BACTERIAL QUALITY OF BEEF CARCASSES AND SANITARY CONDITION OF BUTCHER'S SHOPS IN BASRAH CITY

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ABSTRACT

The microbial quality of beef carcasses, sanitary conditions in butchers' shops and possibility of the presence of human pathogens associated with food poisoning outbreaks such as salmonella and staphylococci was investigated in 160 samples of beef carcasses, cutting blocks, knives, workers' hands and air (32 samples for each) during January and ended in June. All samples examined bacteriologically for aerobic plate count, total coliform count, *Staphylococcus aureus* count and an attempt was made for isolation and identification Salmonella spp.

This study have shown that there was a gradual increment in the count of all microorganisms starting in January and ending with June, the minimum mean of aerobic plate count of beef carcasses was $27.32x10^4$ cfu/cm^2 in January where as the maximum mean was $79.94x10^4$ cfu/cm^2 in June, the minimum mean of total coliform count was $0.67x10^3$ cfu/cm^2 in January and the maximum mean was $1.16x10^3$ cfu/cm^2 in June. The minimum mean of *Staphylococcus aureus* count was $7.36x10^2$ cfu/cm^2 in January whereas the maximum mean was $27.11x10^2$ cfu/cm^2 in June.

The same results were observed in the cutting blocks, knives, and workers' hands concerning the minimum and maximum mean count of the studied bacteria.

Salmonella could not be isolated from any examined samples and the percentage of Coagulase positive *Staph. aureus* which were isolated from beef carcasses, cutting blocks, knives and workers' hands samples was 100%.

INTRODUCTION

Meat is considered as essential foods beings tasty, easily digested and an excellent source of amino acid as well as vitamins and minerals **(1**), it is exposed to biological and chemical contamination and it is regarded as a good medium for the growth of microorganisms as it provides them with nutrient, available water and optimal PH requirement. Hence, it is considered as a source of different sorts of diseases. Further more meat originates from animals which may be infected with many zoonotic diseases that can be transmitted from the animal to human being via meat consumption **(2**).

In fact the predominant source of carcass contamination is the animal, particularly the hide and gastrointestinal tract; additional contamination may be acquired from the processing environment, which can be further exacerbated through poor hygiene practices adopted by plant operatives (**3, 4, 5**) .This may lead to spoilage of meat and act as a public health hazard to consumer. Aerobic plate count at the end of slaughtering operations give an important indicator of hygienic quality of the meat, sanitary condition of slaughtering and handling processes (**6, 7, 8**). It is found to be a suitable indication to assess the number of the organisms and microbial activity **(9).**

On the other hand coliforms count was required due to *Escherichia coli* which is a member of the coliform group. It is common in the faeces of man and animal. Other coliforms such as Aerobacter and Klebsiella are found to be wide spread in soil, water ،and plants **(10).**

Escherichia coli, Aerobacter and Klebsiella are the indicators for the sanitary quality of foods and the presence of one or more of them in great number could easily give rise to public health hazard **(11).** More over Miskimin et al. **(12)** found that *E. coli* count is a suitable indication for the microbiological quality of foods, but to assure safety of a food specific pathogen testing was necessary.

Staphylococci are wide spread in nature and recoverable from many inanimate sources. One of their main hosts is man, and their ready transference from the human body to a variety of foodstuffs accounts for their role as an important cause of food poisoning. By no means all varieties of staphylococcus are concerned. Only those that are coagulase-positive are capable of producing enterotoxins. Food poisoning staphylococci may be isolated from the hands and noses of normal people. Contaminations of towels, tables, knives, dishes, are almost unavoidable. Also staphylococci can be isolated from air, and dust of a contaminated area. Flies in such areas may be infected, and are probably turned into an important source of food contamination by staphylococci **(13).**

Salmonella can gain access to meat at any stage during butchering by unwashed hands, cutting boards, counters, knives, and other utensils cross contamination of carcasses and meat products could continue, during subsequent handling, processing preparation and distribution **(14, 15).**

Therefore the present study was carried out to determine the microbial quality of beef carcasses and sanitary condition of butcher's shops in Basrah city through study the effect of month on the microbial load.

MATERIALS AND METHODS

A total of 160 samples of beef carcasses, cutting blocks, knives, workers' hands and air (32 samples of each) were collected from butchers' shops of Basrah city during three climate periods. The period was started in January and ended in June and all samples examined bacteriologically for aerobic plate count, total coliform count, *Staphylococcus aureus* count and an attempt was made for isolation and identification of Salmonella spp.

Sampling of Beef Carcasses: Sampling of beef carcasses was carried out by using rinse swab method **(16).** For each sample, a sterile tube containing 10 ml of 0.1 % peptone water and another tube containing two cotton swabs perfectly sterilized were employed. At the time of samples collection, a sterile swab was removed from its tube and moistened with peptone water by dipping it into tube containing 10 ml of 0.1 % peptone water. The sterile metallic template was pressed against the surface to be sampled. The tip of moistened swab was rubbed over the area to be sampled and reswabbed by the other dry swab, then both swabs (moist and dry) were broken off into the tube containing peptone water by using the neck of the tube for leverage, taking aseptic precautions, about 2cm above the swab tip. The tube containing the broken off swabs tip was shaken well for about two minute. The carcass area used for sampling was 25 cm2 from thigh.

Sampling of Equipment: The equipment samples in the butchers' shops were knives and cutting blocks. The surface of knives and cutting blocks were swabbed by using rinse swab technique similar to those employed in beef carcasses except the sampling area which was 5 cm2.

Sampling of Workers' Hand: Each worker's hand was sampled by rolling the swab over the fingers using swab technique similar to that employed in sampling of equipment .

Sampling of Air: Samples of air were done by exposure plate method **(17)** which involved exposing 2 standard size petri-plate (85mm in diameter) containing about 20 ml of Nutrient agar for two minute .The petri-plates were placed about one meter upper the ground and one meter away from the carcasses.

Transportation of Samples: The samples, swabs in peptone water tubes and petri-plates duly covered, were transferred by a vehicle immediately to the laboratory by kept them in a cold insulated box, these samples were then subjected to bacteriological examination immediately on

reaching the laboratory .At the time of processing of one sample, the other samples were kept in the refrigerator at 4°C.

Bacteriological analysis: Serial dilution was used to prepare duplicate plates for the determination of aerobic plate counts (APCs), coliforms , and *Staph. aureus.*

APCs were determined by using Nutrient agar (Himeda, India) and plates were incubated at 37C° for 48 h. Then all colonies on plates were counted **(18).**

Colifrom counts were determined by using MacConkey agar (Himeda, India), typical colonies were identified as round, red to pink 0.5-2 in diameter, surrounded with a red to pink halo **(19).**

For the *Staph. aureus* counts ،Mannitol salt agar(Himeda, India)was used and the plates incubated at 37C° for 24 to 48 hr (APHA,1978).All typical colonies on Mannitol salt agar was counted ,selected colonies from the agar surfaces were tested for coagulase activities using rabbit plasma**(20, 21, 22).**

For the enrichment of salmonella, five ml from the original sample was transferred to a 45 ml of tetrathionate broth (oxoid, U.K) and incubated at 37C° for 24 hr. One ml from enrichment culture was transferred to a brilliant green agar (oxoid, U.K) was used for selective plating .Presumptive salmonella colonies selected from each of selective plates were subjected to the biochemical test including: Triple Sugar Iron agar for fermentation and H2S production, Urease broth for urea production and Sulphide Indole Motility medium for indole production were inoculated **(23).** All cultures were incubated at 37ºC for 24 hours.

Statistical Analysis: The results were analyzed by One-way ANOVA test, using statistical package for the social sciences (SPSS, version 9.0). All data were expressed as Mean±Std.Error. Differences between data were compared by least significant deference **(24).**

RESULTS AND DISCUSSION

The aerobic plate count of beef carcasses varied from 3×10^4 to 1.72×10^6, with mean values of $5.84 \times 10^5 \pm 1.21 \times 10^5$ cfu /cm^2 (Table 1). These results were higher than the standard regulation for fresh beef (1×10^4 to 1×10^5 cfu/cm^2) under typical stipulating conditions (**25**). On the other hand, the results of present study was less than what has been recorded by Hoshyare **(26)** who reported that the mean of aerobic plate count in Baghdad was 2.78×10^6 cfu/cm^2 . All samples of beef carcasses had aerobic plate count which is more than 1×10^3 and only 9.38 % of them had the count $>1 \times 10^4$ to $<1 \times 10^5$. These results were lower than the results of Abdul-Wadood **(27)** who found that 80.95% of beef carcasses had aerobic plate count $1X10^4$ cfu/gm.

The difference obtained in the present study may be related to defect in the sanitation, transportation, temperature, handling and ways of presentation of the meat in the butchers' shops.

The data recorded in Table(2) showed that the minimum means of aerobic plate count /cm^2 of beef carcasses was 27.32×10^4 in January while the maximum means was 79.94×10^4 in June These results were in agreement with those reported by other studies (**27;28**) who reported that the minimum means of all examined bacteria of beef carcasses was in January while the maximum means was observed in June, but these results differed from Hoshyare **(26)** who found that minimum means of aerobic plate count of beef carcasses was in January and the maximum means was in April and the count of all examined bacteria showed gradual increase during March ,April ,May and June as compared to the situation in January and February.

The aerobic plate count of cutting blocks, knives, workers' hands and air samples had varied from 0.12×10^4 to 17.80×10^4, 0.03×10^4 to 1.40×10^4, 0.02×10^4 to 0.38×10^4 and 0.08×10^4 to 0.11×10^4. With mean values $2.36 \times 10^4 \pm 0.71 \times 10^4$, $0.46 \times 10^4 \pm 0.07 \times 10^4$, $0.12 \times 10^4 \pm 0.01 \times 10^4$ cfu/cm^2 and 9.79± 0.14 /cm^2 per minute of exposure ,respectively. The same trend of results was observed in the cutting blocks, knives, and workers' hands concerning the minimum and maximum mean counts of the studied bacteria (Table 2). This gradual increment of bacterial count in the present study may be due to gradual elevation of the temperature in these months.

These results seem to be high in compared to the standard (<$1x10^3$ organism/cm^2)as suggested by Patterson (**29**).Up to our knowledge there were few reports concerning the aerobic plate count in cutting blocks, knives and workers' hands in butchers' shops. However, Jawad (**30**) and Abdul-Wadood (**31**) found that the aerobic plate count of knives was $1.69x10^5$ cfu/cm^2 and $13.94x10^4$ ^{cfu}/$cm^{2,}$ respectively. The difference of results may be related to poor sanitation practice in the butchers' shops. In the retail market, Narasimha and Ramesh (**32**) reported that additional contamination is usually taken places with reference to knives, saws, cleavers, slices, chopping blocks, scales, containers, flies, hands and garments of the workers, market operators, and air. Total numbers of microorganisms in a beef may increase due to the pollution of air with microorganisms. The numbers of microorganisms in the air at any given time depend upon a number of factors including movement of people, sunshine, humidity, location, aerosol, and by ventilation (**17; 33**).

The total coliform count/cm^2 of beef carcasses varied from 0.26 $x10^3$ to $1.76x10^3$ with mean values of $0.99x10^3 \pm 0.07x10^3$ cfu/cm^2 (Table 3). These present results were lower than that reported by Hoshyare **(26)** who stated that the mean of total coliform count in butchers' shops in Baghdad was $8.1x10^3$ cfu/$cm^{2.}$ All samples of beef carcasses had total coliform count which is more than $1x10^2$ and only 53.13% of beef carcasses samples had the counting >$1x10^3$ to <$1x10^{4.}$ On the other hand, the minimum means of total coliform count /cm^2 of beef carcasses was $0.67x10^3$ in January and the maximum means was $1.16x10^3$ in June (Table 2)

These results were higher than the result of Goepfert and kim **(34)** who reported that 17.5% of beef carcasses exceed $1x10^3$ cfu/gm. The difference obtained at the present study may be related to the difference in the procedure of coliform counting (Most Probable Number), hygienic measurement, climate, personal hygiene and education. In general the presence of coliforms in the food has been considered by some authors as a criterion for the existence of unsanitary conditions **(12; 25; 35; 36)**. However, the presence of coliforms in the carcasses in the present study may indicate contamination from either faecal or non faecal sources. The high coliform counts of carcasses, when considered along with the total bacterial counts would suggest the presence of unsanitary conditions in the processing plants. The total coliform count/cm^2 of cutting blocks, knives and workers' hands samples had varied from 0.24 $x10^3$ to $1.32x10^3$, 0.22 $x10^3$ to $1.60x10^3$ and 0.20 $x10^3$ to $1.10x10^3$, respectively. With mean values $0.87x10^3 \pm 0.06x10^3$,$0.82x10^3 \pm 0.06x10^3$ and $0.73x10^3 \pm 0.05 x 10^3$ cfu/cm^2 (Table 4).

Staphylococcus aureus count/cm^2 of beef carcasses varied from 0.6 $x10^2$ to $84x10^2$ with mean values of 16.18 $x10^2 \pm 3.22$ $x10^2$ cfu /cm^2 (Table 5). The present results were lower than the result of Hoshyare **(26)** who reported that the mean of *Staph. aureus* count in butchers' shops in Baghdad was $7.19x10^3$ cfu/cm^2 .Four samples of beef carcasses in the present study had *Staph.aureus* count less than 10^2 and only 37.5% of them had counting >$1x10^2$ to < $1x10^3$ (Table 6).These results were higher than the results of Wyatt and Guy **(37)** who found that 5% of beef carcasses exceed $1x10^2$ *Staph. aureus* count. .

The total of *Staph. aureus* count/cm^2 of cutting blocks, knives and workers' hands samples were varied from 18 $x10^2$ to $13.80x10^4$, 0.80 $x10^2$ to $20x10^2$, and 0.60 $x10^2$ to $1.20x10^2$ with the mean values of 15.98 $x10^2 \pm 41.82$ $x10^2$, 7.76 $x10^2 \pm 1.21$ $x10^2$ and 0.89 $x10^2 \pm 0.03$ $x10^2$ cfu/cm^2, respectively, (Table 5).

These results were lower than the results of Abdul-Wadood **(31)** who revealed that *Staph .aureus* count of knives was $13.94x10^4$ cfu/cm^2. The difference in results may be related to the certain bad habits of handlers and absence of the hygienic education concerning good standards of healthy and hygienic care which must be provided by medical officers, public health inspection **(31; 38)**. Mostly raw meats are contaminated with staphylococci at the time of slaughtering or during handling after slaughtering **(39)**.

The beef carcasses, cutting blocks, knives and workers' hands samples showed positive results in coagulase test. These results were in agreement with the results of Abdul-Wadood(**27**) who reported that all isolated (100%) *Staph. aureus* were coagulase positive, but this results differ from the results of Hoshyare**(26)** and Al-kasei **(40)** who found that 97.5% and 93.3% of *Staph. aureus* isolates were coagulase positive .

.Also the minimum means of *Staph. aureus* count /cm^2 of beef carcasses was $7.36x10^2$ in January while the maximum means was $27.11x10^2$ in June (Table 6).

The beef carcasses, cutting blocks, knives and workers' hand samples showed negative results in Salmonella identification testing. The results were in agreement with the results of Lotfi *et al.*, **(41)** and Abd El-Aziz, **(42)** who got negative results in relation to salmonella identification.

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Table (1): The aerobic plate count in examined samples

Source of samples		Aerobic plate count	
	Mean	Range	Std.Error
Carcasses	58.41	$3.00\text{-}172x10^4$	±12.16
Cutting blocks	2.36	$0.12\text{-}17.80x10^4$	± 0.71
Knives	0.46	$0.03\text{-}1.40x10^4$	± 0.07
Workers' hands	0.12	$0.02\text{-}0.38x10^4$	± 0.01
Air	9.79	$0.\ 08\text{-}0.11x10^4$	± 0.14

Results are expressed as mean colony forming units x 10^4 per cm^2.

Table (2): The aerobic plate count of examined samples during six months under the study.

Sources of samples	Aerobic plate count					
	January	February	March	April	May	June
carcasses	27.32^{M} 3-114^{R} ±21.86$^{S}_{.E}$	35.80 3.2-152 ±29.07	63.20 6-146 ±33.41	64.28 3.40-152 ±35.41	71.36 8-166 ±38.23	79.94 3.60-172 ±27.67
cutting blocks	0.52 0.12-0.90 ±0.15	0.75 0.25-1.10 ±0.16	2.10 0.03-5.60 ±0.97	2.22 0.14- 5.80 ±1.00	4.37 0.52-15.80 ±2.89	
knives	0.52 0.03-0.94 ±0.19	0.55 0.03-1 ±0.17	0.65 0.03-1.40 ±0.25	0.654 0.03-1.20 ±0.20	0.68 0.03-1.20 ±0.21	0.74 0.03-1.14 ±0.18
workers' hands	0.05 0.02-0.09 ±0.01	0.12 0.03-0.32 ±0.05	0.125 0.07-0.30 ±0.04	0.13 0.03-.32 ±0.049	0.138 0.03-.38 ±0.06	0.16 0.09-0.34 ±0.04
air	9.32 0.08-0.10 ±0.47	9.35 0.08-0.10 ±0.44	9.81 0.08-0.10 ±0.33	9.88 0.08-0.10 ±0.42	10.04 0.09-0.11 ±0.32	10.16 0.09-0.11 ±0.24

M= means counts, R= range between, S.E= standard error
Results are expressed as mean colony forming units x 10^4 per cm^2.

Table (3): The total coliform count in examined samples

Source of samples		Total coliform count	
	Mean	Range	Std.Error
Carcasses	0.99	0.26-1.76x10^3	±0.07
Cutting blocks	0.87	0.24-1.32x10^3	± 0.06
Knives	0.82	0.22-1.60x10^3	± 0.06
Worker's hands	0.73	0.20-1.10x10^3	± 0.05

Results are expressed as mean colony forming units x10^3 per cm^2.

Table (4): The total coliform count of examined samples during six months

Sources of samples	Total coliform count					
	January	Februar y	March	April	May	June
carcass es	0.67[M] 0.26-1.32[R] ±0.1S.E	0.87 0.28-1.32 ±0.20	1.00 0.28-1.54 ±0.24	1.10 0.66-1.32 ±0.12	1.11 0.56-1.54 ±0.1	1.16 0.3-1.765 ±0.20
cutting blocks	0.63 0.24-1.20 ±0.15	0.79 0.26-1.20 ±0.18	0.38 0.26-1.32 ±0.21	0.94 0.54-1.32 ±0.15	1 0.60-1.20 ±0.11	1.67 0.28-5.40 ±2.71
knives	0.60 0.22-1.10 ±0.19	0.72 0.24-1.10 ±0.17	0.79 0.24-1.32 ±0.20	0.86 0.52-1.20 ±0.13	0.91 0.26-1.20 ±0.17	0.96 0.52-1.60 ±0.13
workers ' hands	0.57 0.20-1 ±0.12	0.12 0.22-1 ±0.15	0.125 0.22-1.10 ±0.16	0.80 0.50-1.10 ±0.11	0.808 0.24-.1.10 ±0.15	0.82 0.50-1.10 ±0.08

M= means counts, R= range between, S.E= standard error
Results are expressed as mean colony forming units x 10^3 per cm^2.

Table (5): *Stapylococcus aureus* count in examined samples

Source of samples		*Staph.aureus* count	
	Mean	Range	Std.Error
Carcasses	16.18	0.6-84x10^2	±3.22
Cutting blocks	15.98	18-1380x10^2	± 41.82
Knives	7.76	0.80-20x10^2	± 1.21
Workers' hands	0.89	0.60-1.20x10^2	± 0.03

Results are expressed as mean colony forming units x10^2 per cm^2

Table (6): The *Staph. aureus* count of examined samples during six Months under the study

Sources of samples	*Staph.aureus* count					
	January	Februar y	March	April	May	June
carcasses	7.36M 0.60-16R ±2.75S.E	13.20 0.60-30 ±5.09	1.3.37 0.64-32 ±5.96	15.32 1-50 ±8.90	16.32 2-36 ±7.28	27.11 2.20-84 ±10.48
cutting blocks	79.20 18.40-180 ±32.39	122.80 36-240 ±36.48	125.20 20-220 ±41.93	140 28-260 ±38.79	151.60 38-260 ±40.58	171.14 30-480 ±58.09
knives	2.96 1.18-6 ±0.87	3.96 0.8015 ±2.76	8.56 2-20 ±3.81	9.32 2.60-18 ±2.98	10.28 2.60-19 ±3.48	10.43 3-18 ±2.60
workers' hands	0.75 0.66-0.88 ±0.05	0.78 0.60-1 ±0.07	0.28 0.60-1 ±0.08	0.94 0.80-1.10 ±0.05	0.97 0.80-1.10 ±0.05	1.03 0.80-1.20 ±0.06

M= means counts, R= range between, S.E= standard error
Results are expressed as mean colony forming units x 10^2 per cm^2

النوعية البكتيرية لذبائح الأبقار والحالة الصحية لمحلات القصابين في البصرة

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الخلاصة

تم استقصاء النوعية الميكروبية لذبائح الأبقار و الحالة الصحية لمحلات القصابين واحتماليه وجود الجراثيم المرضية المرتبطة بتفشي حاله التسمم الغذائي في الإنسان مثل السالمونلا والمكورات العنقودية في مائه وستين عينه من ذبائح الأبقار ، ألواح التقطيع، السكاكين، أيدي العاملين والهواء(32 عينه من كل نموذج).جمعت هذه العينات من محلات القصابين الواقعة في أربعه أسواق محليه في مدينه البصرة (الجمهورية ، البصرة ،العشار و خمسه ميل) ابتداء من شهر كانون الثاني وانتهاء بحزيران 2004. جميع العينات فحصت بواسطة العد الكلي للجراثيم الهوائية ، العد الكلي لبكتريا القولون ،عد المكورات العنقودية الذهبية ومحاولة عزل وتشخيص السالمونلا عدا عينات الهواء التي فحصت بواسطة العد الكلي للجراثيم الهوائية فقط.

أظهرت نتائج هذه الدر اسه بأن هناك زيادة تدريجية في عدد جميع الميكروبات ابتداء من كانون الثاني وانتهاء بحزيران وان الحد الأدنى لمعدل العد الكلي للجراثيم الهوائية لذبائح الأبقار كان $27,32 \times 10^4$ و.ت.م/ $سم^2$ في كانون الثاني بينما الحد الأعلى للمعدل كان $79,94 \times 10^4$ و.ت.م/ $سم^2$ في حزيران وأن الحد الأدنى لمعدل العد الكلي لبكتريا القولون كان $0,67 \times 10^3$ و.ت.م/ $سم^2$ في كانون الثاني بينما الحد الأعلى للمعدل كان $1,16 \times 10^3$ و.ت.م/ $سم^2$ في حزيران.أما معدل العد الكلي لبكتريا المكورات العنقودية الذهبية كان $7,36 \times 10^2$ و.ت.م/ $سم^2$ في كانون الثاني بينما الحد الأعلى للمعدل كان $27,11 \times 10^2$ و.ت.م/ $سم^2$ في حزيران. نفس النتيجة تم ملاحظتها في ألواح التقطيع، السكاكين وأيدي العاملين فيما يتعلق بالحد الأدنى و الحد الأعلى لمعدل عد البكتيريا المدروسة.

لم نتمكن في هذه الدراسة من عزل جرثومة السالمونيلا من جميع العينات، وأن نسبة الكورات العنقودية التي اظهرت نتيجة ايجابية في فحص انزيم التخثر والمعزولة من ذبائح الابقار والواح التقطيع والسكاكين وايدي العاملين 100%.

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