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Bacteriological Investigation of Sudanese Beef

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Abstract

Animals of every kind are in continual contact with microorganisms. Bacteria occur most abundantly in habitats where they find food, bacterial contamination affects human health, and the study will cater for investigation of other meat contaminants in cattle meat. This study is undertaken to fill the gap in this area. Three hundred and twenty four bacterial isolates belonging to twenty seven bacterial genera were recovered from 460 specimens from meat samples and rectal swaps from apparently healthy carcasses from two slaughter houses; Ghanawa-Khartoum (**beef brought from all over the country**) and West Al Gash (Kassala),that for microbial examination . Bacteria were isolated in the period from March, 2011 to June, 2013 involving four seasons. Isolation of bacteria was performed by conventional microbiological methods and identified according to the cultural and biochemical tests. Cambylobacters isolated in accordance with ISO 2006 method and particular attention was made to provide microaerophillic conditions at 42˚C. Statistical analysis of the obtained results showed a significant difference with respect to the seasons for the isolates but no significant difference was indicated among the different types of the carcasses parts from which the specimens were taken. This study explained a high level of bacterial contamination of beef carcasses without identification of the source of contamination. The least encountered isolates were *Clostridium spp.* and *Streptobacillus spp. with prevailed at (*00.74%*).* Although cambylobacters demonstrated a prevalence of 13.33.% in Summer, 2012 nevertheless, their presence of great concern as a zoonotic pathogen. *Arcobacter cryaerophilus* was isolated with a low prevalence however, it`s isolation is of great significance as this species is recently recognized as an emerging pathogen.

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The study recommend that, highly strict measures should be applied to curtail the contamination levels or to lessen it to the minimum, development of methodologies to appropriate management by application of Hazard Analysis Critical Control Point system, and national survey for the identification of meat contaminants should be adopted and executed using both microbilogical culturing methods and molecular biology methods.

Keywords: Bacteriological; Investigation; Sudan; Beef.

1. Introduction

Bacteria are present in all environments where eucaryotic cells live. Animals of every kind are in contact with microorganisms. Bacteria occur most abundantly in habitats provided that they find food, moisture and temperature appropriable for their growth [37]. The micro- flora present at any site in healthy animal is collectively referred to as the normal flora. Many of these microorganisms are anaerobes. Paradoxically, they enjoy a commensal existence with a host dependent upon oxygen for its survival [18]. There is tissue tropism for bacterial colonization; as the tissues vary in constituents resulting in different environments [28],as well temperature variation and humidity are also expected to affect the prevalence and spread of bacterial contaminants. [36] observed a little seasonal variation in the tropical countries however,more infection occurs during the rainy season.Nevertheless, [6] reported that both season and distribution location affected the incidence and the level of contamination of beef and lamb with E.coli in Morocco.This study will cater for investigation of other meat contaminants in cattle meat. Furthermore to evaluate the risk significance of the isolated meat contaminats in colonization of cattle and cattle meat in Sudan.It has been demonstrated that there is a high association between the microbiological contamination of air and carcasses with the movements of workers [30].

2. Materials and Methods

Specimens were collected for this work from 460 healthy slaughtered calves in the period from June (Summer), 2011 up to May (summer), 2012. The specimens were collected from slaughter houses of Western Algash (**Ka**ssala) Kassala State, and Ganawa, Khartoum State, Sudan. Specimens were collected from different parts of each animal carcass including hip (round), short rib (rib), neck (chuck),at the final step of slaughtering and specimens from rectal swaps,which were collected immediately after slaughter. In March, 2012,a hundred rectal swaps specimens were only collected .Fifteen grams of the meat specimen was cut from the carcass using a sterile scalpel and was immediately transferred to a sterile sample bottle and placed in an ice-box.

The refrigerated specimens ($2^0 - 4^0$ C) were then transported to the Veterinary Research Institute laboratory ((VRI)-Sudan) for processing and further work.Samples were prepared in accordance with the specific International Standard[18] . In the laboratory, all transfers, inoculation, culturing and sub-culturing was performed under aseptic conditions. Each meat specimen was gently surface sterilized with a piece of cotton wetted with 90% ethyl alcohol. An equivalent weight (about 10 g.) of each meat sample was finely diced using a sterile scalpel and forceps. Specimens from the prepared sample were then used for inoculation of an enrichment culture. The method used for isolation was the International Standard [18].

A sample was used to inoculate a Bijou containing 10 -15 ml sterilized Brain Heart Infusion Broth (BHI-Broth) for each specimen and twenty replica were made for each specimen for meat samples. For each rectal swap sample, the head of the swap consisting of the cotton plug was broken and placed into a Bijou bottle containing 10 -15 ml sterilized Brain Heart Infusion Broth (BHI-Broth). The inoculated specimens were then placed in a water-path adjusted to 42^{o}C for two hours with mild shaking. Enrichment was performed under anaerobic conditions by covering the cultured material with sterilized Paraffin oil.Microaerophillic conditions for incubation were procured by the candle extinction method microearophillic / mesophiles [39]. A candle extinction jar is a cheap and simple alternative, although it gives slightly higher oxygen pressure [23] ; Campylobacters are described as microaerophillic to distinguish their preferential use of oxygen as a terminal electron acceptor under reduced oxygen tensions [20].

After two hours incubation in the enrichment medium, a loopful was spread in Columbia agar medium supplemented with trimethoprim. Incubation was performed at 42° C in desiccators supplemented with a glowing candle in an incubator to provide microaerophillic conditions. For specimens cultured in microaerobic conditions, a sterile hypodermic syringe was used to obtain a small drop from the culture beneath the oil layer which was then spread onto the Columbia agar trimethoprim supplemented medium. The cultures thus prepared were incubated for 48 hours at 42° C.After 48 hours incubation the Columbia agar supplemented medium the plates were screened for most abundant and rare growth, in each plate, were noted and attempts were made for their identification. Presumptive Campylobacter colonies were sub cultured in Columbia agar and incubated at 48 hrs at 42 ° C under microaerophilic conditions. Pure culture from each isolate was maintained in a Columbia agar slant and was stored in a refrigerator set at 15 ° C. Biochemical and microscopical tests for each isolate was made on a fresh subculture.Microscopical examination for their Gram reaction and shape was done. Further, phenotypic characters included Catalse test, oxidase test, Glucose fermentation and growth atmosphere (aerobic and anaerobic- microaerophilic) as well as growth habit were performed. Other secondary biochemical tests performed included Voges-Proskauer test, nitrate reduction test, indole test and Urease test.

Statistical analysis for the recorded data were subjected toAnalysis of variance(ANOVA) for a completely randomized design and the obtained data were further analyzed using the Chi-Square method as suggested by [15] .

3. Results

Three hundred and twenty four isolates belonging to twenty seven genera were recovered from meat samples and rectal swaps from cattle through all seasons of this study. The prevalence of each bacterial contaminant and its prevalence through all seasons in carcasses and rectal swaps are displayed in Table 1and Table 2 respectively .The highest prevalent genus isolated from meat-cuts, were *Bacillus spp.* **(**30.15%**)** followed by *Staphyllococcus spp(*14.70%). and then *Proteous spp*.(11.76%), while the least prevalent was (0.74%) countered for *Clostridium spp* and *Streptobacillus spp*. The calculated value of Chi-square was greater than the tabulated value under 17 d.f., thereby the genera isolated from meat-cuts prevalent through all seasons are significantly different.

Table 1: Prevalence of bacteria isolated from meat- cuts in all seasons.

Bacteria Isolated	**Total Isolate**	**Relative frequency of isolates (%)**	Chi-Sq.value
Bacillus spp	41	30.15	
Staphyllococcus spp	20	14.70	
Proteous spp	16	11.76	
Enterobacteria spp	10	7.35	
Enterococcus spp.	9	6.62	
Streptococcus bovis	7	5.15	
Micrococcus spp	6	4.41	
Stomatococcus mucilaginous	5	3.68	
Corynobacteria spp.	4	2.94	
Arachnia spp.	3	2.21	
Arcobacter spp	3	2.21	
Acinetobacter spp	2	1.47	
Listeria spp	2	1.47	
Actinomycetes spp.	2	1.47	
Lactobacillus spp	2	1.47	
Pseudomonus spp	2	1.47	
Clostridium spp.	1	0.74	
Streptobacillus spp.	1	0.74	
Total Isolates	136	100.00 %	**161.08**

The result in Table 2. shows that bacteria isolated from rectal swap in all seasons, are nonsignificantly different. The calculated value of Chi-square for genera prevalences isolated from rectal swaps through all seasons was greater than the table value under 23 d.f. This indicated that there was a significant difference in the prevalence of isolates from rectal swaps through all seasons.

Table3. Indicated that Chi-test value (0.66) for these data is less than the table value which indicated a non-significant seasonal load of bacterial contaminants.

Table 4. shows calculated Chi-Square value was greater than the table value indicating that there was a significant difference between meat cuts with respect to contaminant isolates.

Table 2: Prevalence of bacteria isolated from rectal swaps in all seasons.

Bacteria Isolated	No. of Bacteria Isolated	Relative frequency of isolates (%)	Chi-Sq.value-
Bacillus spp	32	17.02	
Streptococcus spp.	19	10.10	
Escherichia spp.	16	8.50	
Lactobacillus spp.	15	7.98	
Neisseria spp.	15	7.98	
Proteous spp.	13	6.92	
Enterococcus spp.	9	4.79	
Enterobacter spp	8	4.26	
Campylobacter jejuni	8	4.26	
Actinomyces spp.	7	3.72	
Archobacter spp	6	3.19	
Clostridium histolyticum	5	2.66	
Corynobacteria spp.	4	2.13	
Micrococcus kristinae	4	2.13	
Kingella kingae	4	2.13	
Pseudomonas spp.	4	2.13	
Yerssinia enterocolitica	4	2.13	
Streptobacillus spp	3	1.60	
Stomatococcus mucilaginous	3	1.60	
Staphyllococcus spp.	3	1.60	
Legionella spp.	2	1.06	
Acinetobacter spp.	2	1.06	
Haemophilus aphorophilus	1	0.53	
Manheimia spp.	1	0.53	
Total isolates	188	100 .00	**91.04**

Table 3: Seasonal Prevalence of bacteria isolated from meat-cuts.

Season	No. of Bacteria Isolated	Prevalence %	Chi-Sq.value
Summer / 2011	41	30.15	
Autmn /2011	45	33.09	
Winter /2012	50	36.76	
Total isolates	136	100.00	**0.66**

Table 4: Prevalence of bacteria isolated from each type of meat-cut in all seasons.

Meat- cut	No. of Bacteria Isolated	**Prevalence %**	**Chi-Sq.value**
Neck	73	53.68	
Ribs	1 5	11.03	
Round	10	07.35	
Shank	38	27.94	
Total isolates 136		100.00	**86.83**

4. Discussion

A total of 460 specimens were screened for presence of microaerophyllic and aerobic bacteria in healthy calves slaughtered for human consumption in Sudan. Of this total specimens 220 specimens were collected as rectal swap specimens and 240 specimens as meat-cuts from different parts of cattle carcasses including neck, ribs,shank and Round.During the seasons (March, 2011 – May, 2012) results obtained show a wide variation of microbial flora associated with cattle meat and the gastrointestinal tract of cattle with respect to seasonality. About seventeen bacterial genera were identified from rectal swaps during summer seasons.*Arcobacter spp* identified in this study, little is known about the mechanisms of pathogenicity or potential virulence factors of this genus. There is evidence that livestock animals may be a significant reservoir of *Arcobacte*r spp[23] .

Isolates identified in specimens collected in Autumn seasons included;seven genera. Similarly, isolates identified in specimens collected in Winter seasons included other seven genera..Seasonal variation of microflora in the rectal swaps is quite expected and is justified by variation between seasons, and within seasons to some extent, of type of fodder, feed and water. This is highly expected as cattle breeding in Sudan is generally carried in an open system which is quite vulnerable to climatic seasonal variations. Furthermore, slaughter houses, in Sudan, receive cattle from different regions of the country, and recently, from Ethiopia. This may also serve as a source of contamination between flocks collected from different regions in enclosures before slaughter, contamination by shedding and contamination of fodder and drinking water. Contamination during slaughter and preparation of meat for marketing is not ruled out.

Studies of rectal microorganisms of cattle are scarce and more research is needed. The large number of microorganisms isolated from rectal swaps in this study is in agreement with the fact that the large intestine contains more than four hundred species[9]. Isolation of the Enterobacteriaceae, *Clostridium spp.*, Lactobacilli and Enterococci isolates ,is in agreement with the findings of Henning and Sude (2001) of the normal fecal flora of cattle. Similarly, isolation of *Corynobacterium spp.*,*Haemophilus spp*., *Actinobacillus spp*.and *Neisseria spp*. from rectal swaps is similar to results reported by[37] .

The isolates identified as *Campylobacter jejuni subspecies jejuni* were isolated in the late summer May /2012 .Considerable numbers of microorganisms were isolated from shank where as few microorganisms were isolated from the round. *Actinobacillus* species are Gram-negative bacteria that inhabit the upper respiratory tract of animals and the oral cavity[31] . Actinobacillosis is a disease of the tongue where the organ become enlarged, firm and contains numerous granulomatous lesions [13]. This species,as far as we know, is reported for the first time in this study in cattle meat in Sudan. This finding is of great implication for cattle production and health in Sudan as well as for Sheep production as actinobacillosisi has also been reported in sheep[41] . The genera were encountered in Winter 2012 from rectal swap specimens were *Pseudomonas spp* and *Lactobacillus spp* isolates in addition to *Enterobacter spp* which are common spoilage bacteria that may be encountered in environmental specimens as well as food item. It has been reported that all forms of these species are specific spoilage organisms of chilled beef during aerobic storage [44] . *Enterobacter sakazakii* was isolated from round specimens in two successive seasons (Atumn and Winter, 2011 and 2012 respectively) nevertheless their isolation does not pose a significant risk to cattle meat contamination by bacteria. The species *Actinomyces bovis* was only encountered in Winter, 2012 from rectal swap specimens. The significance of this finding is that it is more related to the cattle ranching and feeding procedures as infection with *Actinomyces bovis* is reported to occur following injury with a sharp object or hard feed pieces to the oral mucosa (FAO, 2000). *Micrococcus* spp. were isolated from neck, ribs and shank in summer season 2011 and from rectal swap specimens in winter, 2012. *Micrococcus* spp are reported as environmental contaminants or as a normal commensal on animals skin [8]. In the four seasons of this study, members of the genus *Streptococcus* were only encountered in Autumn, 2011 and Winter 2012. Two members of the genus *Neisseria* were isolated in the Summer, 2012. These were *Neisseria mucosa* and *Neisseria haemophillus* in rectal swap specimens. The isolation of these two species is insignificant with respect to meat contamination. *Pasturella haemoltica* species was only isolated during this study in Summer (March), 2013 from rectal swap specimens. *Pasturella haemoltica [P.] haemolytica* is reclassified as *Mannheimia haemolytica* comb. nov. in a conclusions of a polyphasic investigation of the taxonomy of the trehalose-negative *[Pasteurella] haemolytica* compIex (Angen and his colleagues 1999). *Kingella kingae* was encountered once in Summer (March/2012). The species is an emerging pathogen that has been recognized increasingly in recent years as a cause of a variety of pediatric illnesses [39]. *Enterococcus* was isolated in Autumn, 2011 from rectal swap and in Winter 2011 from neck and rectal swap specimens the isolation of this species poses a risk factor as a meat contaminant. *Enterococci* are used as indicators of fecal contamination and they have been implicated in outbreaks of food borne illness [4]. *Enterococcus* species is a fecal normal flora of animal which was previously known as fecal streptococci which commonly contaminate retail meats [20]. *Proteus* spp. were isolated from rectal swap specimens in Summer, 2012; from round, shank and rectal swap specimens in Autumn, 2011 and from round specimens in Winter, 2012. This results is comparable to the findings of [35] who reported a (53.9%) prevalence of *Proteus* spp in 89 out 165 raw beef samples from food establishments, butcher shops and a slaughter houses in Jimma City in Ethiopia, [1] also reported *Proteus* spp as a beef meat contaminant in a study of estimation of bacterial contamination of indigenous bovine carcasses in Khartoum (Sudan). *Stomatococcus mucilaginosus was recoverd from rectal swap and shank specimens in Winter, 2012. The genus Stomatococcus* comprises only one species, *Stomatococcus mucilaginosus is an emerging pathogen* which is a commensal of the normal flora of the human mouth and respiratory tract and may be associated with occasional opportunistic infections [38]. Isolation of

this species is presumably due to contamination from the working environment during slaughter and preparation of carcasses. *Listeria* spp. was only encountered once in Summer ,2011. The genus Listeria comprises two pathogenic species. *Listeria monocytogenes* was recognizing as a causal agent of human and animal listeriosis .However, *L. ivanovii* have not been frequently associated with the human illness and is being recognized as an animal pathogen [15]. As we did not identify the isolates to the species level however, this does not negate the precautions to be taken for the sake of both human and animal health.*Haemophilus aphrophilus*,was isolated once in Summer (March), 2012. This species was transferred to the genus *Aggregatibacter under the name Aggregatibacter aphrophilus.* The organism seems to be a normal component of oral flora and has been reported to cause endocarditis, sinusitis, pneumonia, empyema, soft tissue abscess, meningitis, vertebral discitis and septic arthritis while brain abscess due to *Aggregatibacter* is rare [2]. *Streptobacillus moniliformis* was only isolated once in Summer season March, 2012). *Streptobacillus moniliformis* is reported as the causative agent of both rat-bite fever and Haverhill [25]. As *Streptobacillus moniliformis* is not reported in cattle, most probably the occurrence of this microorganism is due to contamination from the slaughter houses. *Legionell*a was recovered only from rectal swap specimens in Autumn, 2011. In a study of the epidemiology, clinical characteristics, and treatment of Legionnaires' disease *Legionella spp* was recognized as the causative agent of pediatric pneumonia which can be severe and life threatening [17].

Baccilli are the most encountered isolates in this study (Table 1). This finding is comparable to findings of [28] who reported that in a study of bacterial populations associated with meat from the deboning room of a high throughput red meat abattoir counts for *Bacillus cereus., Staphylococcus aureus., Pseudomonas* spp., *Listeria monocytogenes., Escherichia coli* and *Salmonella* spp. from almost all red meat specimens exceeded the microbiological guidelines for raw meat as proposed by the South African Department of Health. *Yersinia*, a very important meat contaminant, was isolated once in Summer (March/2012). The importance of *Yersinia* isolates stems from the fact that the genus *Yersinia* contains three species of medical importance: *Y pestis* , the agent of bubonic and pneumonic plague, and *Y pseudotuberculosis* and *Y enterocolitica*, both of which can result in severe gastroenteritis, with local abscess formation and death as a result of peritonitis [7].

C*orynebacterium spp. was recovered only in season Summer, 2011 from neck specimens , most of the species are of clinical importance* [43]. Recently, the first mesenteric causes lymphadenitis in a cow calf was reported by., [33]. *Clostridium spp* was isolated once in Winter season, 2012 from rectal swap specimens. The results obtained are comparable to results obtained by, [26] who reported recovery of clostridia from beef abattoir environments and/or samples including a wide range of pre- and post slaughter locations and/or samples, with beef hides and feces being identified as the most highly contaminated sites. *C. histolyticum* is an agent of wound infections in both man and animals [42]. *Acinetobacter* spp *were isolated from rectal swap specimen in Summer (March/2012) season* . are widely encountered in the environmental sources including vegetables, pork, beef, and freshwater fish [10]. *Arcobacter cryaerophilus* species was isolated once in Winter, 2012. [32] reported that *Arcobacter* spp were found to be common contaminant of retail raw meats and raw milk in Northern Ireland with up to 34% prevalence in beef meat. These results are incomparable to the results obtained in this study as the prevalence recorded in this study throughout all the seasons was only about 3 %. *Arachnia propionica* was isolated once in Season Autumn, 2011 from a rib specimen. *Arachnia propionica was reclassified* as *Propionibacterium propionicus* comb. nov. [5]. Most probably the isolate originated as a

contaminant from skin during dehiding of carcasses of animals. Seven genera were only isolated from rectal swap specimen and were not encountered in meat cut specimens. Prevalence of bacteria isolated from meat-cuts in all seasons demonstrated a significant difference for the genera isolated from meat-cuts through all seasons of this study (Table 3). Similarly, a significant variation was observed for the genera isolated from rectal swap specimens during all seasons of this study(Table 4). Seasonal variation of bacterial contaminants of beef is expected and may be explained by variation in environmental conditions and their impacts on the slaughterhouse meat processing procedures. In particular, weather conditions particularly those leading to formation of dust storms and rain may be most effective in eliciting contamination to both the premises of the slaughter house and within workers in the slaughter house. Temperature variation and humidity are also expected to affect the prevalence and spread of bacterial contaminants. Although no significant variation was indicated between seasons with respect to isolated bacterial contaminants from meat cuts in (Table 3) however, results indicate a significant variation in the prevalence of bacterial isolates from each type of meat-cut in all seasons (Table 4). Results with respects to seasonal variation obtained in this study are comparable to results published in [36].

Neck and shank meat cuts demonstrated higher levels of contamination than ribs and round meat cuts. These results are in agreement with results obtained by [11] who proposed that carcass hanging may increase the rate of microbial contamination. Microbial contamination is expected to prevail in specimens collected from parts of the carcass near the ground than parts of the carcass which are at an elevated level from the ground. Furthermore, microbial contamination of the lower sites may be due to the effect of tissue tropism described in [18]. Isolation was always made under microaerophillic and thermophillic conditions (42˚C). This explanation is further supported by the fact that there was a non-significant variation of genera isolated in late summer, 2012. In this season the method used for isolation favoured the isolation of Campylobacters more than any of the other contaminating genera. Nine isolates were recovered from rectal swap specimens.

5. Conclusion

To our knowledge all species have been submerged are not cited in literatures as a digestive tract, intestinal and fecal normal flora , as well the findings of this investigation are of medically important that drives researchers to find more information about unknown meat contaminer even digestive system flora or environmental . Possibly these isolates could be environmental contaminants from the working premises, workers or tools used in the slaughtering process. As an example, *Legionella spp*. is a known contaminant associated with water in cooloing towers [22].

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