1 (2.4%)	6 (3.9%)
*S. enterica**	1(1.7%)	-	-	1 (0.6%)
Total (%)	59 (38.3%)	54 (35.1%)	41 (26.6%)	154 (100.0%)

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Table 5 Prevalence of *Salmonella* in meat shops with different risk factors.

Factors	Level	Total	Positive	Percent	95% CI*	*P*-value*
Hygiene	Poor	43	24	55.81	49.1-79.0	0.003
	Good	39	9	23.08	11.1-39.3	
Type of shop	Open	44	24	54.55	38.8-69.6	0.004
	Closed	38	9	23.68	11.4-40.2	
Kind meat sold	1	37	9	24.32	11.8-41.2	0.008
	> 1	45	24	53.33	37.9-68.3	
Knives used	1	30	5	16.67	5.6-34.7	0.001
	> 1	52	28	53.85	39.5-67.8	
Persons handling meat	1	47	10	21.28	10.7-35.7	0.000
	1	35	23	65.71	47.8-80.9	

*CI = Confidence Interval, **P*-value = Pearson Chi^2 test.

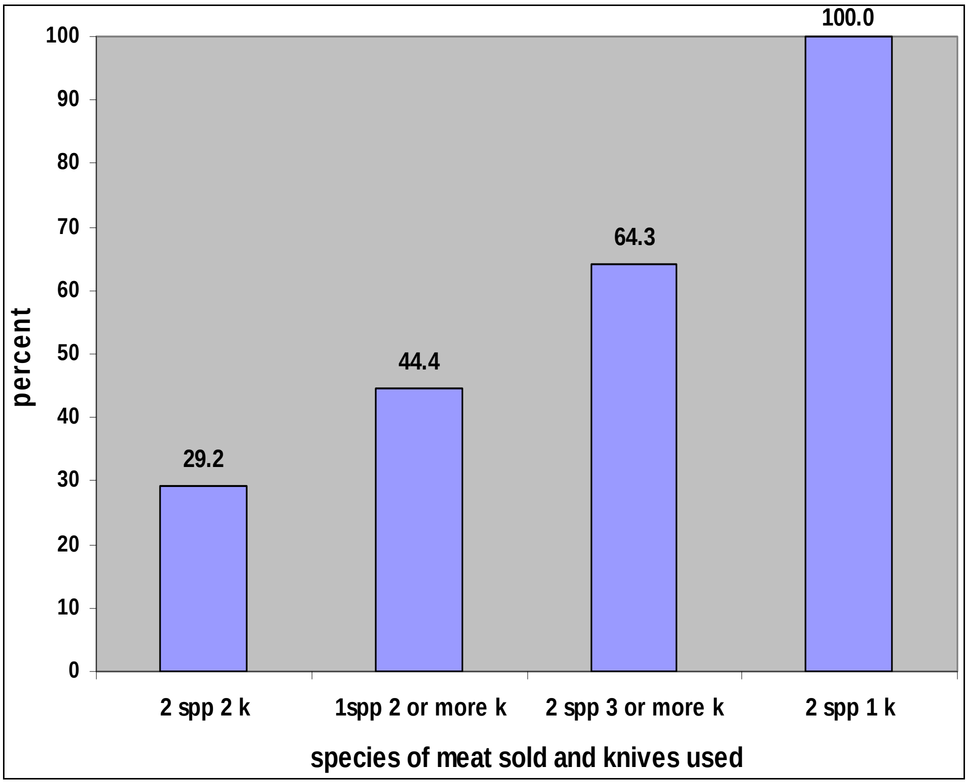

Fig. 3 Prevalence of *Salmonella* in retail meat shops selling different species of meat using different numbers of knives.

Spp. k = Species of meat sold using different number of knives.

Table 6 Potential risk factors associated with higher odds of *Salmonella* in retail meat shops in Kathmandu.

Factor	Level	OR	95% CI	*P*-value
Hygiene	Poor good	4.21	1.47-12.42	0.003
Type of shop	Open closed	3.87	1.36-11.40	0.004
Meat sold	> 1 Single species	3.56	1.25-10.46	0.006
Knives used	> 1 Single knife	5.83	1.78-22.11	0.001
Persons handling meat	> 1 Single	7.09	2.39-21.51	0.000

CI = Confidence Interval, **P*-value = Pearson Chi^2 test.

of shops are shown in Table 8. Six out of nine factors were significantly associated with *Salmonella* proportions in the univariate analysis.

The Chi-square univariate analysis indicates six variables with $P \leq 0.25$ (Table 8) which were further analyzed in a multivariate model. The final multivariate model does contain four risk factors for *Salmonella* contamination in retail meat shops in Kathmandu (Table 8).

The Hosmer and Lemeshow goodness-of-fit test indicated that the model did fit the data adequately (Hosmer and Lemeshow Chi-square = 6.53, P = 0.479). In the final logistic regression model risk factors associated with higher likelihood of *Salmonella* were open shops (OR = 7.66, P = 0.003), multiple knives used (OR = 9.44, P = 0.005) and more persons involved (OR = 10.17, P = 0.001) (Table 9). Checks on the model showed no significant interactions between variables or that they acted as confounders. The discrimination had been plotted with test of ROC curve. In our study the area under the ROC curve was found to be 0.8831 (Fig. 4).

4. Discussion

Results of investigations of environmental swab samples do provide an estimate of the prevalence of

Table 7 Test for trend for *Salmonella* in shops selling several kinds of meats using different number of knives.

Factor	Level	OR	95% CI	*P*-value*
Species of meats sold using different numbers of knives	2 Spp.-2 Knives	1.00		0.004
	1 Spp. ≥ 2 Knives	1.94	0.62-6.07	
	2 Spp. ≥ 3 Knives	4.37	1.15-16.55	

*Pearson Chi^2 (Test for trend).

Table 8 Summary results of the assessment of associations between shop prevalence of *Salmonella* with potential risk factors (Univariate analysis).

Factor	Level	Total	n (+)	% (+)	*P*-value
Hygiene	Good	39	9	23.08	0.003
	Poor	43	24	55.81	
Type of shop	Closed	38	9	23.68	0.004
	Open	44	24	51.55	
Meat sold	Single species	37	9	29.73	0.006
	> 1	45	24	60.00	
Room for evis	Yes	19	9	47.37	0.470
	No	63	24	38.10	
Knife used	Single knife	30	5	16.67	0.001
	> 1	52	28	53.85	
Person handling meat	single	47	10	21.28	0.000
	> 1	35	23	65.71	
Species of meat sold and knife used	2 spp. 2 k	48	14	29.17	0.023
	1spp. 2 or more knifes	18	8	44.44	
	2 spp. 3 or more knifes	14	9	64.29	
	2 spp. 1 k	2	2	100.00	
Species of meat sold	Buffalo	1	0	0.00	0.350
	Chicken	20	7	35.00	
	Goat	10	3	30.00	
	Pork	8	5	62.50	
	Chicken, buffalo	1	1	100.0	
	Chicken, goat	41	16	39.02	
	Chicken, fish	1	1	100.00	
Temperature	< 24 °C	45	17	37.78	0.616
	> 24 °C	37	16	43.24	

*P-value = Pearson Chi^2 and Fisher exact test.

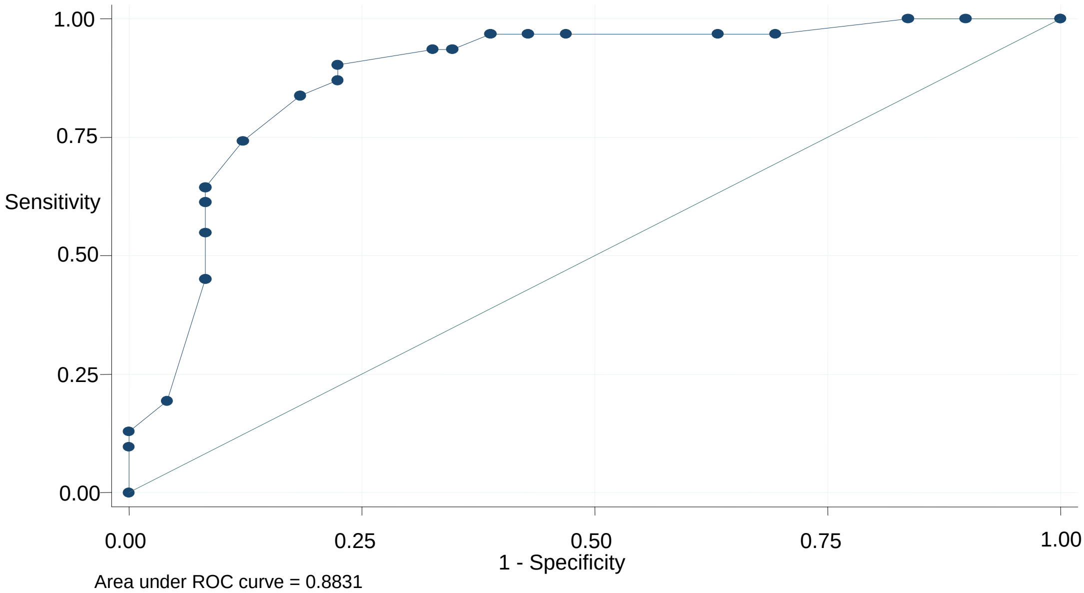

Fig. 4 Receiver operating characteristic (ROC) curves for the specificity and sensitivity of *Salmonella* predictions by risk factors of retail meat shops.

Salmonella in retail meat shops. A shop was considered as positive, if for one out of total of three samples collected in the morning *Salmonella* was confirmed. The same sampling scheme and shop categorization was applied in the evening. The high level of positive shops in the evenings indicated that contamination did accumulate throughout a day and reached peak levels at the end of a day. The eventual prevalence of 40.2% of *Salmonella* in retail meat shops was found to be much higher than the prevalence of 16.4% reported in their study from retail meat shops in India [9]. 31.3% of *Salmonella* in different swab samples was also high compared to 11.4% reported in their study from retail meat shops in Kathmandu [4]. These differences may be the result of different sample types or different methods for the detection of *Salmonella* [10, 11]. High overall prevalences in the shops in all likelihood are related to the poor infrastructure of shops such as lack of dressing facilities, drainage, differentiation between clean and unclean operations, and a general lack of basic maintenance of hygiene and sanitation. It is suggested that contamination levels are further increased due to excessive handling of carcasses, by too many people, by keeping more than two kinds of meats in a shop without proper separation of meat areas in the shops and by a constant flow of contamination from the unsuitable floors of the shops.

The contamination rate of 36.0% on chopping boards and 33.0% on knives can be compared to results of a study carried out in a pork processing plant in Thailand [12]; the authors also found that chopping boards (55.0%) compared to knives (30.0%) were more often contaminated with *Salmonella*. On the other hand, a higher level of *Salmonella* contamination on chopping boards (36.0%) was found, as compared to only 18.8% in retail meat shops in one study in India [13]. In this study, the chopping boards were also found highly contaminated, followed by knives, which can be compared to our study. Also in the Netherlands, contaminated chopping blocks made up about two thirds of all cross contaminations that occurred during meat processing [14].

High contamination of chopping boards, knives and tables in this study indicated improper and ineffective cleaning and disinfection. The rough, porous wooden surface of the boards does play a role in harboring and multiplying the organism better than with the other two sources. In fact, cleaning and disinfection of the wooden chopping boards are not possible for the shops personnel. Almost all chopping boards in this study contained remnants of meat, meat juice and bones, and were rough from immeasurable knife cuts. Simply, wiping off the blood and meat trimmings from the surface of knives with the help of cloth or water is definitely not enough in such a condition. Visual observation can be totally misleading when assessing the smoothness and cleanliness of a surface. The higher proportions of *Salmonella* in samples of retail meat shops in the evening compared to those in the morning (P = 0.001) reflects the spread of contamination throughout a day within the shops.

S. Typhimurium is a common cause of human salmonellosis in many countries [15-19]. The predominant serotype was *S. Typhimurium* (54.5%) followed by *S. Enteritidis* (16.9%), *S. Haifa* (13.6%), *S. Virchow* (10.4%), *S. Agona* (3.9%) and *S. enterica* (0.6%) in our study. In a previous study in meat of different species in retail meat shops in Kathmandu, predominant serotypes reported were *S. Pullorum*, *S. Typhi*, *S. Gallinarum* and *S. Choleraesuis* [4]. The result obtained by previous researchers [20] can be comparable with our study where they also found the predominant serotype as *S. Typhimurium* from the urban water supply system in Nepal. The butchers' shops used water derived from various sources due to scarcity of water in the capital city that can be contaminated out or inside shops. Another study in human blood samples in Kathmandu [21, 22] which reported *S. Typhi* and *S. Paratyhi* as the common serotypes responsible for enteric fever in human. However, none of the isolates from retail shops characterized as *S. Typhi* and *S. Paratyphi* in this study which reflects these two as highly confined serotypes in human in Kathmandu.

S. Agona, *S. Haifa* and *S. Virchow* have been found first time in this study which had never been reported earlier in Nepal. *S. Agona* had been isolated from asymptomatic children in Mexico [23], retail chicken meat in Vietnam [24] and *S. Haifa* from faeces of 3 years old childen having enteritis in Israel [25], fecal samples of a old person having food intoxication in Japan [26] and chicken samples in Ethiopia [27]. These findings clearly indicate the zoonotic importance of these two serotypes.

A higher prevalence of *Salmonella* was found in shops with subjectively assessed poor hygiene compared to those with good hygiene (P = 0.003). The prevalence of 55.8% of *Salmonella* in shops with poor hygiene and 23.1% in shops with good hygiene also was reflected by similar results of prevalences of 62.94% and 32.68% in dirty and clean shops, respectively, of retail meat shops in Hanoi [24].

For the open type of shops, a higher *Salmonella* prevalence was established than for the closed type (P = 0.004) in Kathmandu. A higher prevalence of *Salmonella* in open shops might be due to easy access of flies [28-30] and dust [31, 32] compared to closed shops. The widespread contamination of the different samples in retail meat shops demonstrates that the shops create ample opportunities for the entry and spread of contaminations. Bacteria must be present in water, soil, animal feed, raw meat, offal and vegetables. Invariably, the ultimate source of environmental contamination is faeces [33]. It would have been of interest to investigate the shop's personnel for their *Salmonella* status; considerable *Salmonella* infection rates must be suspected. In open shops, free movement of persons and the touching of meat by different customers with unclean hands as well as dust from the roads are likely further hazards from outside. Inside a shop, cross-contamination of meat is likely due to manipulations and use of utensils on the meat itself. The butchers usually wash the carcasses or parts of it with only small amounts of water, usually in a bucket, and the same water is used for washing knives, hands and even the offal and carcasses/parts. In the closed shops, in contrast, the water used is potable and special provisions exist for washing and cleaning inside the shops.

Keeping and selling different kinds of meat from the same counter in all likelihood did increase further contamination. If stored meat comes in contact with other contaminated meat or with contaminated equipment, cross-contamination is very likely to occur. In this case, the contamination rate will increase with an increasing number of kinds of meat sold in the same shop. There might be other factors in shops which are likely to enhance further cross-contamination.

The use of several knives over a single knife in the shops did increase the prevalence of *Salmonella* in the shops (P = 0.001). This result contradicts findings of some researchers [24] who found higher prevalence for Vietnam in shops that used a single knive. Use of several knives in the shops though does increase the prevalence since handling persons have opportunity to switch between different knives during peak trading hours, with leaving used knives for some time un-attended and un-cleaned. There is no destruction of bacterial cell from such knives, growing of bacterial cell is increased during the day. Such knives play a role in transfer the bacterial cell to the other surfaces as well. Moreover, meat handlers keep their knives above the chopping boards and cover the chopping boards with a cloth. It is not possible to periodically hand-dip in chlorinated water, to wear gloves, or periodically clean and disinfect utensils as it is done in the processing plants. They remove blood and meat from the surface of the knives at will; how finely and frequently material is removed from the surface of knives remains an open question. Cross-contamination may occur when microorganisms are transferred from one surface to another, possibly leading to contamination of otherwise safe meat or clean equipment. Cross-contamination can occur among equipment, meat, the environment, and even employees.

Differences in *Salmonella* prevalences were significant between shops where multiple persons did handle the meat compared to shops that only had a single person (*P* = 0.000); more persons lead to higher *Salmonella* prevalence. Without doubt knowledge of handling meat may not be the same in every person working in the retail shops. More likely, there may be free use of using knives to cut meat of all kinds. During our study we hardly encountered shops where workers washed their hands and utensils in between selling. Dirty or unwashed hands of workers will contaminate meat and equipment. Employees who perform many different tasks in retail meat shops without proper hand washing in between, or who fail to use appropriate utensils (knife used to cut chicken meat might be used to cut goat meat, too) will contaminate meat and equipment. The widespread presence of *Salmonella* in the retail meat shops' environment is clear evidence.

Meats of different animal species are sold in the shops. The highest prevalence of *Salmonella* was found in shops selling pork, followed by shops selling chicken and goat meat. Particularly when chicken and goat meat were sold together in the shops, the prevalence increased. This is likely a result of cross-contamination from the handling of two meats. Moreover, the majority of raw meat requires some forms of preparation (e.g., boning, cutting) prior to selling, this greatly does increase the likelihood of blood/meat juice spillage onto the tables, knives and chopping boards in processing areas of retail meat shops.

The calculation of odds ratios assisted to quantify the relative importance of risk factors. The dimensions of the odds ratio pointed to particular and pressing risk factors. The likelihood of *Salmonella* presence in shops with poor hygiene was four times higher than in the shops with good hygiene (OR = 4.21, *P* = 0.003). In the open type shops *Salmonella* contamination was almost four times higher than in the closed type of shops (OR = 3.87, *P* = 0.004). The shops selling meat of multiple species were almost four times higher in yielding *Salmonella* than the shops selling meat of a single species (OR = 3.56, *P* = 0.006). Shops using several knives during processing were six times higher in getting *Salmonella* than the shops using just a single knife (OR = 5.83, *P* = 0.001). High numbers of people handling the meat put the shops at 7-time higher risk for *Salmonella* than when a single person (OR = 7.09, *P* = 0.000) was handling it.

The logistic regression process served to identify the likelihood of a positive *Salmonella* classification of a retail meat shop by a combination of predictor variables. The backward stepwise elimination process identified a set of four predictor variables as maximum likelihood estimates of the model, being: type of shop, knives, persons, species of meat sold and number of knives in use (Table 9). Results are expressed in terms of odds ratios of the predictor variables; the odds ratios represent the factor by which the odds of the outcome change (from *Salmonella* negative to *Salmonella* positive status) increase for each one-unit change in the predictor. The odds ratios of the predictor variables were 7.66 for type of shop, 9.44 for knives, 10.17 for persons and 5.18 for species of meat sold and number of knives in use. This model by its pseudo R^2 measure explains, that 40.94% of the variation in the outcome (*Salmonella* yes/no) is explained by the chosen model. The pseudo R^2 is equivalent to the Likelihood Ratio Test for a full model (all parameters in the model). Results of the model fitted with four variables (*P* = 0.000) show that the model is highly statistically significant; the four predictors are highly significant predictors. The area under the ROC curve is a measure of discrimination.

It is a measure of the likelihood that a shop with all four predictor variables will have a higher probability to be positive than a shop without all those variables. The risk of a shop to achieve a S*almonella* positive status, when the four variables were present, thus was

Table 9 Final logistic regression model of risk factors associated with *Salmonella* isolations for 82 retail meat shops.

Factor	Level	Odds ratio	*P*-value	95% CI
Type of shop	Open Closed	7.66	0.003	1.96-29.88
Knives	Multiple Single	9.44	0.005	1.94-45.89
Persons	Multiple Single	10.17	0.001	2.58-40.14
Species of meat sold and number of knives in use	Single species of meat using two or more knives	5.18	0.036	1.11-24.14
	Two species of meat using three or more knives	8.15	0.027	1.27-52.54

CI = Confidence Interval, **P*-value = Pearson Chi^2 test.

about 10 times increased when many people rather than a single person was working in the shop and about seven times increased when the shop was of the open rather than the closed type. The variables "knives" and "species of meat sold and numbers of knives in use" in all likelihood are not independent of "persons". Logic tells that more persons will use more knives and the more persons will work, the higher the number of meats is in a shop. For this, persons and "type" in combination are the most significant predictors (risk factors) for a *Salmonella* positive status of a retail meat shop.

5. Conclusions

Being dirty, open, selling several kinds of meat of different species, using multiple knives and involving several persons handling the meat in Kathmandu were identified as risk factors for *Salmonella* contamination. Control measures with better hygienic practices at the shops are asked to reduce the risk of contamination of meat. Implementation and maintenance of a package of integrated hygienic measures, to be monitored regularly, will lower the probability of *Salmonella* contamination in the shops. Incoming meat arriving at the shops may be one source of contamination. Though shops are not organized along hygienic criteria and are never cleaned and disinfected thoroughly; residual contamination on utensils, floors and hands does propel multiplication and spread of organisms during a normal shop day. It is certain that *Salmonella* or other bacteria are present in the environment of the retail meat shops in considerable numbers and that they appear on a regular basis. There is a need to react quickly. All steps in the food chain must be considered while developing control strategy of *Salmonella.* Joining forces of meat handlers, trade associations, academics and government is necessary to minimize the prevalence of *Salmonella* in retail shops.

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