Prevalence and phenotypic antibiotic bioassay of methicillin-resistant *Staphylococcus aureus* in raw meats sold at various retail outlets in the cape coast metropolis of Ghana.

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Abstract

MRSA is an emerging zoonotic organism that has gained attention in public health because of their disease causing abilities. They have been implicated in most food contaminations and foodborne infections. Despite these importance's, little is known on the MRSA situation in the meat consume in the Cape Coast Metropolis of Ghana. This study assessed the MRSA prevalence in meat sold at various retail outlets in the Cape Coast Metropolis. One hundred meat samples were obtained from pigs, chicken, cattle and goats from various retail points in the Metropolis. The total viable count, MRSA isolation and antibiotic susceptibility profiles of the isolates were performed following standard procedures. Overall, a prevalence of 45% (45/100) MRSA isolates was phenotypically detected in this study. Although the sample sizes were different, pork recorded the highest MRSA isolates of 47.6% (20/42). Sixteen (16) out of 45 MRSA isolates were Multi-drug resistant. All the isolates were resistant to all the antibiotics used except for Vancomycin. The prevalence of MRSA isolates in raw meat in most of the retail outlets in this study gives a warning signal for possible occurrence of food borne infections capable of producing outbreaks in the district.

Keywords: Methicillin Resistant *Staphylococcus aureus*, Raw meat, Retail points, Antimicrobial susceptibility.

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Introduction

Staphylococcus aureus is one of the most important organisms that have gain attention because of their role in both hospital and community acquired infections. According to Kluytmans, Methicillin Resistant *Staphylococcus aureus* has been implicated in causing nosocomial and community bacteraemia, food poisoning, pneumonia and wound infections [1]. *S. aureus* resistance to antibiotics is as a results of its mecA gene, which alters the penicillin-binding proteins (PBPs) on their cell wall into Penicillin-binding proteins 2a (PBP 2a), reducing its affinity to all β-lactam antibiotics [2]. Most slaughtering activities in the Cape Coast Metropolis occurs in an almost unhygienic nature which may suggest that, slaughtering an MRSA-positive animal may lead to contamination of both the carcasses as well as the surrounding environments with various MRSA strains. Several studies reiterate the presence of MRSA strains in different foods, meat products and raw meat samples especially Chicken [3-6].

In order to ascertain the public health implications of MRSA-contaminated meats, there should be sufficient and valid data so as to make the vivid assessments, conclusions and the final. recommendations. Despite the aforementioned importance of this MRSA in terms of Public Health, there is scanty information on the MRSA prevalence in raw meat intended for human consumption in the Cape Coast Metropolis of Ghana. This study was undertaken to assess the MRSA prevalence in meat by testing a substantial quantity of meat samples from retail outlets in the Cape Coast Metropolis of Ghana. Also, the antibiotic susceptibility profiles of the MRSA isolates were also determined.

Materials and Methods

Collection of samples

A total of 100 meat samples which included 28 beef samples, 42 pork samples, 10 chevon samples and 20 chicken samples were obtained from five retail outlets (Retail outlet 1 to Retail outlet 5) in the Cape Coast Metropolis of Ghana. The samples were stored in sterile sealed polythene bags and transported on ice to the laboratory for analysis.

Total Viable Count (TVC)

The total viable aerobic bacteria count was performed on Nutrient Agar. For this, meat sample (10 gram meat + 90 ml sterile distilled water) were homogenized in a sterile blender. One ml of this homogenized sample was transferred to a second test tube (test tube contains 9 ml of sterile distilled water) and a series of serial dilutions were performed thereafter. This was followed by culturing on Nutrient agar using the pour plate method and then plates were incubated at 37 for 24-48 h. The colony enumerations were performed thereafter using an electronic colony counter. The counts for each plate were expressed as Colony forming unit of the suspension (CFU/g).

Isolation of Methicillin-resistant *Staphylococcus aureus*

The discrete colonies on the Nutrient Agar were sub-cultured onto selective MRSA agar plates (ORSAB, OXOID, UK). These plates were incubated for 24-48 hours at 37℃ and examined for MRSA isolates.

Antibiotic susceptibility testing

The MRSA isolates that were positively identified using the culture-based methods were subjected to antibiogram characterization. All the MRSA isolates were tested for resistance, intermediate or sensitivity to different antibiotics using the standard disc diffusion method Kirby Bauer. The following antibiotics groups were used; Penicillins (penicillin 15 µg, ampicillin 10 µg, cloxacillin 5 µg), Tetracyclines (tetracycline 30 µg), Folates (cotrimoxazole 25 µg), Macrolides (erythromycin 5 µg), Aminoglycosides (gentamicin 10 µg), Glycopeptides (vancomycin 30 µg), Cephalosporins (cefuroxime 10 µg), Augmentin (30 µg), Carbapenem (meropenem 10 µg), and Quinolones (ciprofloxacin 5 µg). Approximately 3 to 5 isolated colonies from a pure culture were emulsified in sterile nutrient broth and the turbidity of the inoculum was compared with 0.5 McFarland Standard. Mueller-Hinton Agar (Lab M Limited, Lancashire, UK) plates were prepared and a loopful of the inoculum was seeded on the surface of the media. Subsequently, sterile cotton swabs were used to spread the organism evenly on the Mueller-Hinton agar plates. The antibiotic discs were placed on the agar plates using a sterilised forcep. The plates were read after 24 h of incubation at 37ºC under aerobic condition. *S. aureus* ATCC 25923 was used as a quality control strain. The sensitivity of the isolates to various antibiotics were classified in accordance with the guideline of the National Committee for Clinical Laboratory Standards as susceptible, intermediate or resistance for each antibiotic tested by measuring the zone of inhibition around the antibiotic disc [7].

Multidrug resistance pattern of the MRSA

The interim standards defined by the European Centre for Disease Prevention and Control (ECDC), and the Centre for Disease Prevention and Control (CDC) were used for the description of the multidrug-resistant (MDR) profiles of the MRSA isolates. In their standards, non-susceptible to ≥ 1 agent in ≥ 3 antimicrobial categories were considered MDR, while non-susceptible to ≥ 1 agent in ≥ 2 antimicrobial categories were considered extremely drug resistance (XDR) [8]. This was the standard yardstick used for the characterization of the MRSA isolates into MDR profile in this study.

Data analysis

Data was analysed using the statistical software SPSS version 25.0. Descriptive statistics was used to describe the frequency of MRSA from different meat samples, antimicrobial susceptibility pattern and hygienic conditions.

Results

Prevalence of MRSA in raw meat

Overall, 45 MRSA isolates were phenotypically screened obtained in this study, representing an overall prevalence of 45% (45/100). It could be seen that regardless of the specie, MRSA was isolated from its meat sample. In order of highest to lowest, Pork recorded high isolates followed by beef, chicken and chevon (Table 1).

Table 1. *MRSA isolates from raw meats at various retail points.*

	MRSA ISOLATES (n=45)				
Retail Points	**Pork**	**Beef**	**Chevon**	**Chicken**	**TOTAL**
Ab	6	2	2	2	12
Kt	2	4	1	2	9
Yg	5	2	1	1	9
Um	4	4	0	3	11
Ump	3	0	0	1	4
TOTAL	20	12	4	9	45

Key: Ab= Retail outlet 1; Kt= Retail outlet 2; Yg= Retail outlet 3; Um= Retail outlet 4; Ump= Retail outlet 5.

Hygienic Acceptability Value of the Raw Meat Samples

From Table 2, it could be seen that most of the meat samples were satisfactorily good for human consumption. Shockingly, some meat samples had a high total viable count which made them not suitable for consumption as the viable counts were

above the minimum threshold. Again, Retail outlet 1 recorded the highest condemned meat sample (8/32, 25%) followed by Retail outlet 3, Retail outlet 4, Retail outlet 2 and Retail outlet 5 which recorded Zero (0/11) condemned samples.

Table 2. *Meat quality base on total viable count.*

Area	No. of Sample	Satisfactory/passed for consumption (<0.5 million/g)		Acceptable/conditional approval (>0.5 million/g & <2 million/g)		Rejected/condemned (>2 million/g)	
		No.	%	No.	%	No.	%
Ab	32	14	43.8	10	31.3	8	25
Kt	18	7	38.9	9	50	2	11.1
Yg	22	10	45.5	8	36.4	4	18.2
Um	17	11	64.7	4	22.5	2	11.8
Ump	11	8	72.7	3	27.3	0	0

Antimicrobial Susceptibility Patterns of MRSA Isolates

Out of total 45 MRSA isolates subjected for antimicrobial susceptibility test, 45 (100%), 45 (100%), 38 (84.4%), 26 (57.8%), 22 (48.9%), 10 (22.2%), 29 (64.4%), 32 (71.1%), 38 (84.4%) and 33 (73.3%) exhibited resistance to Penicillin, Ampicillin, Cloxacillin, Cotrimoxazole, Tetracycline, Ciprofloxacin, Gentamycin, Meropenem, Cefuroxime and Erythromycin respectively (Table 3).

Table 3. *Antimicrobial susceptibility profile of MRSA isolates from raw meat.*

Antibiotics	Antimicrobial susceptibility test	MRSA Isolates n=45	
		No	Percentage (%)
Penicillin	Resistant	45	100
	Intermediate	-	-
	Susceptible	-	-
Ampicillin	Resistant	45	100
	Intermediate	-	-
	Susceptible	-	-
Cloxacillin	Resistant	38	84.4
	Intermediate	7	15.6
	Susceptible	-	-
Cotrimoxazole	Resistant	26	57.8
	Intermediate	11	24.4
	Susceptible	5	11.1
Tetracycline	Resistant	22	48.9
	Intermediate	6	13.3
	Susceptible	17	37.8
Ciprofloxacin	Resistant	10	22.2
	Intermediate	3	6.7
	Susceptible	32	71.1
Gentamycin	Resistant	29	64.4
	Intermediate	8	17.8
	Susceptible	8	17.8
Meropenem	Resistant	32	71.1
	Intermediate	10	22.2
	Susceptible	3	6.7
Augmentin	Resistant	-	-
	Intermediate	3	6.7
	Susceptible	42	93.3
Vancomycin	Resistant	-	-
	Intermediate	-	-
	Susceptible	45	100
Cefuroxime	Resistant	38	84.4
	Intermediate	6	13.3
	Susceptible	1	2.2
Erythromycin	Resistant	33	73.3
	Intermediate	7	15.6
	Susceptible	5	11.1

The worrying aspect of the current study is that 16 (35.6%) of the isolates were multidrug-resistant (Table 4). Interestingly, all the isolates were susceptible to Vancomycin (Tables 3). Also, almost all isolates were susceptible to Augmentin (42/45, 93.3%).

Table 4. *Multidrug Resistant Pattern of MRSA isolated from raw meat.*

Antibiotics	No of isolates with resistance profile (N) in %	Resistance category
Pen-Clo-Amp	10(22.2)	Drug resistant
Pen-Clo-Amp-Ery	4(8.9)	Multi-drug resistant
Pen-Clo-Amp-Ery-Gen	2(4.4)	Multi-drug resistant
Pen-Clo-Amp-Ery- Gen-Cot	2(4.4)	Multi-drug resistant
Pen-Clo-Amp-Ery-Gen-Cot-Tet	2(4.4)	Multi-drug resistant
Pen-Clo-Amp-Ery-Gen-Cot-Tet-Cip	2(4.4)	Multi-drug resistant
Pen-Clo-Amp-Ery-Gen-Cot-Tet-Cip-Mer	1(2.2)	Multi-drug resistant
Pen-Clo-Amp-Ery-Gen-Cot-Tet-Cip-Mer-Cef	1(2.2)	Multi-drug resistant
Pen-Clo-Amp-Ery-Gen-Cot-Tet-Cip- Mer-Cef-Van	1(2.2)	Multi-drug resistant
Pen-Clo-Amp-Ery-Gen-Cot-Tet-Cip-Mer-Cef-Van-Aug.	1(2.2)	Multi-drug resistance

Key: Pen=Penicillin; Clo=Cloxacillin; Amp=Ampicillin; Ery=Erythromyin; Gen=Gentamycin; Cot=Cotrimoxazole; Tet=Tetracycline; Cip=Ciprofloxacin; Mer=Meropenem; Cef=Cefuroxime; Van=Vancomycin; Aug=Augmentin.

Discussion

Results of this study indicate that fresh raw meat sold at various retail points in the Cape Coast metropolis had some levels of microbial contaminations. The level of total viable count was quite higher in most markets although most were within the normal levels. The higher values could be as a result of contamination from the processing area, equipment used and also the means of transporting the meat to the market centres. The high total bacterial counts recorded in this study also showed the microbial diversity in these markets, condition of the market and the hygienic practice employed by meat sellers. Slaughtering of meat animals under unhygienic conditions, coupled with the use of contaminated water, unsterilized equipment and poor conditions of markets could have resulted in the increased level of total viable count in the meat [9]. Also, from the studies, it can be seen that Retail outlet 1 recorded the highest level of total viable count and this may be due to unhygienic handling of meat right from slaughtering to handling, transportation, and processing [10]. In most of the retail points, meats were seen on the ground and left to the mercies of the environment which can create an avenue for microbial pathogens to proliferate on it. These high bacterial loads could affect the average shelf life of the meats and increase the chances of spoilage.

According to theory of Tiemersma et al., Methicillin resistant *Staphylococcus aureus* (MRSA) has been implicated in food poisoning worldwide, which creates a more public health concern because of the potential for its transmission to humans [11]. Of the various raw meat surveyed, pork had the highest contamination rate in the Cape Coast Metropolis. The results of this study confirm the frequent occurrence of MRSA in pigs at slaughter [12-15]. MRSA is an important medical and public health bacteria because of their disease causing abilities. Research has shown that, staphylococcal toxin dose of less than 1 microgram in contaminated food can produce symptoms of staphylococcal intoxication [11]. With the high level of MRSA in the raw meat samples, the possibility exist that the amount of toxin that will be produced in these meat samples may be greater to even cause food poisoning. Since the bacterium occurs as a normal flora of the human and animal skin, the incidence of MRSA in the meat may suggests excessive human handling as observed in the study area [12]. This may reiterate the findings of Postgate who suggested that *Staphylococcus spp.* can be present on the skin of humans and animals and can be transmitted from person to product through unhygienic practices [13]. *Staphylococcus spp.* have been implicated in infections such as arthritis, black pox, boil, bronchitis, carbuncle, cystitis, endocarditis, meningitis, osteomyelitis, pneumonia, and scalded skin [14].

The emergence of resistant microorganisms such as MRSA associated with livestock and their meat is closely link to the inadequate use of antimicrobial agents in veterinary care. Also, International trade of food animals can facilitate the spread of resistant strains. Antibiotics have long been used to treat and prevent disease, and improve feed efficiency in conventional livestock and poultry production. The final end users of these animals are humans. The increasing dose of these antibiotics in these animals can end up in humans and lead to antibiotic resistance among most of the normal human flora. In relation to resistance patterns, this study was in agreement with the study by who recorded 58% resistant to more than five types of antibiotics [16,17]. The high resistant pattern in this study can be partly being due to antibiotic residue in these livestock and their meat. Unlike other previous studies, all the MRSA isolates in this study were somehow resistant to all the classes of antibiotics used in the study except for vancomycin, augmentin and ciprofloxacin. This study agrees with Sapkota et al. who reported that MRSA were susceptible to Ciprofloxacin and Vancomycin [18]. The present study showed the resistance of MRSA to penicillin, ampicillin, tetracycline, cefuroxime, gentamycin, meropenem and cotrimoxazole. This agrees with the findings of who reported resistance of MRSA to penicillin (94%) and tetracycline (73.8%) around Addis

Ababa [19]. This also confirms the fact that MRSA is resistant to all β- lactam antibiotics.

Conclusion

Although phenotypically detected, the higher prevalence of MRSA isolates from raw meat in most of the retail outlets in the Cape Coast metropolis gives a warning signal for possible occurrence of food borne infections capable of producing outbreaks. The distribution pattern of MRSA on positive carcasses has to be confirmed with more carcasses, potentially supported by the quantification of MRSA in the carcass samples and by using Molecular means.

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