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Prevalence and Antibiotic Resistance of *Staphylococcus aureus* Isolated from Beef Carcasses at Abattoirs in North West Province

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ABSTRACT *Staphylococcus aureus* is notorious for causing human diseases, and is primarily associated with the consumption of contaminated meat and meat products. The aim of this paper was to determine the prevalence and the antibiotic resistant profiles of *Staphylococcus aureus* on beef carcasses isolated from different abattoirs in North West Province. A total of 600 swab samples were collected from beef carcasses, and cultured on Mannitol salt agar (MSA). The isolates were confirmed by morphological identification and biochemical tests. A total of 159 (26.5%) samples were contaminated with *S. aureus*. All the *S. aureus* isolates showed high susceptibility to Chloramphenicol (30μg), Gentamicin (10μg) and Tetracycline (10μg). However, all isolates were highly resistant to Penicillin (10μg), Ampicillin (30 μg) and Oxytetracycline (10μg). The study confirms the presence of *S. aureus* in beef carcasses, which might be a potential threat to the consumer's health.

INTRODUCTION

Staphylococcus aureus is one the most prevalent causes of clinical infections globally and has garnered substantial public attention due to increasing mortality associated with multidrug resistance (Kwon et al. 2006). Moreover, it is one of the most common causes of foodborne infections in most of the countries around the world (Kwon et al. 2006; Pereira et al. 2009). It is well known that the organism produces various extracellular active substances, such as coagulase, hemolysins, nuclease, acid phosphatase, lipase, protease, fibrinolysin, enterotoxins and toxic shock syndrome toxins. These active substances are thought to contribute to the pathogenicity of the organism (Aydin et al. 2011). It has been reported that strains of *S. aureus*, which had strong proteolytic activity, were isolated from birds like chickens suffering from edematous and necrotic dermatitis (Karmi 2013).

In meat, *S. aureus* can grow to sufficient level to allow a toxic dose of enterotoxin to be produced before consumption. Therefore, the level of contamination of meat is to be kept as low as possible during the production process, because of the enterotoxin thermo-stability (Euzeby 2003; Jay et al. 2005). The presence of *S. aureus* in products for human consumption is important to the food industry, as some strains are the cause of foodborne intoxication (Ruhe and Menon 2006; Aydin et al. 2011). The presence of *S. aureus* in meat is often attributed to inadequate hygiene during handling by the individuals involved in the production of meat (Pereira et al. 2009). Contamination with *S. aureus* is important in the evaluation of the safety and hygienic quality of meat, and also in determining the origin of food poisoning (Sasidharan et al. 2011; Karmi 2013).

Antibiotics are used to treat bacterial infection (Er et al. 2013). Despite obvious benefits, improper use of different classes of antibiotic causes bacterial resistance against infectious diseases causing agents (Nisha 2008; Er et al. 2013). The prevalence of antimicrobial drug resistance among foodborne pathogens is increasing due to its use in human therapy and animal farming for therapeutic purposes. Therefore, it is more difficult to treat multidrug resistant *S. aureus* as compared to drug susceptible strains (Saleha and Zunita 2010). Methicillin resistant *S. aureus* (MRSA) is responsible for a huge number of hospital-acquired infections. MRSA is

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equally associated with human and veterinary infections (Sasidharan et al. 2011).

Both developed and developing countries are largely affected by foodborne infections. Foodborne diseases not only affect people's health and well-being, but also have economic impacts on the individuals and the countries (Sherertz et al. 2001). The objective of this study was to determine the prevalence of *S. aureus* on beef carcasses using biochemical tests and also to determine their antibiotic resistant profiles of isolates from different abattoirs in North West Province, South Africa.

MATERIAL AND METHODS

Area of the Study

This study was carried out in the North West Province of South Africa, which lies between latitude 25 and 28 degrees south of the equator and 22 and 28 degrees longitude east of the Greenwich meridian and the its temperatures ranges from 17° to 31°C in summer and from 3° to 21 °C in winter. This province was officially created after the end of the apartheid era in 1994, and includes parts of the former Transvaal and Cape Province, as well as most of the former Bantustan of Bophuthatswana. It has four districts, namely, Bojanala Platinum, Ngaka Modiri Molema, Dr. Ruth Segomotsi Mompati and Dr. Kenneth Kaunda. Samples were collected from four abattoirs in the province and in consideration of confidentiality the abattoir are herein referred to as abattoir A, B, C and D in this paper.

Sample Collection

A total of 600 samples were collected with swabs from the beef carcasses. 500 samples were collected from three high throughputs, whilst the remaining 100 were collected from a low throughput as shown in Table 1. Bio-culture transport media swabs were used to collect samples from the inside and the outside of the beef carcasses after evisceration. The swabs were rubbed on the sites continuously for 30 seconds and transferred to a capped tube with transport media. The swabs were immediately transported on ice to the Animal Health Microbiology Laboratory at the North West University Mafikeng Campus for analysis.

Table 1: Abattoirs, number of samples and number of visits

Abattoir	*Abattoir classification*	*(n)*	*N of visits*
A	Low throughput	100	3
B	High throughput	100	1
C	High throughput	200	1
D	High throughput	200	2

N=number of samples

Bacteria Identification

Cellular Morphology

The morphology of presumptive *S. aureus* isolates was determined by Gram-staining using a standard technique. All yellowish colonies that were Gram-positive cocci were subjected to the catalase test (Fig. 1).

Fig. 1. Golden yellow colonies of *S. aureus*

Biochemical Methods Used for Identification of Isolated Bacteria

The Catalase Test

The catalase test is important in distinguishing Streptococci (catalase-negative) Staphylococci, which are catalase positive. The test was performed according to Haghkhah (2003). A bacterial colony was transferred to a glass slide with a wire loop and one drop of three percent hydrogen peroxide was added to the culture. The presence of bubbles was indicative of the catalase enzyme in the bacteria, the positive samples were presumptively considered to be *Staphylococcus* spp.

Coagulase

Two coagulase methods (coagulate tube and slides) were used to differentiate *S aureus* from coagulase negative staphylococci, and the main

aim of using two different methods was for validation of the results. Testing was performed according to the tube and slide methods (Goja et al. 2013).

Analytical Profile Index (API) Staph

The samples that tested positive for the coagulase test were subjected to API Staph test. The test was performed according to the manufacturer's instructions (BioMérieux, Marcy-L'Etoile / France). A bacterial suspension was added to a set of wells containing dried substrates for 26 colorimetric tests. After 24 hours of incubation at 37°C and the addition of a few other reagents, the reactions were read according to the reading table and the identification was obtained by using API web TM identification software.

Antibiotic Susceptibility Test

The antibiotic susceptibility test was performed according to the according to the Kirby-Beuer disk method (Magiorkas et al. 2012). Fresh overnight cultures were prepared and used for antibiotic sensitivity tests, and the discs diffusion method was used. A 6 mm filter paper disk impregnated with a known concentration of an antimicrobial compound was placed on a Mueller Hinton Agar (Biolab, supplied by Merck) plate and incubated aerobically at 37°C for 18 to 24 hours (Ateba et al. 2010). After 24 hours, the antimicrobial begins to diffuse into the surrounding agar. The rate of diffusion through the agar is not as rapid as the rate of extraction of the antimicrobial out of the disk. Therefore, the concentration of antimicrobial is highest closest to the disk. An inhibition zone diameter was measured and values obtained from the National Committee on Clinical Laboratory Standards (NCCLS 2006) were used to interpret the results obtained. Six antibiotic disks were used for sensitivity tests, namely, chloramphenicol, gentamicin, penicillin, ampicillin, oxytetracycline and tetracycline. Some of these antimicrobial agents selected in previous studies (Ateba et al. 2010) have shown that large numbers of bacteria isolated from communal farms in the North West province were resistant to them (Table 2).

Table 2: Antibiotics used for *S. aureus* with its disc content

Antibiotic	*Abbreviation*	*Antibiotic concentrations disc(ìg)*
Ampicillin	AMP	(10 ìg)
Tetracycline	TE	(30 ìg)
Chloramphenicol	C	(30 ìg)
Gentamycin	CIP	(10 ìg)
Oxytetracycline	OX	(30 ìg)
Penicillin	PG	(10 ìg)

RESULTS

Results of the Gram Staining and Biochemical Test

Out of the total of 600 samples of swabs from beef carcasses, 159/600 (26.5%) were positive for *S. aureus.* 159 isolates satisfied all the identification criteria and were used for subsequent analysis. The results demonstrated the presence of *S. aureus* in all beef samples regardless of the abattoir settings.

The Gram Staining

The 600 colonies were subjected to a Gram staining and the cells were analyzed under a microscope. The cells that were gram positive were appeared dark purple. However, if the cell was gram negative, it was pink. The gram stain was also used to identify bacterial shape, as shown in Table 3, and 484 colonies were gram negative and with cocci shape. Those colonies were subjected to suitable biochemical methods (catalase, coagulase and API-staph).

Detection Using Biochemical

The Catalase Test

This test was performed to differentiate *Staphylococcus* and *Streptococcus* species, as both are Gram-positive cocci bacteria. *Staphylococcus* spp are catalase positive whilst *Streptococcus* spp are catalase negative. Out of 484 samples, only 419 reacted positively by forming bubbles on the slide.

Coagulase

Both coagulate tube and slides were used to differentiate *S. aureus* from coagulase negative staphylococci. From a total of 419 colonies, 159 reacted positively to this test. This test led to API staph test, which was used as an additional confirmation.

API Staph

After getting positive results from the coagulate test, all the samples were further tested

Table 3: Different types of biochemical tests and sensitivity test

Abattoir	*G-stain*		*Catalase*		*Coagulase*		*API-staph*		*Sensitivity*	
	(n)	*+ve*	*(n)*	*+ve*	*(n)*	*+ve*	*(n)*	*+ve*	*(n)*	*+ve*
A	100	74	74	72	72	53	53	48	48	48
B	100	68	68	57	57	24	24	20	20	20
C	200	166	166	128	128	54	54	41	41	41
D	200	176	176	162	162	53	53	50	50	50
Total	600	484	484	419	419	159	159	159	159	159

n= Number of samples, +ve= positive samples

using API Staph and all (159) colonies tested positive (Fig. 2).

Fig. 2. Bacteria identification using API staph

Overall and Abattoir Specific Prevalence

A total of 159 samples were positives for *S. aureus* after they were subjected to morphological identification, gram staining and biochemical test as shown in Table 4. The prevalence of *S. aureus* at the abattoir is shown in Table 3, of the 100 samples from abattoir A that were examined, 49 (49%) were contaminated with *S. aureus*. All the samples were collected from after washing carcasses. The distributions of contamination were noted during marking (abattoir A). It was observed that marking workers extensively handle carcasses during pushing them on line. At abattoir B, prevalence rate was twenty percent of the 100 samples examined. The procedure for collecting samples was the same as the one for abattoir A. While abattoir C, *S. aureus* was isolated from 41/200 (20.5%) samples. At abattoir D, *S. Aureus* was isolated from 50 samples out of 200 samples (25%). The collection of the samples was the same as the one in abattoir A.

Table 4: Prevalence of pathogenic S. aureus isolated from beef carcasses

Abattoirs	*Analysed samples*	*+ve samples*	*Prevalence (%)*
A	100	48	48
B	100	20	20
C	200	41	21
D	200	50	25
Total	600	159	26.5

+ve= positive samples

Antimicrobial Susceptibility of *S. aureus*

A total of 159 confirmed *S. aureus* isolates obtained from the study were subjected to antibiotic sensitivity tests. The antibiotic susceptibility profiles were determined using six antimicrobial agents. The antimicrobial sensitivity and resistance pattern of all *S. aureus* isolates were studied (Table 5). The results of the antimicrobial susceptibility test showed that isolates were highly susceptible to oxytetracycline

Table 5: Antimicrobial susceptibility profile of *S. aureus* isolated from beef carcasses

Antimicrobial	*Drug concentration (ì g)*	*Susceptibility*		*Resistance*		*Intermediate*	
		(n)	*(%)*	*(n)*	*(%)*	*(n)*	*(%)*
Oxytetracycline	10	50	8	5	8	5	-
Ampicillin	10	25	16	10	10	10	-
Penicillin	10	25	24	15	15	15	-
Tetracycline	30	22	22	13	13	13	12.6
Chloramphenicol	30	16	28	18	18	18	-
Gentamycin	30	12	52	33	33	33	-

n= Number of samples

(31.4%), ampicillin (15.7%), and penicillin (15.7%). 17.6 percent and 13.8 percent of isolates showed resistance to chloramphenicol and tetracycline, respectively. The highest resistance obtained was against gentamicin (32.2%).

Antibiotic Test

The highly susceptible *S. aureus* was found more on chloramphenicol (30 μg), gentamicin (10 μg) and tetracycline (30μg), however, these isolates were highly resistant to penicillin (μg), ampicillin (30 μg) and oxytetracycline (10 μg). The susceptibility of antibiotic drugs were categorized as followed resistant, intermediate and susceptible (Table 6). A total of 26.5 percent (n=159) were contaminated with *S. aureus*. All the *S. aureus* isolates showed high susceptibility to chloramphenicol (30μg), gentamicin (10μg) and tetracycline (10μg), however, all isolates were highly resistant to penicillin (10μg), ampicillin (30 μg) and oxytetracycline (10μg) (Fig. 3).

Fig. 3. Inhibition zones for antimicrobial drugs

DISCUSSION

The objective of this study was to determine the prevalence of *Staphylococcus aureus* on beef carcasses isolated from different abattoirs in North West Province. The motivation behind was that it is a well-established fact that contaminated food is the main source of transmission of *S. aureus* leading to mortalities and morbidity, and the pathogen is one of the major causes of enteric disease in developing countries (Balaban and Rasooly 2000). *S. aureus* contamination of beef carcasses during slaughter is a major problem that leads to the final products that are unsafe and also cross-contaminating of other food products. The overall prevalence of 26.5 percent of *S. aureus* on beef carcasses was found in this study from four different abattoirs in North West Province. Abattoir A at the highest of forty-eight percent (48/100) followed by abattoirs D with twenty-five percent (50/200), then abattoir C at 20.5 percent (41/100) and the lowest prevalence was abattoir B at twenty percent (20/100) prevalence. This finding is high compared to other studies (Heo et al. 2008) that reported an eleven percent prevalence in Korea from raw beef, and the prevalence of *S. aureus* from humans was 52.3 percent, cattle 37.5 percent, sheep 42.5 percent and goats thirty percent. An overall isolation rate of 44.8 percent from a study conducted in Nigeria by Adamu et al. (2010) was higher than the prevalence rate obtained in this study.

The primary habitat of *S. aureus* is the mucous membranes of the human nasopharynx and animal skin (Genigeorgis 1989). *S. aureus* is also present in soil, water sources, dust and air (Hamann 1986). The presence of *S. aureus* in foods is often related to improper handling by personnel, who are frequently contaminated with these microorganisms (Hatakka et al. 2000). The presence of *S. aureus* in beef commonly indicates contamination that may be directly introduced into the carcases by workers, who have skin lesions containing *S. aureus*, or by sneezing or coughing (Jay 1986), or indirectly through working surfaces and knives (Yeh 2004). During sample collection it was additionally noted that some of the abattoirs hygienic standards were poor and approximately fifty percent of the human population carry *S. aureus* as commensals (Arbuthnott 1990). Other contamination sources of *S aureus* are soil, water, dust and air. Also, the different stages of slaughter also affect the prevalence and bacterial load of *S. aureus* on the carcass (Hansson 2001). Opinion on the effect of washing, on the distribution of bacteria is divided, with some researchers reporting no effect, while others report significant distribution of bacteria (Gorman et al. 1995). Based on observations made throughout the collection of samples, the researchers report that improper hygiene and poor abattoir management practices contributed to the presence of *S. aureus* on the carcasses.

A further objective of the study was to determine the antibiotic resistance profiles of *S. aureus*. Several *S. aureus* isolates from meat

samples were known to be frequently resistant to antibiotic therapy due to their capacity to produce an exopolysaccharide barrier and because of their location within micro abscesses, which limit the action of drugs (Gundocan et al. 2006; Virdis et al. 2010). In addition, because ninety-five percent of antibiotics given to livestock are excreted unchanged, bacteria living on people who have regular contact with animal waste or in the environment in close proximity to animal waste are constantly exposed to antibiotics and may develop resistance (Choi 2007). These people can then spread the resistant bacteria off the farm on their shoes or bodies. Consumer exposure to raw meat products could carry similar risks of infection with antibiotic resistant bacteria as are seen in farm and meat processing plant workers. Cross-contamination has been shown to allow bacteria from several animals to spread to processing plant surfaces (Fluckey 2009). *S. aureus* was resistant to multiple classes of commonly used antibiotics is a serious health problems (Tenover 2006). The *S. aureus* incidence at a considerable high percentage indicates the alarming situation both for abattoirs and for public health.

A total of 159 confirmed isolates of *S. aureus* were subjected to sensitivity test. All isolates were found resistant to penicillin, ampicillin and oxytetracycline as shown in Table 5, which means that the isolates are multiple drug resistant (MDR). The isolates from this study were resistant to penicillin, which is in agreement with a previous report (Ateba et al. 2010). According to the study by Kumar (2010), *S. aureus* was resistant to penicillin and as shown by Acco et al. (2003), seventy percent strains of *S. aureus* isolated from food handlers were resistant to penicillin, while in Brazil, Da Silva et al. (2004) found that sixty-four percent of the strains of *S. aureus* isolated from goat mastitis were susceptible to penicillin. From this study, *S. aureus* is resistant to penicillin, and this agrees with the results acquired by Kumar (2010). Also, in the study conducted in Hawassa area in South Ethiopia (Daka et al. 2012), the isolates were resistant to ampicillin and penicillin, which is an agreement with this study. However, *S. aureus* from this study were found to be the highly susceptible to chloramphenicol, gentamicin and tetracycline. From the study that was conducted in Nigeria (Adamu et al. 2010) with healthy humans and animals, isolates were resistant to gentamycin and chloramphenicol.

The resistance to most antibiotics rises, and modulation of virulence factor expression by antibiotic treatment may be of increasing importance (Gemmel et al. 2006). The increasing incidence of bacterial infections due to *S. aureus* and the rise in MRSA is of great concern. The antibiotics that were susceptible to chloramphenicol, gentamicin and tetracycline can be used as the effective drugs against staphylococcal infections (Table 5).

CONCLUSION

Staphylococci of animal origins exhibit resistance to a number of antimicrobial agents. Nevertheless, most of the published resistance rates of *S. aureus* from animals are lower than comparable resistance rates from clinical human isolates. There are a number of promising new drugs in clinical trials or are under development, including oxazolidinones, ketolides, glycylcyclines, but also new cephalosporins and fluoroquinolones. The finding of this study revealed that carcasses from the abattoirs were contaminated with pathogenic bacteria *(S. aureus*). The possible source of contaminants is due to the unhygienic manner of handling meat from the slaughters.

RECOMMENDATIONS

The slaughterhouses should be routinely investigated for hygiene of workers in connection with meat production and distribution must be routinely medically examined. The findings indicate that multidrug resistant *S. aureus* should be added to the list of antimicrobial resistant pathogens that routinely contaminate the food supply.

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