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Research Article

Occurrence of Multidrug-Resistant Bacteria on Meat-Contact Surfaces and Hands of Meat Sellers: A Public Health Concern in Dutsin-Ma, Katsina State, Nigeria

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ABSTRACT

Antibiotic-resistant bacteria (ARB) on food and food-contact surfaces pose a global public health risk in the supply chain. This study assessed the antibiogram of Escherichia coli and Staphylococcus aureus recovered from meat contact surfaces in Dutsin-Ma, Katsina State, Nigeria. Forty-five swab samples were collected from table surfaces, palms, and knives in meat retail outlets across four locations: Wednesday Market, Hayin Gada, Abuja Road, and the Federal University Dutsin-Ma (FUDMA) Takeoff Site Market. Bacterial counts were performed on nutrient agar, and the isolates were identified by Gram staining and biochemical tests. Antibiotic susceptibility patterns were assessed using the agar disk diffusion method, and isolates resistant to \geq 3 classes of antibiotics were considered multidrug-resistant (MDR) strains. The highest mean bacterial counts were 1.14×10⁵ CFU/mL on tables (Wednesday Market), 1.33×10⁴ CFU/mL on palms (Darawa), and 1.38×10⁴ CFU/mL on knives (FUDMA Market). Among the 77 isolates recovered, S. aureus (55.8%) and E. coli (44.2%) were predominant. All E. coli isolates were resistant to amoxicillin and septrin (trimethoprim-sulfamethoxazole), but were susceptible to augmentin (amoxicillin-clavulanic acid) (61.8%) and ciprofloxacin (88.2%). S. aureus exhibited resistance (100%) to zinnacef but was susceptible to gentamicin (46.5%) and ciprofloxacin (48.8%). Multidrug-resistant (MDR) strains accounted for 59.7% of isolates, with S. aureus (58.1%) and E. coli (61.8%). This study highlights the prevalence of MDR E. coli and S. aureus on meat contact surfaces, posing a public health threat to the food supply chain. Routine ARB surveillance of local food markets is urgently needed in the study locality.

Keywords: Meat; Meat-contact surfaces; Antibiotic-resistant bacteria; E. coli; S. aureus

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INTRODUCTION

Meat is a nourishing food that offers numerous nutrients needed for human well-being. It contains protein, fat, and essential vitamins, making it an important food with numerous health benefits (Momtaz *et al.*, 2013a; 2013b; 2013c; Rahimi *et al.*, 2014; Hemmatinezhad *et al.*, 2015). However, meat provides a nutritious and favorable environment that is prone to contamination by pathogenic microorganisms (Mladenović *et al.*, 2021). The unsanitary conditions in slaughterhouses in developing countries have further undermined the safety of the meat procured from local meat retailers, leading to numerous outbreaks of foodborne illnesses caused by consuming contaminated meat (McEvoy *et al.*, 2003; Momtaz *et al.*, 2013a; Momtaz *et al.*, 2013b; Momtaz *et al.*, 2013c; Rahimi *et al.*, 2014; Bello *et al.*, 2015; Hemmatinezhad *et al.*, 2015).

Furthermore, antibiotic-resistant bacteria (ARB) on food-contact surfaces is a growing global public health concern (Miranda *et al.*, 2009). Over the past two decades, many studies have reported the recovery of pathogenic strains in food-producing animals and various food-related environments known to cause foodborne illnesses (Majowicz *et al.*, 2009). Bacteria such as *Escherichia coli* and Staphylococcus aureus have been reported to be among the major bacterial pathogens associated with contaminated food products of zoonotic origin (Abebe et al., 2020). Approximately 600 million people, equivalent to 7.69% of the world's population, fall ill from consuming contaminated food every year. This results in an estimated 420,000 deaths annually, accounting for 7.5% of all deaths globally (WHO, 2015; Ritchie and Roser, 2018). The World Health Organization (WHO) reported that approximately 30% of the population in developed countries experiences foodborne illnesses annually, while it was estimated that up to 2 million deaths occur each year in developing countries due to these diseases (WHO, 2015; Abunna et al., 2016).

Nigeria, the largest country in sub-Saharan Africa, faces foodborne-related challenges that are often not reported except in cases of outbreaks (Odo et al., 2021). For instance, in January 2018, the Nigerian Centre for Disease Control (NCDC) was informed of a botulism outbreak linked to food consumption in Abuja (Okunromade et al., 2020). In addition, numerous studies have reported the recovery of foodborne pathogens that pose significant public health risk, such as E. coli 0157:H7, Salmonella spp., Shigella spp., S. aureus, and Clostridium spp. from fruits, vegetables, and readyto-eat foods sold on the streets, markets, schools, major cities, and fast-food restaurants in Nigeria (Nwachukwu et al., 2008; Akindele et al., 2016; Ogidi et al., 2016; Akpoka et al., 2019; Oyedele et al., 2020). Moreover, retail food and meat are often kept at ambient temperature for long, facilitating the multiplication of pathogenic bacteria. In Europe, the detection of E. coli is commonly employed as a marker to assess the quality of food and antibiotic resistance in the food supply chain (European Food Safety Authority, 2008). Thus, this study aimed to evaluate the microbiological quality of meat contact surfaces viz sampling butchers' palms, meat retailing table surfaces, and knives used at selected meat retail outlets in Dutsin-Ma, Katsina State, Nigeria.

MATERIALS AND METHODS

Study Area and Sampling Locations

Dutsin-Ma is the administrative capital of Dutsin-Ma Local Government Area in Katsina State, Nigeria. It is situated at latitude $12^{\circ}27'17''$ N and longitude $7^{\circ}29'29''$ E. Dutsin-Ma has a land area of 527 km² with a projected population of 264, 588 residents in 2021 (Lawan *et al.*, 2023). The town is surrounded by a savanna landscape characterized by flat plains, scattered hills, and the nearby Dutsin-Ma Dam, which provides irrigation for agricultural activities in the area. The sampling locations

included Wednesday market, Hayin Gada, Abuja Road, Federal University Dutsin-Ma take-off site market, and Darawa outlets

Sample Size and Sample Collection

A total of 45 swab samples were randomly collected from 5 meat retail outlets: 9 samples were collected from each location i.e. (5 swab samples from the retail table surfaces, 2 swab samples from the butchers' palms, and 2 swab samples from the butchers' knives, respectively, in September 2023. The surfaces of the meat retail tables, palms, and knives were swabbed using sterile swab sticks moistened with sterile physiological saline. The samples were immediately transported to the laboratory for microbiological analyses at the Department of Microbiology, Federal University Dutsin-Ma, Katsina State, Nigeria.

Isolation and Characterization of Bacterial Isolates A tenfold serial dilution of each sample was prepared using sterile distilled water, after which 1 mL of each dilution from 10¹ to 10³ was transferred into separate sterile petri dishes. Sterile Eosin Methylene Blue agar (EMB), Mannitol Salt agar (MSA), and Nutrient agar (NA) prepared according to the manufacturer's protocol, were poured into each petri dish and allowed to set. The plates were then incubated at 37 °C for 18 to 24 hours. Visible colonies on NA were counted and recorded as the total viable count from each sample. Presumptive S. aureus and E. coli isolates were selected based on mannitol fermentation and the production of a green metallic sheen on MSA and EMB, respectively, followed by Gram staining. Pure isolates were stored on sterile nutrient agar slants at 4-6 °C for biochemical characterization.

Biochemical Characterization

The presumptive pure bacterial isolates were Gram-stained, and subjected to biochemical tests for the characterization of *S. aureus* and *E. coli* as previously described (Alabi *et al.*, 2023).

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing (AST) was conducted using the modified Kirby-Bauer disk diffusion technique. Gram-positive and Gramnegative antibiotic disks (Abtek Biological, UK) were used. The Gram-negative disks included: septrin (25 μ g), sparfloxacin (5 μ g), gentamicin (30 μ g), ciprofloxacin (5 μ g), chloramphenicol (30 μ g), tarivid (5 μ g), streptomycin (25 μ g), pefloxacin (5 μ g), augmentin (30 μ g), and amoxicillin (25 μ g) , while the gram-positive disks included pefloxacin (5 μ g), ampiclox (15 μ g), gentamicin (10 μ g), erythromycin (15 μ g), zinnacef (30 μ g), amoxicillin (30 μ g), rocephin (30 μ g), ciprofloxacin (5 μ g), streptomycin (25 μ g), and septrin (25 μ g).

The bacterial inocula were prepared using sterile distilled water and standardized to match the 0.5

McFarland standard. Aliquots from each inoculum were evenly spread on Muller-Hinton agar (MHA) using sterile swab sticks and allowed to dry. Afterward, antibiotic disks were aseptically placed on the plates, and incubated at 37°C for 24 hours. Zones of inhibition were measured and interpreted using the CLSI 2020 breakpoints (Humphries *et al.*, 2021).

RESULTS

The mean bacterial counts from various sampling locations revealed high levels of contamination with pathogenic bacteria. The highest $(1.14 \times 10^5 \text{ cfu/mL})$ mean bacterial count was found on tables from the Wednesday Market, while the lowest count $(6.8 \times 10^3 \text{ CFU/mL})$ was recorded on knives from Darawa and the palms of retailers from Abuja Road outlets (Table 1).

The overall occurrence of *E. coli* was 75.6%, while *S. aureus* was 95.6% on meat contact surfaces, knives, and butcher palms. The prevalence of *E. coli* was

44.2% (34/77), while *S. aureus* was 55.8% (43/77), as shown in Table 2.

Resistance to multiple antibiotics was observed among the E. coli and S. aureus isolates recovered from tables, palms, and knives. Escherichia coli exhibited a high level of resistance to septrin (100%), sparfloxacin (94.10%), and chloramphenicol (82.40%), but low resistance to ciprofloxacin (11.80%). Similarly, S. aureus exhibited high levels of resistance to zinnacef (100%), septrin (97.70%), ampiclox (93.02%), rocephin (93.02%), pefloxacin (90.70%), and amoxicillin (86.05%), however, a lower resistance (51.2%) to ciprofloxacin was observed among isolates of S. aureus (Table 3).

Although some of the isolates were susceptible to all the antibiotics tested, a significant percentage exhibited multiple resistance phenotypes to all the antibiotics (Table 4). Overall, 46 % of the bacterial isolates recovered from the meat contact surfaces in this study were MDR (Table 5).

Table 1: Mean bacterial count from sam	ples collected across different sampling locations

Locations/ Contact-surface	Number examined	Mean colony count (CFU/mL)
Darawa		
Table	5	3.26×10 ⁴
Palm	2	1.33×10 ⁴
Knife	2	6.8×10 ³
Wednesday market		
Table	5	1.14×10 ⁵
Palm	2	1.46×10 ⁴
Knife	2	1.94×10 ⁴
Hayin Gada		
Table	5	1.94×10 ⁴
Palm	2	7.4×10 ³
Knife	2	2.88×10 ⁴
FUDMA Take-off site market		
Table	5	8.6×10 ³
Palm	2	1.54×10 ⁴
Knife	2	1.38×10 ⁴
Abuja Road		
Table	5	8.6×10 ³
Palm	2	6.8×10 ³
Knife	2	8.6×10 ³

Table 2: Frequency of occurrences of *E. coli* and *S. aureus* on meat contact surfaces

Locations		E. coli		S. aureus	
	Number	Positive	Negative	Positive	Negative
	Examined	Sample	Sample	Sample	Sample
		(%)	(%)	(%)	(%)
Darawa	9	6 (66.7)	3 (33.3)	9 (100)	0 (0)
Wednesday market	9	9 (100)	0 (0)	9 (100)	0 (0)
Hayin Gada	9	7 (77.8)	2 (22.2)	9 (100)	0 (0)
FUDMA take-off Campus market	9	4 (44.4)	5 (55.6)	7 (77.8)	2 (22.2)
Abuja Road	9	8 (88.9)	1 (11.1)	9 (100)	0 (0)
Total	45	34 (75.6)	11 (24.4)	43 (95.6)	2 (4.4)

Antibiotics	<i>E. coli</i> (n=34)		Antibiotics	3)	
	Resistant (%)	Susceptible		Resistant (%)	Susceptible (%)
		(%)			
Septrin	34 (100)	0 (0.00)	Pefloxacin	39 (90.70)	4 (9.30)
Chloramphenicol	28 (82.40)	6 (17.60)	Gentamicin	23 (53.50)	20 (46.50)
Sparfloxacin	32 (94.10)	2 (5.90)	Ampiclox	40 (93.02)	3 (6.97)
Ciprofloxacin	4 (11.80)	30 (88.20)	Zinnacef	43 (100)	0 (0.00)
Amoxicillin	34 (100)	0 (0.00)	Amoxicillin	37 (86.05)	6 (13.95)
Augmentin	13 (38.20)	21 (61.80)	Rocephin	40 (93.02)	3 (6.97)
Gentamicin	18 (52.90)	16 (47.10)	Ciprofloxacin	22 (51.20)	21 (48.80)
Pefloxacin	22 (64.70)	12 (35.30)	Streptomycin	26 (60.50)	17 (39.50)
Tarivid	26 (76.50)	8 (23.50)	Septrin	42 (97.70)	1 (2.30)
Streptomycin	17(50.00)	17(50.00)	Erythromycin	36 (83.70)	7 (16.30)

Table 3: Antibiotic susceptibility patterns of E. coli and S. aureus from meat-contact surfaces

Table 4: Phenotypes of Multidrug Resistant Bacterial Isolates in the Study

Bacteria	Number	Resistant phenotypes
Escherichia coli	2	AMP, CPR
	7	AMP, GEN, PEFX, CPR, SXT
	5	AMP, GEN, CPR, SXT, CH
	2	AMP, SXT
	5	AMP, GEN, AUG, CH
	21	
Staphylococcus aureus	5	AMP, GEN, ERY, SXT, APX
	7	AMP, GEN, ERY, STR, SXT, APX
	4	AMP, GEN, SXT
	3	AMP, ERY, STR
	6	AMP, GEN, ERY, STR, SXT, APX
	25	

Keys: AMP (ampicillin), GEN (gentamicin), PEFX (pefloxacin), ERY (erythromycin), SXT (septrin), APX (ampiclox), CPR (ciprofloxacin), CH (chloramphenicol)

Bacteria	Number recovered	Number of MDR	Percentage (%)
E .coli	34	21	61.8%
S. aureus	43	25	58.1%
Total	77	46	59.70

DISCUSSION

In this study, we reported the recovery of MDR pathogenic bacteria from meat-contact surfaces. The total bacterial count obtained revealed a significantly high mean colony count of bacterial isolates in all the samples collected. Foodborne infections are a significant cause of morbidity and mortality globally (WHO, 2015; Abunna *et al.*, 2016). The results obtained revealed a high level of contamination of meat contact surfaces with *E. coli* and *S. aureus* isolates in all the study areas. *In this study, S. aureus had a higher frequency of*

occurrence (95.6%) than E. coli (75.6%). In comparison, our finding is higher than that of Greeson et al. (2013), who reported E. coli (72.2%) and S. aureus (24.6%) from Riyadh, Saudi Arabia. The high occurrence of Staphylococcus species on meat-contact surfaces may be attributed to the fact that Staphylococcus species are a part of the skin's natural flora and can easily be shed on surfaces in meat retail outlets. In addition, Staphylococcus species can persist and survive various unfavourable conditions, including food processing and handling environments. This is consistent with previous studies where foodborne Staphylococcus species were recovered from food and food retailing environments (Nwachukwu *et al.*, 2008; Akindele *et al.*, 2016; Ogidi *et al.*, 2016; Akpoka *et al.*, 2019; Oyedele *et al.*, 2020).

Furthermore, the recovery of E. coli (75.6%) on meat-contact surfaces is worrisome and may be attributable to the unhygienic conditions in slaughterhouses which can significantly undermine the safety of meat procured from meat retailers, as reported elsewhere (McEvoy et al., 2003; Bello et al., 2015). Sebsibe et al. (2020) reported the recovery of E. coli (20.2%) and E. coli O157:H7 (5.4%) from samples collected in abattoir environments and meat retail outlets, respectively, in Jimma, Southwest district of Ethiopia. In contrast, this is lower than our findings in the current study. Sebsibe and colleagues noted that the high contamination of abattoir environments and meat was attributable to little or no formal education and training on food safety, and unwholesome practices among butchers working in slaughterhouses (Sebsibe et al., 2020). Our findings may in part be due to the poor monitoring of activities in abattoirs by environmental health workers, poor hygiene practices by the local meat retailers, and abattoir conditions in the study locality.

The antibiotic susceptibility patterns revealed that the recovered isolates were predominately antibiotic-resistant strains. The recovered E. coli exhibited a high level of resistance to amoxicillin (100%), septrin (100%), sparfloxacin (94.10%), and chloramphenicol (82.40%). It is worth noting that these antibiotics are extensively used in clinical and veterinary practice to manage bacterial infections. The overuse and misuse of these antimicrobial agents may drive the emergence of resistant strains in the study locality due to selective pressure (Wang et al., 2017; Wang and Hu, 2022). Similarly, S. aureus also exhibited multiple resistance to antibiotics in the current study. The antibiotics to which resistance was most commonly observed were; zinnacef (100%), septin (97.70%), ampiclox (93.02%), rocephin (93.02%), pefloxacin (90.70 %), and amoxicillin (86.05%). The high antibiotic resistance particularly to β -lactam antibiotics among the recovered S. aureus isolates suggested that they may have acquired β -lactam resistance genes through horizontal gene transfer (Adesoji et al., 2019). Zehra et al. (2019) reported a high resistance (90.97%) to β -lactam antibiotics among methicillin-resistant Staphylococcus aureus (MRSA) recovered in retailed meat from Punjab, India (Zehra et al., 2019).

In addition, 59.70 % of all the bacterial isolates recovered in this study were identified as MDR strains. Among the MDR isolates detected, 58.1% were *S. aureus* and 61.8% were *E. coli* isolates. This

high occurrence of MDR bacteria on meat contact surfaces poses a significant public health threat to meat consumers as MDR bacterial infections have become increasingly difficult to treat amidst the global antimicrobial resistance crisis. The high level of antibiotic resistant bacterial contamination is not unexpected, given the unsanitary habits of the meat vendors. Many of the vendors engage in behaviors that compromise food safety, such as leaving meat uncovered, and using unclean knives and utensils, leading to flies infestation. Additionally, cross-contamination by flies may contribute to the dissemination of MDR bacterial strains. These practices are common in many local markets in the country and may contribute to the widespread ARB contamination in retailed meat (Baah et al., 2022).

Our findings are in tandem with the study by Jaja et al. (2020), where they reported high levels of antibiotic-resistant S. aureus and E. coli in retailed meat in South Africa. Meat contamination was attributed to the transfer of pathogenic microorganisms from animal guts to carcasses during processing in abattoirs and unapproved processing environments meat which is subsequently transferred to consumers (Jaja et al., 2020). This highlights the need for robust routine surveillance, improved antibiotic stewardship, improved sanitation, and hygiene practices in the meat industry to mitigate the spread of ARB in the local food supply chain.

CONCLUSION

This study found high levels of pathogenic MDR bacteria on various meat contact surfaces in the study area. The isolates were resistant to commonly used antibiotics in clinical practice. This portends a significant public health threat and underscores the need for surveillance of ARB in the local meat supply chain.

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