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Bacteriological Examination of Fresh Raw Beef on Retail in the Open Market in Owo Metropolis, Ondo State, Nigeria

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Abstract

Beef meat is an essential source of human nourishment universally. The processing of beef often leads to the introduction of fecal bacteria from the food animal. Some of the introduced microorganisms have been traceable to food-borne illnesses and the spread of antimicrobial resistant bacterial species that are inherently more challenging and costlier to treat. The study aimed to evaluate the bacteriological quality of fresh raw beef marketed in Owo, Ondo State, Nigeria. Samples of procured fresh raw beef were examined applying standard bacteriological techniques for total aerobic count (TAC), total coliform count (TCC), variety of bacteria and susceptibility to antimicrobial agents. The TAC and TCC were much in excess of the set limits. *Klebsiella* species 118 or 35.0% of all 337 isolates was the most predominant microorganism. *Staphylococcus aureus* 33.5% was next in frequency of isolation. Other microorganisms recovered

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were *Escherichia coli* 16.6%, *Preteus* species 10.1%, *Pseudomonas aeruginosa* 2.7%, *Enterococcus faecalis* 1.5% and *Salmonella enterica* 0.6% accounted for the least frequently isolated microorganism. The counts and variety of microorganisms that accompanied the beef samples clearly indicates a gap in hygiene ideals for handling beef that can become channels for the transmission of food-borne illnesses and source of spread of multidrug resistant bacteria to humans. This thus, necessitates the review of the processing of raw beef and monitoring enforcement of strict sanitation codes for safe beef to consumers.

1. Introduction

Beef meat is a major source of relevant nutriment that provides proteins, amino acids, vitamins and trace elements [1-3]. The processing of meat, transportation, storage and retail activities readily lead to the introduction of microorganisms [2,4-6]. The ability of meat to transfer pathogenic bacterial species to humans has been documented [7,8]. Meat has been shown to be an important highway from which humans can be infected with pathogenic bacteria [3,9]. The desire to keep meat free from the risk of food-borne illness has therefore, become more strident than ever [4,8]. A number of pathogenic bacteria seen in fresh raw beef across the globe include *Staphylococcus aureus* as the most dominant microorganism [10,11]. Notable food-borne diseases have been traceable to meat processed from animal carriers of *Salmonella* species and *Escherichia coli* especially serotype 0157 [12,13], as well as the most frequent causes of food-borne diseases in the United States [14]. The widespread application of antimicrobial agents in animal production is attributed to increased bacterial resistance in such food animals and the resultant existence of palpable apprehension worldwide on the impacts of these practices (7,15-19]. The emergence of pathogenic bacterial species with multiple-drug resistance, MDR genes in meat and meat products come with the attendant negative bearing on the management of such bacterial infections if transmitted to humans creates a dilemma of epic proportions [5,20-23]. Mitigating the effect of bacterial infection with the administration of antimicrobial agents has been the bedrock of modern medical care in dealing with most bacterial infections [24]. The current trend in the declining effectiveness of antimicrobial agents has dimmed the hope for their continued successful use and a key point for concern to healthcare providers across all nations [27,28]. Multidrug resistant, MDR bacteria are responsible for causing a significant number of deaths all over the world with more devastating effects in poorer countries [29]. It is projected that MDR bacterial species will be the topmost killer by the year 2050 [30]. This study intended to examine the nature and public health implications of bacterial flora accompanying fresh raw beef sold in the open market in Owo metropolis, Ondo State, Nigeria.

2. Materials and Methods

2.1. Study Design

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The study was cross-sectional, conducted on fresh raw beef in the open market in Owo metropolis during a ten-day period between the hours of 10.00 am and 11.00 am in February 2024 to evaluate the bacteriological quality of fresh raw beef on retail.

2.2. Sample collection

A minimum of 100 g of fresh raw beef muscle obtained from a selected retailer into sterile polypropylene autoclavable bags and stored in a cold chamber. The samples were thereafter, transported to the laboratory for examination within two hours.

2.3. Examination of samples

Aseptically, 10 g of beef sample removed with sterile blade and a pair of forceps, and weighed into a stomacher (Seward, United Kingdom) containing 90 mL of 1% buffered peptone water, BPW (Oxoid CM 1049) with inoculation of 0.1 mL onto dried plates of blood agar (Oxoid CM0085), MacConkey agar (Oxoid CM0007) and mannitol salt agar (Oxoid CM 271). Further ten-fold serial dilutions were done from the beef homogenate stock (10^{-2}) to give 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, from which 0.1 mL was plated onto dried plates of nutrient agar (Oxoid CM 0003) and MacConkey agar in replicates including from the stock homogenate. The plates were thereafter, incubated at 37ºC for 24-48 hours. Colonies appearing on plates showing with 3-30 colonies counted and mean from the two plates recorded as count for that dilution. The other inoculated agar plates were assessed for colonial morphology, characterization tests and identification following the scheme described by Cowan [31] Susceptibility of isolates to antimicrobial agents was determined by Kirby-Bauer disc diffusion technique applying the guidelines of the Clinical Laboratory Standards Institute [32]. Methicillin resistance was determined with oxacillin 1.0 µg disc as marker of methicillin resistance (ORSA/MRSA) and the double disc synergy test to detect the production of extended spectrum beta-lactamases, ESBLs in enterobacterial isolate

3. Results

The examination of 120 beef samples yielded 337 different microorganisms with aerobic bacterial count (TAC) range of 6.2-170.2 x 10^4 (mean ± SD = 41.13 ± 56.51) cfu/g and total coliform count, TCC of 2.7-98.7 x 10^4 (mean SD = 23.94 ± 29.03) cfu/g. The TAC in 70% samples were ≥10^5 cfu/g and 60% of TCC (Table 1). The most frequently isolated microorganism was *Klebsiella* species 118 isolates representing 35.0% of all isolates (Table 1). The next most dominant microorganism was *Staphylococcus aureus* with 113(33.3%). Other microorganisms were *Escherichia coli* 56(16.6%), *Proteus* species 34(10.1%), *Pseudomonas aeruginosa* 9(2.7%) *Enterococcus faecalis* 5(1.5%) and *Salmonella enterica* 2(0.6%) was the least dominant isolate

Table 2 represents the susceptibility of the isolated microorganisms to antimicrobial agents. All the isolates were resistant to tetracycline and 9.4% *Staphylococcus aureus* were susceptible to amoxicillin and 49.6% to amoxicillin-clavulanate. *Enterococcus faecalis* were not susceptible to amoxicillin and gentamycin, 40% inhibited by amoxicillin-clavulanate. Inhibition by other agents

ranged from 60-80%. Susceptibility of *Escherichia coli* and *Klebsiella* species to gentamycin varied from 39.3% to 90.0%. Susceptibility to perfloxacin, sparfloxacin and ciprofloxacin ranged from 41.6-88.2%% against *Pseudomonas aeruginosa* and *Proteus* species. Cefotaxime inhibited 33.3% of *Pseudomonas aeruginosa* and 88.2% of *Staphylococcus aureus* isolates.

Methicillin resistant *Staphylococcus aureus* strains were 1.8% of the isolates. The detection of extended spectrum beta-lactamase, ESBL enzymes in the enterobacterial isolates (Table 3) showed 1.8% of *Escherichia coli*, 2.5% *Klebsiella* species, 2.9% *Proteus* species produced ESBLs.

Table 1: Distribution of microorganisms in raw beef

Isolate	No. of cases (%)
Staphylococcus aureus	113(33.5)
Klebsiella species	118(35.0)
Escherichia coli	56(16.6)
Proteus species	34(10.1)
Pseudomonas aeruginosa	9(2.7)
Enterococcus faecalis	5(1.5)
Salmonella enterica	2(0.6)

Table 2: Antimicrobial susceptibility pattern of isolates

	Antimicrobial agent (%)							
Isolate	TE	A	AMC	CN	PEF	SPX	CPX	CTX
Staphylococcus aureus	0.0	19.4	49.6	74.5	80.5	83.1	81.4	7 8.8
Escherichia coli	0.0	0.0	0.0	39.3	62.5	64.3	62.5	55.4
Klebsiella species	0.0	0.0	0.0	90.0	62.5	63.3	61.7	5 6.7

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Proteus species	0.0	0.0	2.9	85.3	88.2	85.2	79.4	67.6
Pseudomonas aeruginosa	0.0	0.0	0.0	50.0	41.6	41.6	58.3	33.3
Enterococcus faecalis	0.0	0.0	0.0	0.0	80.0	80.0	60.0	60.0
Salmonella enterica	0.0	50.0	100.	100.	100.0	100.0	100.0	50.0

Key**:**

Tetracycline (TE), Amoxicillin (A), Amoxicilin-clavulanate (AMC), Gentamycin (CN), Perfloxcin (PEF), Sparfloxacin (SPX), Ciprofloxacin (CPX), Cefotaxime (CTX).

Table 3: Detection of ESBLs in isolates

Isolate	Positive cases (%)
Escherichia coli (n=55)	1 (1.8)
Klebsiella species (n=118)	3 (2.5)
Proteus species (n=314)	1 (2.9)
Pseudomonas aeruginosa (n=9)	0 (0)
Salmonella enterica (n=2)	0 (0)

4. Discussion

Fresh raw beef marketed in the open market in Owo metropolis contained aerobic and coliform counts in excess of the level 10^4 cfu/g recommended as upper limits [33-35]. The total aerobic count, TAC and total coliform counts, TCC were also respectively higher than the suggested baseline limits. This mirrors the poor microbiological quality of the beef samples and a reflection of non-adherence to strict cleanliness ideals required for handling fresh raw meat and meat products. This represents a direct signal for conceivable harm from such meat sources [36], since meat from muscles of healthy animals do not contain microorganisms. Even though, the number of microorganisms in fresh beef does not directly reflect the risk of harm to health [21,37],

microbial count of 10^6 cfu/g or higher was recorded in 70% of the samples which is the threshold that regularly signify meat spoilage [33]. Bacterial counts of 10^7-10^8 cfu/g are linked constantly with the onset of alteration in organoleptic properties such as appearance, odour and flavour [21]. Lower numbers of microorganisms in meat and meat products are advantageous in maximizing the shelf life and diminishing the possibility for food-borne illnesses [9]. The beef samples revealed a variety of bacterial species that are relevant not only in their potential to initiate diseases in humans through food as a major source of attention worldwide but also in zoonotic infections [8,9,38]. The most predominant microorganism isolated was *Klebsiella* species (36.0%), as a member of the enterobacteria, they are always present in waste materials, natural bodies of water and abattoirs. Their detection in beef is critical for risk assessment of the source and cleanliness of beef [39]. Members the genus especially *Klebsiella pneumoniae* is habitually associated with a high degree of notoriety for antimicrobial resistance in a wide range of human infections as well as a major microorganism in hospital acquired infections, HAIs [40]. The interaction between humans and animals predictably leads to exchange of microorganisms and when this involves multidrug resistant bacteria, the outcome can have confounding impact on the health of humans [41]. *Staphylococcus aureus* (33.5%) was the next in frequency as a colonizer of a wide variety of domestic and wild animals that is commonly found in meat products [11,42], with the potential for transmission into humans [43-45]. *Staphylococcus aureus* is of exceptional importance in man as a commensal in some humans and a key pathogen that can initiate an extensive range of infections in humans, some of which could lead to high rate of mortality [46-48]. *Escherichia coli* (16.6%) was the third most frequent bacterium. Studies have shown that this microorganism gains access into beef from cattle feces during evisceration and handling [8,49]. *Escherichia coli* contaminating beef may thus become transmissible to humans. A large number of *Escherichia coli* serotypes are capable of producing verotoxin involved in food-borne illnesses that range from mild to intense bloody diarrhea [8,50]. *Proteus* species were 10.1% of all the isolates. This microorganism inhabits the intestinal tract of many animals and man with a wide array of species. *Proteus* species are opportunistic pathogens with the ability to cause several types of human infections when transmitted to the appropriate body site [51]. The detection of *Proteus* species in beef is a sign that they originate from the feces of the animal or introduction during handling and therefore, a signal of potential health risk [52]. A low proportion of *Pseudomonas aeruginosa* (2.7%) encountered in the study is consistent with observation from other studies [53]. In spite of the low prevalence rate of *Pseudomonas, aeruginosa* and being a psychrotrophic bacterium that can thrive between 0-4^{o}C and a key microorganism in the putrefaction and spoilage of meat and meat products kept at low temperatures [54]. The organism is of great significance in causing HAIs and multidrug resistance of the organism in most clinical settings prompted the World Health Organization, WHO to classify it as an organism of critical priority for concerted effort to curtail or eradicate the infection caused by the organism [55,56]. *Enterococcus* species typically get into meat from feces as a classical intestinal bacterium. The detection in meat is an indicator of very low bacteriological purity and unsanitary handling techniques [21]. The regular detection of *Enterococcus* species in beef is disconcerting due to its ability to initiate infections with highly multidrug resistant strains, highlights the important need for beef to be available to consumers from secured sources [57-59]. The contamination of meat and meat products with antimicrobial resistant, AMR bacteria derived

from the intestinal tract of animals has elicited sharp criticisms on the use of antimicrobial agents in livestock [60]. This encourages the emergence of resistant strains through selective pressure and the propensity to cause severe nosocomial infections [61,62]. The recovery of *Salmonella enterica* - a non-typhoid salmonella, NTS from 0.6% of the samples and a key causative agent of food-related illnesses [12,63,64] with a significant number of infections in the United States of America [65]. There is no doubt, therefore, that *Salmonella enterica* infection may be common in Nigeria due to poorer asepsis levels and ineffective monitoring of beef processing and handling practices. *Salmonella enterica*'s infection rate of 4.2% reported in abattoir workers in Nigeria [66] does not account for the level of infection traceable to meat products in the consuming public. Resistance of the isolates to tetracycline implies that infection with any of the isolates are untreatable with this agent. The regular use of tetracycline at sub-therapeutic doses in animals feedlots is suggested a major contributory factor in the high rate of resistance seen with tetracycline and source of peril to humans and animals alike and the environment as well [67-69]. One strain (50%) of *Salmonella enterica* was susceptible to each of amoxicillin and cefotaxime, and susceptibility to other agents was 100%. Susceptibility of the isolates to other agents ranged from 41.6-100% with perfloxacin sparfloxacin, ciprofloxacin and lower against cefotaxime with susceptibility ranging from 33.3-78.8% for *Staphylococcus aureus*. Only isolates of *Staphylococcus aureus* (49.6%) were susceptible to amoxicillin-clavulanate, 2.9% of *Proteus* species and 100% *Salmonella enterica* were also respectively susceptible. Each of which points to the variability of antimicrobial agents needed for managing infections with these microorganisms. Two (1.8%) *Staphylococcus aureus* were methicillin resistant, MRSA strains. This should generate intense interest, as MRSA infections are some of the most difficult to treat bacterial infections [70]. Studies have reported that MRSA strains have a lower prevalence in livestock than in humans as this study has revealed [46,47,71,72]. Reports indicate that these strains derive their source from the livestock rather than from humans [73], which firmly places the focus on food asepticism [74]. The detection of MRSA in beef calls for process review from the slaughterhouse to the retail outlets [75,76]. Extended spectrum beta-lactamase, ESBLs detection for MDR organisms amongst the Gram-negative isolates ranged from 1.8% of *Escherichia coli* strains to 2.9% of *Proteus* species. These microorganisms are a cause of variety of human infections and multidrug resistant strains implicated in infections of opportunities [77]. The lower rate of ESBL observed in this study may echo the source of beef obtained mainly from the traditionally bred stock where the animals roam-free and substantially free from the administration of antimicrobial agents. The presence of ESBLs producing bacteria in animal-sourced food emphasizes the pressing need for new and innovative strategies to combat bacterial resistance [78].

5. Conclusion

The presence of very high bacterial loads in fresh raw beef meat marketed in Owo metropolis is a source of serious unease as this may serve as a veritable vehicle for the transmission of food-borne ailments that are associated with MDR organisms with heightened prospects for longer duration of illness, higher healthcare costs and elevated mortality rates. There is therefore, the need for a complete overhaul of the steps adopted in fresh raw beef processing and handling to the retail outlets in Nigeria.

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