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MULTIPLE DRUG RESISTANCE *STAPHYLOCOCCUS. AUREUS* ISOLATED IN FOODS OF ANIMAL ORIGIN IN NAIROBI, KENYA
J. M. Mathenge, HND, MSc, J. M. Mbaria, BVM, MSc, PhD, Department of Public Health Pharmacology and Toxicology, University of Nairobi, P.O. Box 29053, Nairobi, Kenya, P. O. Okemo, BSc, MSc, PhD, Department of Microbiology, Kenyatta University, P.O. Box 43844-00100 Nairobi, Kenya, P. M. Ng'ang'a, BSc, MSc, Division of Vector-Borne and Neglected Tropical Diseases, Ministry of Health, P.O. Box 19982–00202, Nairobi, Kenya and M. M. Gicheru, BVM, MSc, Department of Zoological Sciences, Kenyatta University, P.O. Box 43844-00100 Nairobi, Kenya.

MULTIPLE DRUG RESISTANCE *STAPHYLOCOCCUS. AUREUS* ISOLATED IN FOODS OF ANIMAL ORIGIN IN NAIROBI, KENYA

J. M. MATHENGE, J. M. MBARIA, P. O. OKEMO, P. M. NG'ANG'A and M.M. GICHERU

ABSTRACT

***Background*: *StaphylococcuS. aureus* is the most important agent, which is known to cause a wide range of diseases in both human and animals. Extended use and misuse of antibiotics in agriculture, stock farming and in the treatment of human diseases, has contributed to the rapid increase of the number of bacteria that are resistant to antimicrobial agents.**

***Objective*: To determine the occurrence of *S. aureus* in foods of animal origin and their reactions to commonly used antibiotic.**

***Design*: A descriptive, cross-sectional, quantitative study.**

***Setting*: Central Business District of Nairobi (CBDN) and its environment.**

***Subject*: Samples of meat (n=280) and dairy products (n=140) were randomly purchased from various butcheries and supermarkets. Additional 251 samples of various pork products were also collected randomly from a nearby pig processing plant for comparison purposes. Baird-Parker agar with 2% egg yolk tellurite emulsion was used as growth medium for isolation of *S. aureus*. The typical culture confirmed positive of *S. aureus* were tested for antibiotic susceptibility to eight commonly used antibiotics using the disc diffusion method.**

***Result*: Occurrence of *S. aureus* was 36.2% (152/420) and 39.4% (99/251) from the food outlets and meat processing factory respectively. Proportions of contamination from the two sources were not significantly different (p=0.400). Significantly, more contamination was observed in meat products (40.7%) compared to dairy products (25.0%) (p=0.001). Penicilin G (246; 99.6%) was the most resisted antibiotic followed by Ampicillin (230; 93.1).**

***Conclusion*: The results of this study confirms that multi antibiotics resistant *S. aureus* strains are present not only in hospital setups, but also widespread in foods of animal origin.**

INTRODUCTION

Worldwide, *S. aureus* is recognised to cause a wide variety of diseases in humans and animals, in varying degrees of severity(1). The microorganism is known to produce a wide variety of exotoxins and other determinants of virulence that contribute to its pathogenicity. Staphylococcal enterotoxins (SEs) are recognised as being the most important virulence factors involved in cases of food poisoning in humans. Most of the common skin infections are caused by these pathogens. Conditions such as tissue infections, wound and joint may progress with these bacteria if not treated(2). Food containing one or more preformed staphylococcal enterotoxins when ingested may cause food poisoning. Symptoms of SFP emerge within a few hours (from 30 min to 8 h) and may include vomiting, nausea, diarrhoea and abdominal cramps(1).

In recent times, bacterial antimicrobial resistance is of global concern. This has been aggravated by the fact that only few new antimicrobial agents come out of research in pharmaceutical products(3). Extended use and misuse of antibiotics in agriculture, stock farming and in the treatment of human diseases, has contributed to the rapid increase of the number of bacteria that are resistant to antimicrobial agents. In the last decade, bacteria isolated from foods have shown a considerable increase in resistance against most antibiotics including methicillin(4).

Antibiotic-resistant *S. aureus* isolates pose a severe challenge to both veterinary and human health professions, because they have a negative impact on therapy(5). *S. aureus* can express antibiotic resistance genes either in chromosomic or in plasmidic DNA. In the case of plasmidic resistance, *S. aureus* cannot conjugate with another cell because of lack of ''sexual pili'' which are usually present in Gram negative bacteria. Fagic transduction or membrane binding are necessary in such a condition(6). Increased frequency of methicillin resistant *S. aureus* (MRSA) associated with nosocomial infections, and their tendency to be multidrug resistant (MDR), has been reported leading to reduced effectiveness of antibiotics and growing healthcare costs, making the pathogen a major public health concern(7). Conventional foods, such as meat, milk, and vegetables can be potential sources of *S. aureus*. However, published reports on the antibiotic resistance of *S. aureus* isolates in different kinds of foods in Kenya are limited. The present study was performed in order to investigate the antibiotic resistance of *S. aureus* isolated from foods of animal origin collected from various supermarkets and other food outlets in Nairobi, Kenya.

MATERIALS AND METHODS

Samples collection: Six hundred and seventy one **(**671) samples of food including meat and dairy products were used in this study. Out of these, 420 samples comprising of meat products (n=280) were purchased randomly from various butcheries and supermarkets (80 beef chunks, 40 pork, 60 fish, 40 sausage, 60 poultry carcasses). Likewise, dairy products (n=140), which included 40 raw milk, from Supermarket dispensers, 60 packet of yoghurt and 40 samples of pasteurized milk were purchased from, retail shops and supermarkets in Nairobi City and its surrounding. Additionally 251 samples were collected from a meat processing plant for comparison purposes. These comprised of 79 finished pork products, 19 cooked salami, 20 fresh sausages, 20 uncooked ham, 20 Hot dogs, and 172 raw pork. All samples collected were aseptically, placed in sterile plastic bags, and transported to the laboratory in cool boxes at 4^{o}C.

Isolation and identification of StaphylococcuS. aureus: For each sample, 10 grams of the product were weighed and transferred into a sterile plastic bag and 90ml of 0.1% sterile peptone water added. Each sample was homogenised for 2min in a stomacher (Elekta Ltd., Japan 400). Homogenates of 0.1mls of the sample were pipeted and spread plated on Baird-Parker agar with egg York Tellurites Emulsion (Himedia M 043). For the dairy products, samples of approximately 10 ml were collected using sterile sample collection bottles and 0.1ml aseptically pipeted and spread on Baird-Parker agar. After incubation at 37^{o}C, suspected *S. aureus* colonies which had convex, black, shiny appearance with narrow white margin surrounded by clear zone were identified and preserved. Five typical preserved colonies with similar morphologies were isolated and cultured separately in blood Agar plates. These colonies were confirmed to be *StaphylococcuS. aureus* through Gram staining, coagulase test, catalase test, DNase test, anaerobic utilisation of glucose and use of mannitol as described by Bennett and Lancette (2001). All the isolated bacteria were kept frozen at -70^{o}C until the time of use.

Determination of antimicrobial resistance properties of S. aureus: Mueller-Hinton agar (HiMedia Laboratories, Mumbai, India) was prepared as per the manufacturer's instructions. The medium was cooled to 45-50^{o}C and poured into the Petri dishes to a depth of approximately 4mm, set on a level surface and allowed to cool. After solidification, the plates were stored at 4^{o}C until the time of use. Colonies from overnight culture of staphylococcal isolates were transferred to 5 ml tube of sterile normal saline. A sterile swab was dipped into the inocula and, care taken to express excess broth from the swab, by pressing and rotating the swab firmly against the side of the tube above the level of the fluid then a sterile Mueller-Hinton agar plate was swabbed with the inoculums. The plate was rotated through an angle of 60^{o} as the swab was rubbed over the surface of the medium three times after each application. The swabbed plate was left for four minutes to dry at room temperature with the lid closed.

The antibiotic impregnated disks used were Penicillin G (1ug), Minocycline (30ug), Erythrmycin (15ug), Methicillin (5ug), Co-trimoxazole (25ug), Choramphenicol (30ug), Ampicillin (10ug) and Lincomycin (2ug). The methods were done in accordance with the National Committee for Clinical Laboratory (NCCLS, 2006). Discs were warmed to room temperature, and then dispensed on the agar surface. They were gently pressed down on the surface of the Mueller-Hinton Plates with sterile forceps. The plates were incubated within 30 min for 18-24h at 37^{o}C. After incubation, the diameters of complete zones of inhibition were noted, measured in millimeters and interpreted as per the NCCLS (2006) standards. A standard strain of *S. aureus* (ATCC 25923) was used as a control. For typing purposes, a code profile was established based on antibiotic susceptibility of each isolate (NCCLS, 2006).

Statistical analysis and presentations: Data were entered using Microsoft Excel® and analysed by IBM SPSS Statistics® 21.0 (IBM Corporation, New York). Statistical analyses involved computation of appropriate descriptive statistics. Associations between the variables were assessed by use of Chi-square ($\chi 2$) tests. The threshold for statistical significance was set at $p<0.05$.

RESULTS

Isolation of S. aureus: Of the 671 samples examined, 37.4% were contaminated with *S. aureus (Table 1)*. Overall, 36.2% and 39.4% of the samples collected from, the food outlets and processing factory were contaminated with *S. aureus,* respectively. The proportions of contamination of animal products from the two sources were not significantly different (p=0.400). Analysis of *S. aureus* contamination by type of product revealed that significantly more contamination was observed in meat and meat products (40.7%) than in the dairy products (25.0%) at p=0.001.

Antibiotic resistance pattern of *S. aureus* isolated showed various results with the highest percentage of resistance to Penicilin G (246; 99.6%) followed by Ampicillin (230; 93.1%) (Figure 1). Additionally, antibiotics which were least resistant (<50%) included cotrimoxozole (89; 36%), Methicilin (67; 27.1%), Minomycin (60; 24.3%), Lincomycin (39; 15.8%) and erythromycin 31(12.6%). The highest sensitivity was observed in chloramphenical (52.2%) with a low prevalence of resistance of 4% for all the *S.aureus* isolates. Low levels of resistance were also observed with only 10(4.0%), 31(12.6%) and 39(15.8%) *S. aureus* isolates being resistant to, chloramphenical erythromycin and lincomycin respectively. Methicillin resistance was found in 67(27.1%) *S. aureus* isolates.

Table 1

Distribution of S. aureus contamination

Characteristic	Total number of *S. aureus* samples	-ve (n=420)	+ve (n=251)	p-value
Source				
Seller	420	268(63.8%)	152(36.2%)	0.400
Processor	251	152(60.6%)	99(39.4%)	
Type of animal product	(n=671)	(n=420)	(n=251)	
Meat (products)	531	315(59.3%)	216(40.7%)	0.001
Dairy products	140	95(67.9%)	35(25.0%)	

Figure: 1

Antibiograms pattern for S. aureus isolates from foods of animal origin

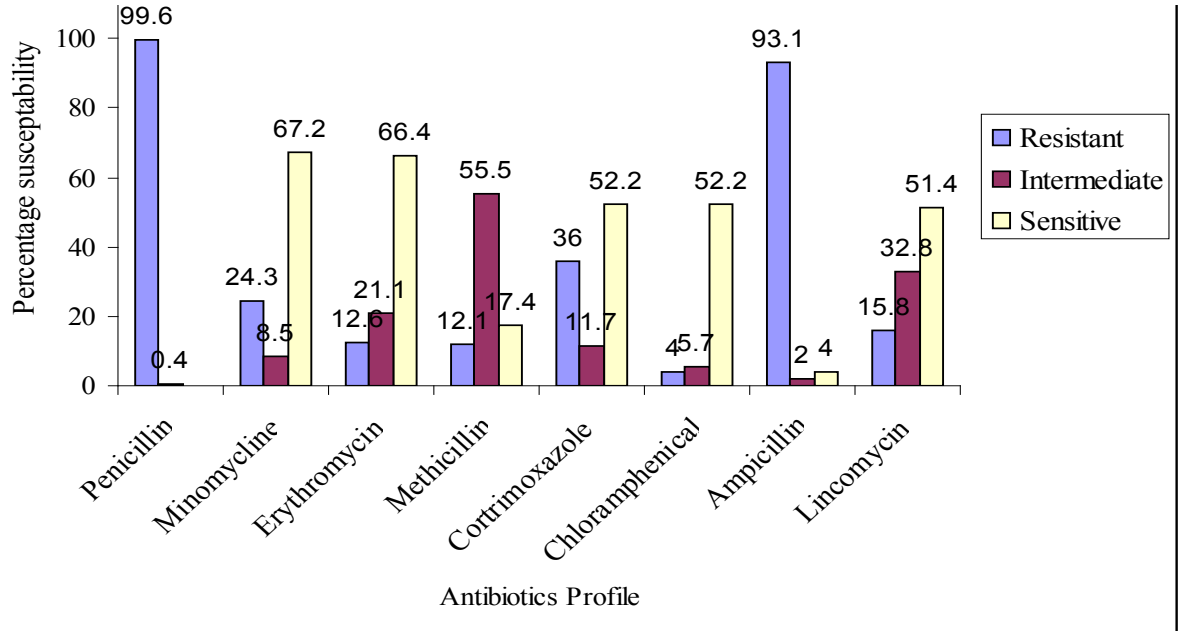

Table 2 gives a summary of Multi antibiotic resistance (MAR) of *S. aureus* phenotype isolated from samples of food of animal products the study area. The most predominant multi antibiotic resistant phenotype were Pen/Amp and Pen/Cot/Amp in 49 (19.8%) and 32 (32%) respectively. Other phenotypes include Pen/Met/Amp, Pen/Min/Amp with 11.75% each and Pen/Cot/Met/Amp with 10.5%. Only one phenotype Pen/Min/Ery/Cot/Amp/Lin (1; 0.4%) was observed from the study.

Table 2

Multiple antibiotic resistant phenotypes for S. aureus

Phenotype	No. of strains (n=247)	Percentage
Met	1	0.4
Pen	6	2.4
Pen/Amp	49	19.8
Pen/Amp/Lin	13	5.3
Pen/Cot	2	0.8
Pen/Cot/Amp	32	13.0
Pen/Cot/Amp/Lin	3	1.2
Pen/Cot/Chl/Amp	2	0.8
Pen/Cot/Chl/Amp/Lin	1	0.4
Pen/Cot/met/Amp	26	10.5
Pen/Ery/Cot/Amp	9	3.6
Pen/Ery/Cot/Amp/Lin	1	0.4
Pen/Ery/Met/Cot/Amp	5	2
Pen/Lin	3	1.2
Pen/Met/Amp	29	11.7
Pen/Met/Amp/Lin	1	0.4
Pen/Met/Cot/Chl/Amp	3	1.2
Pen/Min	2	0.8
Pen/Min/Amp	29	11.7
Pen/Min/Amp/Lin	6	2.4
Pen/Min/Chl/Amp	3	1.2
Pen/Min/Cot/Amp	2	0.8
Pen/Min/Ery	1	0.4
Pen/Min/Ery/Amp/Lin	7	2.8
Pen/Min/Ery/Amp	6	2.4
Pen/Min/Ery/Cot/Amp/Lin	1	0.4
Pen/Min/Ery/Cot/Lin	1	0.4
Pen/Min/Lin	1	0.4
Pen/Min/Met/Amp	1	0.4
Pen/Cot/Met/Amp/Lin	1	0.4

Pen=Pencilin; Min=Minomycin;
Ery=Erythromycin;
Cot=Cotrimoxazole;
Amp=Ampicilin;
Lin=Lincomycin;
Met=Methicilin;
Chl=Chlorampenical.

DISCUSSIONS

Ability to develop and expand resistance to a broad spectrum of antibiotics by *S. aureus* has been demonstrated(8). Use of antibiotics for both prophylaxis and as growth promoters in animals, contributes to the development of antibiotic resistant bacteria(9). Virulence of *S. aureus* has increased in recent years due to extensive use of antibiotics, which allows the bacteria to acquire multi resistance genes. This makes the organism to resist almost all antibiotics families(10). Multi-drug resistance *S. aureus* in food samples have been reported in various studied(11). In the present study the highest percentage of resistance was against Penicillin G (99.6%) followed by Ampicillin (93.1%). The results of Penicillin being the most resisted is not surprising since the drug has for a long time been used for the treatment of both human and animal diseases. Isolates of *S. aureus*, which are Penicillin resistant, have been reported in various food sources such as meat, dairy products and poultry(12).

Staphylococci resistance to several therapeutically useful antibiotics, including, Novobiocin, Fusidic acid, Rifampicin, and streptomycin is thought to be derived from chromosomal mutation (13). This study observed the resistance patterns of *S.aureus* to eight antimicrobial agents, with 247 (96%) isolates being resistant to at least one antibiotic. *S. aureus* isolates were resistant to three, (42.1%) four, (24.3%) and two (22.7%) antibiotics respectively. The analysis revealed that additionally, seven (2.8%) and nineteen isolates (7.7%) were resistant to one and five antibiotics respectively with only one isolate resistant to six antibiotics. The resistance pattern of *S. aureus* isolated from commercial dairy products to 12 antimicrobial agents showed that most of the *S. aureus* isolates (95.0%) were resistant to one or more antimicrobial agent(14). Three isolates (15.0%) were resistant to a single antibiotic and seven isolates (35.0%) showed resistance to two antimicrobial agents. Multi resistance, which was defined as resistance to three or more of antimicrobial agents tested, was found in 45.0% of *S. aureus* isolates. A total of 23.9% clinical *S. aureus* isolates were shown to be resistant to at least one or more antimicrobial agents, but 9.9% of clinical isolates showed multiple resistances (≥3 antibiotics(15).

Results have indicated that resistance (resistance and intermediate resistance) of the antibiotic, Ampicillin was the most common (54.3%)(16). *S. aureus* is known to produce β-lactamase, which breaks down the β-lactam ring and makes most of the antibiotics ineffective. Developments of resistance to families in these antibiotics can results in infection with strains of *S. aureus* that are difficult to treat. Penicillin resistant *S. aureus* in foods is becoming extremely common in many countries. Reports by many other investigators have shown similar evidence(17,18).

From the current study, results demonstrated that the most predominant multi antibiotic resistant phenotype were Pen/Amp and Pen/Cot/Amp in 49 (19.8%) and 32 (32%) respectively with only one phenotype resistant to Pen/Min/Ery/Cot/Amp/Lin (1; 0.4%) obtained. In Ethiopia, the most predominant multi antibiotic resistance phenotypes demonstrated for *S. aureus* were PG/AP/AC/E/CRO/Ox and PG/AP/Ox in 19.2% and 20.5% of the isolates, respectively(19). In Kenya, most frequent resistance was in Penicillin (72.2 %) followed by Trimethoprim + Sulfamethazin (59.2 %); tetracycline (57.9 %); Chloramphenicol (46.8 %) Erythromycin (21.3 %); and Methicillin (7.8 %)(20). Multi drug resistances in poultry have been reported in various countries such as Italy, United States and Ireland (11). Antibiotics such as Penicillin, Tetracycline, and Oxytetracycline are the most prevalently used in veterinary medical treatment and animal feed additives. This can provide selective pressure in the development and propagation of resistant bacteria (21). Dissemination of resistant into humans is through contact with animal or consumption of foods products with resistance strains of *S. aureus*.

Erythromycin resistance was low in comparison with the other antibiotics tested. Despite the fact that 31(12.6%) of *S. aureus* isolated from foods of animal origin samples were resistant to erythromycin, it was evident from our results that this antibiotic was not frequently used in animals by large-scale farmers. Strains of *S. aureus* obtained from chicken (33.3%), minced calf meat (14%) and chicken carcass resistant to erythromycin had been reported(18). The occurrence of erythromycin resistance among poultry *S. aureus* was 24% and that the *ermA* gene was found in most erythromycin resistant *Staphylococci* in Denmark(12). Their results sugest that *eamA* genes might be introduced into the community through food chain. Chloramphenical was the drug to which small proportion of the isolates were resistant 10 (4.0 %). Similarly, a previous study reported 15.7% to 23.8% of *S. aureus* isolated from meat and dairy products resistant to Sulfamethoxazole respectively(22,12). Chloramphenical is not commonly used in livestock farming in Kenya, which reduced much contact with this antibiotic hence, reduction of resistance. The results presented herein are similar to previous studies in which Gram-positive bacteria were found to be generally susceptible to Vancomycin, Methicillin, Chloramphenicol, Cephalotin and Ciprofloxacin(17, 18). These drugs are no longer used in veterinary medicine in many countries, which may account for the observed results(23).

In conclusion, many of the strains in this study have multiple resistances to antibiotics and almost 100% resistance to penicillin G (99.6%) was

observed, followed by Ampicillin (93.1%), and Cotrimoxazole (36%). High levels of multi antibiotic resistance to two drugs (22.7%), three (42.1%) and six (0.4%) was obtained. Based on this, antibiotic resistance of *S. aureus* in food is increasing, which is a concern to human health in Kenya. The Kenyan government should therefore enforce laws that prohibit indiscriminative use of antibiotics to prevent development of multi antibiotic resistant strains of *S. aureus* in food. Genes coding for antibiotic resistance will be reported in future communication.

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