



Original Research Article

COMPARATIVE ANTIBIOTIC SUSCEPTIBILITY PROFILES OF BACTERIAL ISOLATES FROM RAW MEAT AND COWHIDE SOLD IN ILE-IFE METROPOLIS

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ABSTRACT

Raw meat and ready-to-eat cowhide (ponmo) are two dietary intakes with different nutritional values. However, their consumption keeps increasing worldwide despite their nutritional differences. Their microbial quality is therefore important considering the public health significance of both meat and “ponmo”. This study aimed to evaluate raw meat and ready-to-eat cowhide for microbial quality and to determine the susceptibility profiles of the isolates to selected antibiotics. Two grams (2g) sample (meat or cowhide) was aseptically cut into an Eppendorf bottle containing 45 mL sterile distilled water and vortexed using a vortex mixer. A loopful of the supernatant was streaked on Salmonella-Shigella agar, MacConkey agar, Cetrimide agar, Mannitol salt agar and blood agar. Following a 24 h incubation at 37 °C, distinct pure colonies were identified using conventional standard biochemical tests. The isolates were evaluated for antibiotic susceptibility using the disk diffusion technique. Similar bacterial genera namely: *Escherichia* spp, *Klebsiella* spp, *Proteus* spp, *Staphylococcus* spp, *Micrococcus* spp, *Bacillus* spp, *Salmonella* spp, were isolated from both samples. While *Staphylococcus* spp (29%) was the predominant isolate in raw meat, followed by *E. coli* (19%), both were the predominant isolates with 29% occurrence each in cowhide. Ciprofloxacin was the antibiotic of choice for isolates from both sources with the least degree of resistance. Isolates from raw meat displayed high resistance to tetracycline, while isolates from cowhide displayed high resistance to cefotaxime and ceftriaxone. The study concluded that both raw meat and cowhide have equal chances of harbouring similar pathogenic bacteria with similar susceptibility to the same antibiotics.

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INTRODUCTION

Raw meat and ready-to-eat cowhide are two dietary intakes with differing nutritional values. Proximate analysis has revealed the presence of protein, carbohydrate, fibre, fats, as well as metabolizable energy in both diets, although at varying degrees [1,2]. However, the variation in bioavailability of the essential nutrients has been attributed to the treatment

employed during their transformation [3]. Transformation of cowhide to ready-to-eat form requires several stages that can impact distinct properties in terms of coloration and essential nutrients [4]. Similarly, it has been reported that the use of various cooking techniques, such as grilling, frying, and microwaving, to turn raw meat into more appealing, edible, and ready-to-eat forms results in the generation of free radicals and

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the loss of important fatty acids and other nutritional qualities, such as vitamins and minerals [5,6].

Aside from the variation in their essential nutrient composition, both are prone to microbial contamination, which may lead to their spoilage and infections in consumers. Microbial spoilage of meat and meat products is encouraged because they provide a favourable growth environment for a wide range of microorganisms, including the pathogenic ones [7]. Before spoilage, organisms like *Salmonella*, *Staphylococcus*, *Campylobacter*, *Bacillus*, *Listeria* and *Clostridium* are the prevalent genera of bacteria found in meat. However, while *Cladosporium*, *Penicillium*, *Geotrichum*, and *Mucor* are among the mould species that can be found in meat, *Cryptococcus* sp and *Candida* species are examples of yeasts [8]. Microbial spoilage of meat and cowhide may manifest by changes in flavor, tenderness, juiciness, odor and texture due to the formation of new compounds because of protein and lipid breakdown [9].

Despite the fact that both meat and cowhide are prone to microbial contamination, they differ in their microbial qualities. For instance, Mensah-Agyei *et al.* [2] reported the presence of *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* spp, *Pseudomonas aeruginosa*, and coagulase-negative staphylococci in contaminated cowhide, while Magdalene *et al.* [10] reported the isolation of *Bacillus* spp, *Escherichia coli*, *Proteus* spp and *Staphylococcus aureus*. However, *Klebsiella pneumoniae*, *Enterobacter* spp, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella* spp, *Serratia marcescens*, *Pseudomonas* spp, *Proteus vulgaris* have been reported to be associated with contaminated raw meat by Okonko *et al.* [11] while Olise *et al.* [12] reported the existence of *M. luteus*, *E. coli*, *K. pneumoniae*, *S. aureus*, *P. vulgaris*, *S. epidermidis*, *S. marcescens*, *B. subtilis*, *E. aerogenes*, *P. aeruginosa* and *S. pyogenes*.

Nonetheless, the association of pathogens with contaminated meat and cowhide can lead to foodborne infections. There have been reports of outbreaks of infections associated with contaminated meat worldwide. For example, there had been reports of a nationwide outbreak of Listeriosis in the United States due to contaminated meat [13] and outbreaks of *Salmonella* infections attributed to beef [14]. There have been several other studies on outbreak investigations for which meat and associated products have been reported as the culprit [15-18].

Additionally, in 2019, the Nigerian food and drug regulatory body, the National Agency for Food and Drug Administration and Control (NAFDAC), issued a warning against the consumption of cowhide due to concerns about potential chemical contamination that could pose a health risk to end users [19]. A similar warning was raised in 2022 by the same Agency.

Although there have been reports on the microbial qualities of raw meat and cowhide, similarities or otherwise, of their microbial contents, as well as the susceptibility to antibiotics of

the associated bacteria, have not been reported, hence this study.

MATERIALS AND METHODS

Collection of Samples

This was done according to the method of Awojinmi *et al.* [20] with modification. A total of fifty (50) samples each of raw meat and ready-to-eat cowhide (ponmo) were purchased from different local markets and abattoirs in Ile-Ife, Osun State. To avoid contamination from external sources, the sellers were asked to put the raw meat samples and ponmo directly into a sterile container (Zip-lock polythene packs) provided, after which the samples were transported in a cool box with ice packs to the Department of Pharmaceutical Microbiology Laboratory for further processing and analysis within 1-4 hours of collection. Samples of raw meat and cowhide collected are as shown in Figure 1.

Isolation method

Isolation was done using the modified procedure of Olise *et al.* [12]. Briefly, about 2 g meat sample was aseptically cut into an Eppendorf bottle containing 45 mL sterile distilled water and vortexed using a vortex mixer. A loopful of the supernatant was streaked on *Salmonella-Shigella* agar, MacConkey agar, Cetrimide agar, Mannitol salt agar and blood agar. Following a 24 h incubation at 37 °C, distinct colonies were sub-cultured and purified for further identification using conventional standard biochemical tests [21,22]. The procedures were also repeated on cowhide samples.

Antibiotic Susceptibility Test

This was determined using the Kirby-Bauer disc diffusion technique [23]. Briefly, colonies of each organism were dissolved in 10mL sterile distilled water in a McCartney bottle and standardized to 0.5 MacFarland standards using sterile distilled water. The resulting bacterial suspensions were uniformly spread on the Mueller-Hinton agar surface with the aid of sterile swab sticks. Antibiotic discs containing Tetracycline 10 µg, Cefotaxime 30 µg, Cotrimoxazole 25 µg, Ciprofloxacin 5 µg, Gentamicin 10 µg, Chloramphenicol 10 µg, and Ceftriaxone 30 µg were placed in contact with the organism on each plate. The plates were incubated at 37 °C for 24 h, after which the inhibition zone diameter was measured. The results were interpreted into resistant, intermediate and susceptible using the Clinical and Laboratory Standard Institute guidelines [24].

Statistical analysis

The results of the experiments, performed in triplicate, were presented as mean with SEM. Analysis was done using GraphPad Prism 5.



Samples of raw meat sold in Ife metropolis



Samples of cowhide (ponmo) sold in Ife metropolis

Figure 1: Samples of raw meat and cowhide sold in Ife metropolis

Table 1: Percentage distribution of bacterial isolates from raw meat and cowhide.

Organisms	Percentage distribution (%) in raw meat (n = 68)	Percentage distribution (%) in cowhide (n = 62)
<i>Escherichia</i> spp	19.0	29.0
<i>Staphylococcus</i> spp	29.0	29.0
<i>Klebsiella</i> spp	12.0	12.9
<i>Proteus</i> spp	16.0	11.3
<i>Bacillus</i> spp	6.0	9.6
<i>Micrococcus</i> spp	12.0	4.8
<i>Salmonella</i> spp	6.0	3.2
Total	100.0	100.0

Table 2: Percentage distribution of antibiotic resistance profiles of bacterial isolates from raw meat.

Organisms	BA (%)	CF (%)	C (%)	CP (%)	CR (%)	TE (%)	GM (%)
<i>Proteus</i> spp (n = 11)	2(18)	3(27)	2(18)	0(0)	3(27)	9(81)	3(27)
<i>Staphylococcus</i> spp (n = 20)	8(40)	8(40)	10(50)	0(0)	7(35)	18(90)	6(30)
<i>Micrococcus</i> spp (n = 8)	3(38)	3(38)	4(50)	1(13)	3(38)	7(88)	4(50)
<i>Escherichia</i> spp (n = 13)	5(38)	3(23)	3(23)	0(0)	3(23)	11(85)	2(15)
<i>Klebsiella</i> spp (n = 8)	3(38)	1(13)	1(13)	0(0)	1(13)	7(88)	4(50)
<i>Salmonella</i> spp (n = 4)	2(50)	1(25)	0(0)	0(0)	2(50)	4(100)	2(50)
<i>Bacillus</i> spp (n = 4)	2(50)	1(25)	2(50)	0(0)	1(25)	4(100)	0(0)

Key: BA: Cotrimoxazole; CF: Cefotaxime; C: Chloramphenicol; CP: Ciprofloxacin; CR: Ceftriaxone; TE: Tetracycline; GM: Gentamicin

Table 3: Percentage distribution of antibiotic resistance profiles of bacterial isolates from cowhide (ponmo).

Organisms	BA (%)	CF (%)	C (%)	CP (%)	CR (%)	TE (%)	GM (%)
<i>Proteus</i> spp (n = 7)	1(14)	7(100)	0	0	7(100)	5(71)	3(43)
<i>Staphylococcus</i> spp (n = 18)	8(44)	18(100)	3(17)	0	18(100)	12(67)	13(72)
<i>Micrococcus</i> spp (n = 3)	0	2(67)	0	0	2(67)	1(33)	1(33)
<i>Escherichia</i> spp (n = 18)	12(67)	16(89)	2(11)	1(6)	17(94)	11(61)	10(56)
<i>Klebsiella</i> spp (n = 8)	5(63)	8(100)	0	0	8(100)	8(100)	7(88)
<i>Salmonella</i> spp (n = 2)	1(50)	2(100)	0	0	2(100)	2(100)	2(100)
<i>Bacillus</i> spp (n = 6)	3(50)	6(100)	1(17)	2(33)	6(100)	4(67)	6(100)

Key: BA: Cotrimoxazole; CF: Cefotaxime; C: Chloramphenicol; CP: Ciprofloxacin; CR: Ceftriaxone; TE: Tetracycline; GM: Gentamicin.

Table 4: Comparative percentage distribution of antibiotic resistance profiles of bacterial isolates from raw meat and cowhide (ponmo).

Organisms	BA (%)		CF (%)		C (%)		CP (%)		CR (%)		TE (%)		GM (%)	
	R/M	C/H	R/M	C/H	R/M	C/H	R/M	C/H	R/M	C/H	R/M	C/H	R/M	C/H
<i>Proteus</i> spp	18	14	27	100	18	0	0	0	27	100	82	71	27	43
<i>Staphylococcus</i> spp	40	44	40	100	50	17	0	0	35	100	90	67	30	72
<i>Micrococcus</i> spp	38	0	38	67	50	0	13	0	38	67	88	33	50	33
<i>Escherichia</i> spp	38	67	23	89	23	11	0	6	23	94	85	61	15	56
<i>Klebsiella</i> spp	38	63	13	100	13	0	0	0	13	100	88	100	50	88
<i>Salmonella</i> spp	50	50	25	100	0	0	0	0	50	100	100	100	50	100
<i>Bacillus</i> spp	50	50	25	100	50	17	0	33	25	100	100	67	0	100

Keys: R/M: Raw Meat; C/H: CowHide; BA: Cotrimoxazole; CF: Cefotaxime; C: Chloramphenicol; CP: Ciprofloxacin; CR: Ceftriaxone; TE: Tetracycline; GM: Gentamicin.

Table 5: Percentage distribution of resistance profiles of bacterial isolates from raw meat and cowhide (ponmo) to each antibiotic.

Antibiotic	Percentage resistance by isolates from Raw meat n = 68		Percentage resistance by isolates from Cowhide (Ponmo) n = 62	
	R/M	C/H	R/M	C/H
Ciprofloxacin	0	1.5	0	4.8
Chloramphenicol	0	32	0	10
Gentamicin	0	31	0	69
Cotrimoxazole	0	37	0	50
Tetracycline	0	88	0	71
Ceftriaxone	0	29	0	98
Cefotaxime	0	29	0	97

RESULTS

Table 1 shows the percentage distribution of bacterial isolates in raw meat and cowhide (ponmo). While *Staphylococcus* spp was the prevalent isolate in raw meat, followed by *Escherichia* spp, the two genera have the same percentage of occurrence in cowhide (ponmo). *Salmonella* spp was the least isolated in both raw meat and cowhide (ponmo), with a percentage occurrence of 6% and 3.2%, respectively. Other isolates present in both raw meat and cowhide (ponmo) were *Klebsiella pneumoniae*, *Proteus mirabilis*, *Bacillus* spp., and *Micrococcus luteus*.

Table 2 shows the percentage distribution of resistance profiles of bacterial isolates from raw meat to selected commonly used antibiotics. All the isolates have zero resistance to ciprofloxacin except in *Micrococcus* spp with 13% resistance. However, *Salmonella* spp and *Bacillus* spp displayed 100% resistance to tetracycline. Moreover, resistance to other antibiotics varied among the species of isolated bacteria in the study.

The percentage distribution of antibiotic resistance profiles of bacterial isolates from cowhide (ponmo) is shown in Table 3.

All the isolates showed 100% susceptibility to ciprofloxacin except in *Escherichia* spp, with 6% resistance. Also, all the isolates displayed 100% resistance to cefotaxime and

ceftriaxone except *Micrococcus* spp, which displayed 67% resistance to each of cefotaxime and ceftriaxone; and *Escherichia* spp with 89% and 94% resistance to cefotaxime and ceftriaxone, respectively. However, resistance to other antibiotics varied among the species of isolated bacteria in the study.

Comparative percentage distribution of antibiotic resistance profiles of bacterial isolates from raw meat and cowhide (ponmo) is shown in Table 4. All the isolates in raw meat and cowhide (ponmo) had zero resistance to ciprofloxacin, except in *Micrococcus* spp isolates from raw meat with 13% resistance, as well as *Escherichia* spp and *Bacillus* spp isolates from cowhide (ponmo) with 6 and 33 % resistance, respectively. However, isolates from raw meat displayed less resistance to cefotaxime and ceftriaxone than isolates from cowhide (ponmo). On the other hand, isolates from cowhide (ponmo) displayed less resistance to chloramphenicol than those from raw meat. Nonetheless, *Salmonella* spp from both raw meat and cowhide displayed an equal degree of resistance to cotrimoxazole and tetracycline, while *Bacillus* spp from both sources displayed an equal degree of resistance to cotrimoxazole.

The percentage distribution of resistance profiles of all the bacterial isolates from raw meat and cowhide (ponmo) to each

antibiotic is as shown in Table 5. More of the isolates from cowhide were resistant to the effect of ciprofloxacin, gentamicin, cotrimoxazole, ceftriaxone and cefotaxime, while more of the isolates from raw meat were more resistant to the effect of chloramphenicol and tetracycline.

DISCUSSION

Cow meat and cowhide are two dietary intakes with differing nutritional compositions. Although both have been reported to be rich in proteins, fat, carbohydrates and fibre, the percentage of protein in cowhide is low, relative to fresh cow meat [25]. Despite this difference in nutritional composition, the consumption of meat and meat products, including the ready-to-eat cowhide, is increasing daily due to its appealing taste and affordability [9,10]. However, meat and meat products, including cowhide, are prone to microbial contamination, which can be attributed to processing and manipulations to which they are subjected before they reach the final consumers [12]. Other major factors that have promoted bacterial contamination of meat and cowhide are the sanitary conditions of abattoirs and their surrounding environments [26]. Raw meat and its products may become a risk for human health when they harbour many important pathogenic microbes, such as *Salmonella* spp., *E. coli*, *Campylobacter jejuni*, *S. aureus*, *Campylobacter coli*, *Yersinia enterocolitica*, and *Listeria monocytogenes* [12]. Considering the public health significance of meat and its products, the microbiological quality of meat and meat products is therefore very important [27].

In this study, the evaluation of 50 samples each of raw cow meat and ready-to-eat cowhide for their microbial quality resulted in the isolation of 68 and 62 bacterial species, respectively. All the bacterial species isolated can broadly be classified into 7 genera, namely *Escherichia*, *Klebsiella*, *Proteus*, *Micrococcus*, *Staphylococcus*, *Salmonella* and *Bacillus*. However, while *Staphylococcus* spp (29%) was the predominant isolate, followed by *Escherichia* spp (19%) in raw cow meat, both *Escherichia* spp (29%) and *Staphylococcus* spp (29%) were the predominant isolates in ready-to-eat cowhide. *Salmonella* spp was the least isolated in raw cow meat and cowhide, with 6 and 3.2 % occurrences, respectively. The findings in this study agree with the reports of other workers. For instance, the predominance of *Staphylococcus* spp in raw cow meat as found in this study agrees with the reports of Ahmad *et al.* [28] and Soyiri *et al.* [29]. Similarly, the study's findings regarding the incidence of *Escherichia* spp isolates in ready-to-eat cowhide are consistent with those of Olukitibi *et al.* [30], Keta *et al.* [31], and Mensah-Agyei *et al.* [2]. The finding, however, disagrees with the report of Magdalene *et al.* [10], where *S. aureus* was reported as the predominant bacterial isolate associated with cowhide.

The presence of *Staphylococcus* spp in both raw meat and cowhide (ponmo) can be attributed to the organism being a common food contaminant which can originate from food handlers, environment or post-process contamination through

direct contact or respiratory secretions [32]. However, *Staphylococcus* spp is one of the most resilient non-spore-forming pathogens, largely due to its ability to remain dormant for a long time in a dry state [32].

The presence of *Escherichia* spp, *Klebsiella* spp and *Proteus* spp in this study is an indication of squalid sanitary conditions as well as a filthy environment where the raw meat and cowhide (ponmo) were processed and hawked. *Escherichia* spp indicates faecal contamination of the water used for processing and preparation of both the raw meat and cowhide. Contamination of raw meat and cowhide during preparation may arise from the use of contaminated and untreated water [33]. The presence of *Bacillus* spp in raw meat and cowhide (ponmo) may be due to contamination from soil, water, food and animal faeces which are the natural habitat of *Bacillus* spp. Two major consequences of microbial contamination of raw meat and its products are spoilage and infections in consumers. Spoilage may manifest in raw meat and cowhide in the form of visual, textural and organoleptic changes [34]. In spite of the reports of a worldwide increase in consumption of meat and cowhide, a considerable portion of meat and meat products are lost yearly due to microbial spoilage [35].

Aside from spoilage, consumption of contaminated meat and its products can lead to infections (foodborne) in consumers. Although a variety of bacteria have been implicated in foodborne diseases, there are only a few species, such as *Bacillus cereus*, *Clostridium botulinum*, *Campylobacter*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella*, cause many health problems. Others include *Vibrio cholera*, *Aeromonas hydrophila* and *Yersinia enterocolitica* [36]. Many of these organisms have been isolated from both raw meat and cowhide in this study. Apart from the fact that all the isolated bacterial species in this study differ in their pathogenicity, they also differ in their degrees of susceptibility or resistance to antibiotics.

In this study, all the isolates from raw meat samples displayed diverse degrees of resistance to all the selected antibiotics used. Resistance to tetracycline was the highest, with a 81 - 100% range. However, all the isolated bacteria were highly susceptible to ciprofloxacin, except for *Micrococcus* spp, with 13% resistance.

Similarly, all the isolates from cowhide were susceptible to ciprofloxacin, except for *Escherichia* spp with 6% resistance and *Bacillus* spp with 33% resistance. However, resistance to cefotaxime and ceftriaxone was the highest with 67 – 100 % range.

Comparatively, ciprofloxacin was the antibiotic of choice to which many of the isolates from raw meat and cowhide were susceptible, with 1.5% of the total isolates from raw meat and 4.8% of all the isolates from cowhide being resistant. This finding agrees with the report of Olise *et al.* [12] where all bacterial isolates from the meat samples showed 100% sensitivity to antibiotics like ofloxacin, ciprofloxacin, and gentamicin, while many of the bacterial isolates were found to be non-susceptible to chloramphenicol, ampiclox, and septrin.

The broad-spectrum antibiotic ciprofloxacin, a fluoroquinolone, works by blocking the DNA gyrase, also referred to as topoisomerase II and topoisomerase IV [37]. Ciprofloxacin resistance can be caused by changes in target enzymes, changes in drug penetration, and the acquisition of plasmids that protect cells from the lethal effects of ciprofloxacin [38]. Other mechanisms by which antibiotic resistance can manifest include enzymatic inactivation through hydrolysis (e.g. via β -lactamase) or enzymatic modification of the target sites (for example, aminoglycoside resistance); prevention of the access of drugs to the target; or efflux pump mechanism [39].

CONCLUSION

The study concluded that both raw meat and ready-to-eat cowhide can harbor similar pathogenic bacteria that are similar in their susceptibility to the same antibiotics. Also, both raw meat and ready-to-eat cowhide have equal chances of predisposing their consumers to similar infections, especially the foodborne infections.

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AUTHORS' CONTRIBUTION

MOO: Design and execution of the work, analysis of the results, writing of the manuscript and revision of the manuscript; FOA: Isolation as well as characterization of isolates from meat samples, writing of the manuscript; OOA: Collection of meat samples, Isolation as well as characterization of isolates from raw meat samples; FAA: Collection of cowhide (ponmo) samples, Isolation as well as characterization of isolates from cowhide samples; DIO: Isolation as well as characterization of isolates from cowhide (ponmo) samples, writing of the manuscript.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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