Pakistan is a tropical country, with ambient temperatures which are conducive for the growth of microorganisms, which can rapidly render the meat unsafe for human consumption (Bughti et al., 2017; Doulgeraki et al., 2012). Episodes of food borne illnesses are frequently been reported in the Pakistan, but due to lack of a surveillance network, the exact magnitude of the problem in the country remains unknown. Commonly, cow, buffalo, sheep, goat, camel, and poultry meat are used as a source of protein (FAO, 2013). As general practice, raw meat is sold in open market. Particularly in Peshawar and country as a whole, majority of the population consumes meat slaughtered and butchered in small local shops where the maintenance of hygiene is always questionable (Akhtar, 2015). Meat produced for the domestic market is sold as hot meat directly to the consumers on retail meat shops. The poor
hygiene and sanitation prevailing in the abattoirs as well as
the shops encourage microbial contamination, survival and
growth in meat as well. The higher microbial load in the
meat shops is due to floor dressing and neglect of hygiene
(Bhandare et al., 2007).

The most common pathogenic bacterial species found
in meat are Escherichia coli, Staphylococcus aureus, Listeria
monocytogenes, Salmonella, Aeromonas spp., Arobacter spp.,
Bacillus cereus, Campylobacter spp. and Clostridium botuli-
num (Cho et al., 2012; Javed, 2016; Kamboh et al., 2017).
These organisms are involved in meat poisoning. Meat may
become contaminated by these pathogenic bacteria either
endogenously or by subsequent postmortem contamination
from blood, gastrointestinal contents, feet, hide or skin,
water, knives, instruments used in slaughter hall, vehicles,
 personals and airborne materials (Sheridan et al., 1992).
The unhygienic conditions of the slaughter houses, butcher
shops, handling of meat, hot environmental condition and
packing of meat further provide the source of contamination
(Bhandare et al., 2007). It is generally agreed that the
internal tissues of healthy slaughtered animals are free of
bacteria at the time of slaughter, assuming that the animals
are not in a state of exhaustion. On examination of fresh
meat and poultry products at the retail level, various num-
bers and types of microorganisms are found (Ahmad et al.
2013). The intestinal contents along with the usual heavy
load of microorganisms may be deposited onto the surface
of freshly dressed carcasses. Especially important in this
regard is the paunch or rumen of ruminant animals, which
typically contains 10^{10} bacteria per gram. In the case of
red meats, lymph nodes are usually embedded in fat often
contains large numbers of organisms, especially bacteria. If
they are cut through or added to portions that are ground,
one may expect this biota to become prominent. Hands
of handlers are the source of human pathogens to freshly
slaughtered meat. Even when gloves are worn, organisms
from one carcass can be passed onto other carcasses (FAO,
2013). Further that containers, where meat cuts are placed
may be expected to become contaminated with the organ-
isms. This tends to be a primary source of microorganisms
to ground or minced meats. Further the handling and storage
environment circulating air are significant sources of
organisms to contaminate meat surfaces of all slaughtered
animals (Ozlem, 2005).

Keeping in view the above facts, the present study was
therefore designed to determine the microbiological load
in meat obtained from local open market in Peshawar. In
this study we determined whether pathogenic bacteria are
present or not. And to compare the bacterial load, among
the meat of different animal species viz., cattle and buf-
falo. Further that, the present investigation regarding the
meat, provide basic knowledge about the pathogenic bac-
teria and their harmful activities against public health. The
study also helps in recognition of bacterial species which are
responsible for spoilage of meat in study area.

MATERIAL AND METHODS

COLLECTION OF MEAT SAMPLES
A total of 52 meat samples, 26 each from cattle and buffa-
loes were collected from different retail shops and slaughter
houses of Peshawar under sterile conditions. Each sample
represents the cumulative sampling of various carcass sites
collected randomly in polythene bags and then brought to
the Veterinary Research Institute (VRI), Peshawar, Khyber
Pakhtunkhwa, Pakistan for further processing of bacterio-
logical investigations. Furthermore, during sample collect-
ion, cleanliness status of retail shop and its surroundings
was also noted, and it was categorized either hygienic on
unhygienic.

PROCESSING OF MEAT SAMPLES
The collected meat samples were minced/grinded individ-
ually by using grinder/ scissor into very fine pieces, then
10g of the minced meat were properly mixed and added
to 90 ml peptone water (DifcoTM) by stirring with a stirrer
or shaking in vortex mixer. The above solution was further
prepared into tenfold dilutions. The dilutions 10^{-4}, 10^{-5}
and 10^{-6} were used for bacteriological investigation. Three
samples, each comprising of 50µl from each dilution were
streaked over on individual plates of general and selective
media for isolation, characterization and quantitative study
of bacterial organisms according to procedures of Haque
et al. (2008). The identification of isolates was made through
morphological, staining and cultural characteristics on
culture media under the microscope with the help of oil
immersion objective (X100). Further recognition of the
bacterial species was made through different biochemical
tests (Manoj et al., 2017).

STATISTICAL ANALYSIS
With the help of Microsoft Excel and Analytical Software
“Statistix8.1” data was processed to calculate the means;
while Duncan Multiple Rang Test was applied to compare
the differences between cattle and buffalo meat for bacte-
rial load and bacterial isolates.

RESULTS
A study was carried out on isolation and characterization
of pathogenic bacterial species from meat of domestic an-
imals. In this regard, seven bacterial species were recog-
nized. The bacterial species identified from meat samples
of animals in Peshawar were Escherichia coli, Staphylococ-
cus aureus, Pseudomonas aeruginosa, Bacillus cereus, Listeria
monocytogenes, Salmonella enteritidis and Campylobacter je-
junii. Morphologically, the bacterial species varied from co-

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Percentage Incidence of Bacterial Organisms in the Meat

The data regarding percentage prevalence of bacterial species in meat samples of cattle and buffaloes are presented in Table 1. Of the 52 samples studied, 51 samples were found positive for bacterial contamination. The higher prevalence (100%) of bacterial organisms was recorded in the meat of buffaloes.

Bacterial Load in Meat Samples of Cattle and Buffaloes

The data regarding bacterial mean population in meat samples of different animal species are given in Table 2. The large number of colonies (g⁻¹) and mean bacterial counts (g⁻¹) were recorded in the meat samples of buffaloes. In g⁻¹ meat sample of buffaloes, the mean number of 330 colonies was counted while quite higher number of bacterial cells (6.6×10⁸) was also counted in the meat samples of buffaloes as well. Comparatively lower mean number of colonies and bacterial counts g⁻¹ were detected in the meat samples of cattle.

Table 2: The mean bacterial population (bacterial load) in meat samples of cattle and buffaloes obtained by log cfu g⁻¹

<table>
<thead>
<tr>
<th>Animals species</th>
<th>Cattle (log cfu g⁻¹)</th>
<th>Buffaloes (log cfu g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>7.5 A</td>
<td>7.8 A</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>5.3 B</td>
<td>6.4 A</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>4.7 C</td>
<td>4.8 C</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>3.9 D_b</td>
<td>4.2 C</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>3.5 D_b</td>
<td>4.3 C</td>
</tr>
<tr>
<td>Salmonella enteritidis</td>
<td>4.9 Cb</td>
<td>5.1 Ba</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>3.9 D_b</td>
<td>4.6 C</td>
</tr>
</tbody>
</table>

A-D Means followed by different lettering in a column showing significant difference (p < 0.05).

Comparison of mean population of individual bacterial species in meat samples: The comparison of mean population of individual bacterial species was demonstrated among the meat samples of the animals (Table 3). Non-significant differences (p > 0.05) in the mean populations of Escherichia coli and Pseudomonas aeruginosa were observed between the meat samples of cattle and buffaloes. However, the mean population of Staphylococcus aureus, Salmonella enteritidis, Bacillus cereus and Campylobacter jejuni were recorded higher (p < 0.05) in the meat samples of buffaloes as compared to cattle.

Table 3: The mean comparison of population of individual bacterial species in meat samples of cattle and buffaloes obtained by log cfu g⁻¹

<table>
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<tr>
<th>Bacteria species</th>
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<td>4.6 C</td>
</tr>
</tbody>
</table>

A-D Means followed by different lettering in a column showing significant difference (p < 0.05).

Mean bacterial load in meat samples collected under different conditions: The data about mean bacterial load in the meat samples examined under different conditions are summarized in Table 4. Generally, no any suitable places for slaughtering, processing and selling of meat is available in all four provinces of Pakistan. People are slaughtering and selling meat beside the roads, small streets, open shops, small cott, and semi-open slaughter houses, under such conditions, definitely meat could be contaminated with different bacterial population. During present study fewer than two different conditions, the investigation on meat samples contamination with bacterial species was carried out. One condition was considered to be hygienic where minimum hygienic facilities were available in semi-open.
slaughter houses while other was considered to be unhygienic, where open streets, sabzi mandi shops, cabins etc were brought in use. In hygienic conditions, significantly higher bacterial cells \((3.23\times10^5 \text{ g}^{-1})\) was counted in the meat samples of buffaloes while in the same conditions, the lower count of bacterial cells \((3.19\times10^4 \text{ g}^{-1})\) was recorded from the meat samples of cattle. Furthermore, in the case of un-hygienic conditions, significantly higher differences in the mean population of bacterial cells were recorded as 4.0\times10^5 \text{ g}^{-1} in buffaloes and lower mean population of bacterial cells \((3.73\times10^4 \text{ g}^{-1})\) was recorded in the meat samples of cattle. Significantly higher bacterial population \((3.6\times10^5 \text{ g}^{-1})\) was recorded in the meat samples of buffaloes as compared to cattle. While lower mean count of bacterial cells \((3.19\times10^5 \text{ g}^{-1})\) was recorded in the meat samples of cattle. The mean log 10 of aerobic plate was counted as 7.26 cfu g\(^{-1}\), and that of total \(\text{Coliforms}\) count while \(\text{Escherichia coli}\) was counted as 4.11 \text{log}^{10}\text{ cfu g}^{-1} and 3.03 \text{log}^{10}\text{ cfu g}^{-1}, respectively. All samples (100) were found positive for \(\text{Coliforms}\), however, 49.0% was found positive for \(\text{Escherichia coli}\) and 3.0% for \(\text{Salmonella}\). Haque et al. (2008) reported the mean values of TVC of slaughter yards and meat stalls were the log of 6.03 and log 6.53 respectively, whereas the TCC showed log of 4.85 and 3.82 respectively and that of TSC were 3.31 and 3.82 respectively. The mean values of TVC in brisket, neck and thigh regions of slaughter yards were log of 6.11, 6.01, and 6.31 while in meat stalls were log of 6.48, log 6.30, log 6.84 respectively. The TCC values of slaughter yards showed the log of 4.77, 4.36, and 5.12 whereas in meat stalls demonstrated logs of 4.94, 4.68, and 5.42 respectively. In the case of TSC values, the mean values were the log of 3.83, 3.07, 4.06, 3.96, 3.37, and 4.22 respectively. The results demonstrated the fact was that the unhygienic and poor sanitary conditions under where the meat and meat products were handled and processed were not acceptable from sanitary point of view. The statistical analysis showed that TVC and TCC obtained from meat samples of different markets and different regions of the carcass exhibited significantly (P < 0.01) variation in counts at regional level whereas TSC did not present any remarkable regional variation. A significant correlation (P<0.01) in between TVC and TCC was found and similar significant correlation (P<0.01) was also recorded in TCC and TSC, but surprisingly, no significant correlation was observed in between TVC and TSC.

**DISCUSSION**

The meat of cattle is observed to be the least contaminated than buffalo meat sample studied. Among bacterial species isolated from the meat samples of cattle and buffaloes, the higher bacterial load of \(\text{Escherichia coli}\) was recorded in the meat samples of animals and the lowest bacterial load of \(\text{Bacillus cereus}\) was recorded in meat samples of all animals investigated. However, some variation in bacterial population was observed among the meat samples of animals (Tables 2 and 3). Roberto et al. (2006) reported the aerobic mesophilic bacteria with values that ranged from 5.5 to 6.9 \text{log}^{10}\text{ cfu g}^{-1}, indicating the higher contamination takes place during the slaughtering and processing stages. Fung et al. (1980) defined the number of bacterial contamination that ranged from 5.0 to 6.0 \text{log}^{10}\text{ cfu g}^{-1} of aerobic microorganism’s was recorded as high population of bacterial organisms considered to be not acceptable for consumption while the values up to 4.0 \text{log}^{10}\text{ cfu g}^{-1} of bacterial population in meat samples could be considered to be acceptable for consumption. However, Jay (1996) reported that the total count of aerobic mesophilic bacteria between 5.0 and 7.0 \text{log}^{10}\text{ cfu g}^{-1} for raw meat are considered to be normal and the values above this range could spoil the meat and cause unpleasant odour. All samples presented a total number of \(\text{Coliforms}\) but 96.6% presented as fecal \(\text{Coliforms}\) in between 2.3 and 5.0 \text{log}^{10}\text{ NMP g}^{-1}. Uzeh and Adeniji (2006) reported that \(\text{Pseudomonas aeruginosa}, \text{Bacillus cereus}, \text{Staphylococcus aureus}\) and \(\text{Escherichia coli}\) isolated from both raw meat and tsire-suya. The total viable bacterial count varied from 2.0\times10^2 to 289\times10^2 cfu g\(^{-1}\) for the raw meat while 7\times10^2 to 171\times10^2 cfu g\(^{-1}\) for the tsire-suya. The \(\text{Coliforms}\) count varied from 4\times10^2 to 71\times10^3 cfu g\(^{-1}\) for raw meat and 1\times10^2 to 42\times10^3 cfu g\(^{-1}\) for tsire-suya, while \(\text{Staphylococcus aureus}\) count varied from 1\times10^2 to 60\times10^2 cfu g\(^{-1}\) for tsire-suya. In all cases, the bacterial count was higher in raw meat than tsire-suya. Therefore, the bacterial species and their population recorded in meat samples by the above workers are in line to the numbers measured in the present study. However, the results of the present study regarding bacterial load/population and prevalence of bacterial species in meat samples do agree with the findings of the above authors. Further that we have recorded similar trend and pattern of contamination as recorded by the earlier mentioned workers in their studies.

Similar kind of investigation was carried out by Yadav et al. (2006) who recorded the bacterial load in 100 sheep carcasses collected from retail meat shops of domestic markets. The mean \text{log}^{10}\text{ of aerobic plate was counted as 7.26 cfu g}^{-1}, and that of total \(\text{Coliforms}\) count while \(\text{Escherichia coli}\) was counted as 4.11 \text{log}^{10}\text{ cfu g}^{-1} and 3.03 \text{log}^{10}\text{ cfu g}^{-1}, respectively. All samples (100) were found positive for \(\text{Coliforms}\), however, 49.0% was found positive for \(\text{Escherichia coli}\) and 3.0% for \(\text{Salmonella}\). Okodugha and Aligba (1991) determined a TVC of 6.11 \text{log} cfu cm\(^2\) from beef at retail outlets. Also mentioned that after evisceration, the TVC increased. At the abattoir the mean TVC was counted as 5.51 ± 0.36 \text{log} cfu cm\(^2\), whereas the mean obtained from shops was 5.83 ± 0.42 \text{log} cfu cm\(^2\). While Okudugh and Aligba (1991) determined a TVC of 6.11 \text{log} cfu cm\(^2\) from beef at retail outlets. Also mentioned that after evisceration, the TVC increased. At the abattoir the mean TVC was recorded as 6.06 ± 0.53 \text{log} cfu cm\(^2\) while at shops it was counted as 6.48 ± 0.27 \text{log} cfu cm\(^2\).
log cfu cm² after evisceration of sheep carcasses, which increased 0.45 log units as compared to the post flaying level of contamination at the abattoir. Similarly, Gill and Baker (1998) also noted an increase in total counts by 0.30 and 0.64 log units, respectively after evisceration of sheep carcasses. Most butchers and shop keepers used to clean the meat by washing through simple tap water or by rubbing with a piece of cloth, both of these conditions were found to be highly contaminated.

In the light of their study, Sofos et al. (2000) explained that live animals and the environment serve as main sources for pathogenic microorganisms, which contaminate carcasses during the slaughtering process and meat products during processing, storage and handling. The decontamination processes, include animal cleaning, chemical dehairing at slaughter, spot-cleaning of carcasses before evisceration by knife-trimming or steam and vacuum, spraying, rinsing, or deluging of carcasses before evisceration and/or before chilling with water or chemical solutions (e.g., organic acids, trisodium phosphate, etc.) or steam could help to reduce the number of population of bacterial organisms. The processes applied at various concentrations or intensities, pressures (2-20 bar), temperatures (15-80 °C) for different lengths of time (5-20 sec), individually or in sequential combinations and under hygienic conditions also decrease the bacterial population.

CONCLUSIONS

From the present study, it is concluded that meat samples, irrespective of animal species, have higher prevalence of bacterial contamination had probably because of poor environmental conditions prevailing in the area. It was further observed that the meat samples of buffaloes were highly contaminated with bacterial organisms as compared to cattle. To avoid high level of bacterial contamination in meat, slaughtering as well as meat handling practices should be improved. To reduce the higher levels of bacterial count in meat, a good infrastructure such as dressing facility, drainage differentiation between clean and unclean operations and maintenance of hygiene and sanitation should be constructed and provided to every slaughter house.

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CONFLICT OF INTEREST

There is no conflict of interest.

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Jay JM (1996). Microorganism in fresh ground meats: the


