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QUANTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY PROFILES OF *STAPHYLOCOCCUS AUREUS* AND *BACILLUS CEREUS* STRAINS ISOLATED FROM BILTONG

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ABSTRACT

Biltong, an intermediate moisture meat product from South Africa, has been regarded as microbially safe because of its low water activity, low pH as well as the presence of curing salts. However, in this study, *Staphylococcus aureus* and *Bacillus cereus* counts from the biltong samples, in most cases, were above the infective dose limit and the South African national guidelines. Antimicrobial resistance of *S. aureus* and *B. cereus* on selected antibiotics was determined. Both species (*S. aureus* and *B. cereus*) were susceptible to oxytetracycline (74%; 80%), limcomycin (40%; 57%), bacitracin (65%; 58%), penicillin (53%; 63%), oxacilin (50%; 63%), methicillin (93%; 97%) and tetracycline (83%; 97%), respectively. *S. aureus* and *B. cereus* were resistant to nalidixic acid, and in general 47% and 33%, respectively, of the total tested strains of *S. aureus* and *B. cereus* were partially susceptible. It was encouraging to observe bacterial strains susceptible to antimicrobials used therapeutically in humans. However, the presence of nalidixic acid-resistant strains of *S. aureus* and *B. cereus* on biltong is of concern as resistance might spread to other bacteria and ultimately consumers.

PRACTICAL APPLICATIONS

Biltong is a traditional South African spiced intermediate moisture meat (IMM) product with increasing popularity as a commodity worldwide. However, biltong production in different regions of South Africa (S.A.) is often a home industry, and the safety of this commodity is of concern. Therefore, biltong safety has to be ensured before it can be exported anywhere in the world. Currently, studies conducted in S.A. investigating the safety of biltong were mainly in the northern region. Thus, more studies are required to update the current knowledge on the prevalence of bacterial foodborne pathogens associated with this product as well as its potential in spreading antibiotic resistance. This is the first report on the presence of nalidixic acid-resistant strains of *S. aureus* and *B. cereus* on biltong (produced in the central region of S.A.). This study contributes new knowledge to food processors in the central region of S.A. relating to the production of safe dried meat products.

INTRODUCTION

Biltong, an intermediate moisture meat (IMM), is regarded as a microbial safe meat product because of its low water activity, pH and the presence of curing salts (Jay *et al*. 2005). However, the numerous steps used during the production of this product may be a source of contamination if not executed properly (Calicioglu *et al*. 2002). Previous studies have shown that raw meat may contain some microbial strains that may be detrimental to both the consumers and the product (Nel *et al*. 2004; Shale *et al*. 2005). Recently, a study by Naidoo and Lindsay (2010b) indicated biltong as a potential vehicle for foodborne pathogens. Previous studies revealed that commercial biltong may contain high levels of spoilage organisms

as well pathogens such as *Salmonella* and toxin-producing *Staphylococci* spp. (Wolter *et al*. 2000; Mhlambi *et al*. 2010; Naidoo and Lindsay 2010a,b). These facts have implications for foodborne illnesses, which are mostly treated with antibiotics. In addition, studies have reported that antibiotic-resistant bacteria can survive on raw meat, leading to the transfer of these bacteria to the person who consumes the product (Phillips *et al*. 2002).

Antimicrobial resistance has emerged as a major public health concern as a consequence of the selective pressure exerted by the widespread use of antibiotics in medicine, agriculture and veterinary practices as well as of growth promoters in animal husbandry (Lowy 2003). The public health concern was exacerbated by possible treatment failures of hospitalized patients with certain kinds of infections and increased healthcare cost as newer and more expensive antibiotics would be needed to treat infections (Moreno *et al*. 2000; Normand *et al*. 2000; Berger-Bächi and McCallum 2006; Doyle and Erickson, 2006; Stevens *et al*. 2006; Uhland and Higgins 2006; Verhoef and Fluit 2006; Garofalo *et al*. 2007; Pesavento *et al*. 2007).

The improper use of antibiotics, such as inclusion in animal feed at low levels for growth promotion and at intermediate levels to prevent disease (Pezzotti *et al*. 2003; Florea and Nightingale 2004), can lead to the spread of antibiotic resistance (AR) (Summers 2002; Lowy 2003). This is of concern because studies have shown that bacteria can survive on raw meat and methods used to preserve biltong are sometimes insufficient (Phillips *et al*. 2002; Naidoo and Lindsay 2010a,b). Summers (2002) reported that consumption of antibiotics does not only affect pathogens but also commensal bacteria. The latter has to develop resistance to survive, thus becoming a resistance reservoir. This leads to the transfer of resistance genes from commensal bacteria to pathogens when they enter the body again (Summers 2002; Mathur and Singh 2005, Normanno *et al*. 2007; Pesavento *et al*. 2007). Further studies showed that pathogens and other bacteria can be transmitted from animals to human beings via the food chain, and if these bacterial strains possess antibiotic resistant genes, then AR can be spread from animals to people (Singer *et al*. 2003; Phillips, 2007).

According to Wright and Sutherland (2007), nosocomial organisms such as *Staphylococcus aureus*, commonly associated with infections in the community and hospitals, readily acquire antibiotic-resistant gene clusters through resistance plasmids and other mobile genetic elements. Naidoo and Lindsay (2010b) found *Bacillus* and *Staphylococci* as dominant populations of microflora on biltong. *Staphylococci* are among the most important nosocomial pathogens because of the diversity and the severity of the infections they cause, including a variety of toxin-mediated diseases, such as gastroenteritis (Jones 2001; Brown and Ngeno 2007; Normanno *et al*. 2007; Stradén *et al*. 2009).

The increase in the number of *S. aureus* strains that exhibit antimicrobial resistance properties has led to the potential risk of transmitting the same properties to the human microbiota via foods such as meats, or of inducing infections that are difficult to treat (Teuber 1999; Van Der Mee-Marquet *et al*. 2004; Normanno *et al*. 2007; Pereira *et al*. 2009). The presence of *S. aureus* on biltong suggests poor hygiene of food handlers as humans are primary sources of *Staphylococcus* spp. *Bacillus cereus*, a spore-forming rod that is ubiquitous in the environment and has been quantified on a wide variety of foods including meats (Ozkocaman *et al*. 2006). AR in *B. cereus* has been reported to be due to the presence of β-lactamases (Fenselau *et al*. 2008). Spices used in the curing of meat products have been classified as vectors of various microorganisms such as *B. cereus*, thus implicating possible health problems and transfer of resistance to humans (Banerjee and Sarkar 2004).

Multiresistance has developed and has spread among the bacterial community in a very short evolutionary time. Resistance formation has been fostered by the large and often injudicious consumption of antibiotics, especially broad spectrum drugs, in medicine and as food additives in agriculture. Currently, studies carried out on biltong sold in Bloemfontein were on the isolation and identification of yeasts, and no studies have yet been carried out on the presence of bacteria. Against this background, the aim of the study on which the article is reporting was first to quantify *S. aureus* and *B. cereus* found on biltong sold in Bloemfontein and to establish the prevalence of resistance to antimicrobial substances on *Staphylococcus* and *Bacillus* strains isolated from biltong.

MATERIALS AND METHODS

Sampling

The study was conducted in the Motheo District of Bloemfontein (Mangaung), a city in the Free State Province of South Africa. Biltong samples were collected in Bloemfontein over a period of 3 months between the dry and wet seasons. Sample collection occurred over three rounds (rounds 1, 2 and 3) to determine if there was a change in microbes present on biltong samples. Selected outlets were divided into four groups: supermarkets, butcheries, stalls (mini-market outside malls) and kiosks (inside malls). The study was conducted at least in duplicate. All samples were stored in a cooler box to minimize any microbial proliferation during transportation and samples were analyzed immediately upon arrival at the laboratory. This study was divided into two parts: the first part focused on the quantification of *S. aureus* and *B. cereus* strains isolated from biltong, while the second part focused on antibiotic susceptibility profiles of *S. aureus* and *B. cereus* strains isolated from biltong.

Laboratory Analysis

Ten grams of each sample was weighed off and added to 90 mL of sterile peptone buffered water (Biolab, Pretoria, South Africa), and homogenized for 2 min using a stomacher (Stomacher 400 circulator laboratory blender; Thermo Fisher Scientific, Gauteng, South Africa) (Nortjé *et al.* 1999; Fang *et al.* 2002). Serial dilutions up to 10^{-6} were prepared. Aliquots (0.1 mL) of the solutions were spread-plated and incubated on the surface of different solidified selective media in duplicate and incubated as detailed next (Herbert 1990; Martinez-Tomé *et al.* 2000).

Total Viable Counts

For the enumeration of total viable counts (TVCs), plate count agar (PCA) plates (Merck, Gauteng, South Africa) were incubated at 35C for 48 h (Houghtby *et al.* 1993; Vorster *et al.* 1994).

S. aureus. Baird-Parker agar (BPA, Oxoid CM275, Bloemfontein, South Africa) with 50 mL egg yolk telluride emulsion (Merck) was used for the enumeration of coagulase-positive *Staphylococci* from biltong. BPA contains lithium chloride and tellurite to inhibit the growth of alternative microbial flora, while the included pyruvate and glycine promote the growth of *Staphylococci*. Typical *S. aureus* colonies will appear black with white margins surrounded by clear zones. Samples were incubated for 48 h at 35C, according to the method of Nikanen and Aalto (1978). *S. aureus* ATCC 25923 was used as a positive control and a blank BPA plate was used as a negative control. All *S. aureus* colonies were confirmed using the rapid latex agglutination test (Slidex Staph Plus test kit, Bio Merieux, Marcy l'Etoile, France) (Personne *et al.* 1997; Van Griethuysen *et al.* 2001). The test was performed according to the manufacturers' instructions. The principle of this test is based on latex particles being sensitized with human fibrinogen and monoclonal antibodies simultaneously, to detect clumping factor, staphylococcal protein A and group-specific antigens on the *S. aureus* cell surface. A test would be considered positive if there is a visible agglutination of the latex particles and clearing of the background in the test reagent and no agglutination in the presence of only the control reagent.

B. cereus. To achieve enumeration of *B. cereus*, a blank *B. cereus* agar plate was used as a negative control, while *B. cereus* ATCC 14579 was used as a positive control. *B. cereus* selective agar (BCSA) plates) (Oxoid) (Scharlau, South Africa) were used for the enumeration of *B. cereus*. BCSA consists of the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-glycopyranoside and polymixin B. Polymixin B allows growth of *B. cereus* only and inhibits growth of unwanted species. The chromogenic substrate is cleaved by the enzyme β-glucosidase present in *B. cereus*, resulting in the formation of peacock blue colonies. The plates were incubated at 30C for 24 h and examined for typical *B. cereus* colonies, which were characterized by peacock blue colonies, with egg yolk precipitate (Nortjé *et al.* 1999). Confirmation of colonies was performed according to the procedure of Holbrook and Anderson (1980).

Antibiotic Susceptibility Test

Antibiotic susceptibilities for *Staphylococcus* spp. and *B. cereus* isolates from the enumeration studies were determined by disk diffusion method on a Mueller–Hinton medium (Difco, Bloemfontein, South Africa) in accordance with the Clinical and Laboratory Standards Institute 2006). Subsequent to 24 h incubation, colonies from each selective medium (containing *Staphylococcus* spp. and *B. cereus*) were transferred to about 5 mL saline solution to achieve a suspension equivalent to 0.5 McFarland standards. A sterile cotton swab was used to apply the inoculum evenly on the agar surface. The test agar plates were allowed to dry for 15 min, and antibiotic disks were embedded aseptically on each plate, with their centers at least 30 mm apart. The test agar plates were then incubated overnight at 37C. The zones showing growth inhibition were measured and an instruction sheet was followed for zone size interpretation. The following antibiotic disks were used: methicillin (5 μg), oxacillin (1 μg), penicillin (10 μg), tetracycline (30 μg), lincomycin (2 μg), chloramphenicol (30 μg), oxytetracycline (30 μg), bacitracin (10 μg) and nalidixic acid (30 μg) (Merck) (Banerjee and Sarkar 2004; Gundogan *et al.* 2005; Kaçmaz and Aksoy 2005; Uhland and Higgins 2006; Pesavento *et al.* 2007).

The disk diffusion method indicates susceptibility of the challenged organism to the tested antibiotic by a clear zone of inhibited growth around the filter paper disks. Upon contact with the agar surface, the antibiotic drug diffuses into the medium. The growth of the organism is inhibited until a critical concentration (minimal inhibitory concentration) is reached. The diameter of the resulting zone is considered proportional to the degree of susceptibility and allows categorizing the organism into susceptible (S), intermediating (I) or resistant (R) when comparing with international guideline tables (Huys *et al.* 2002).

RESULTS

TVCs

TVCs of samples collected from selected supermarkets, butcheries, stalls and kiosks in the city of Bloemfontein ranged from 10^4 to 10^6 colony forming units (cfu)/g. For all collected samples, the mean values for supermarkets were 1.4×10^6, 5.9×10^5 and 2.5×10^5 cfu/g, respectively, in

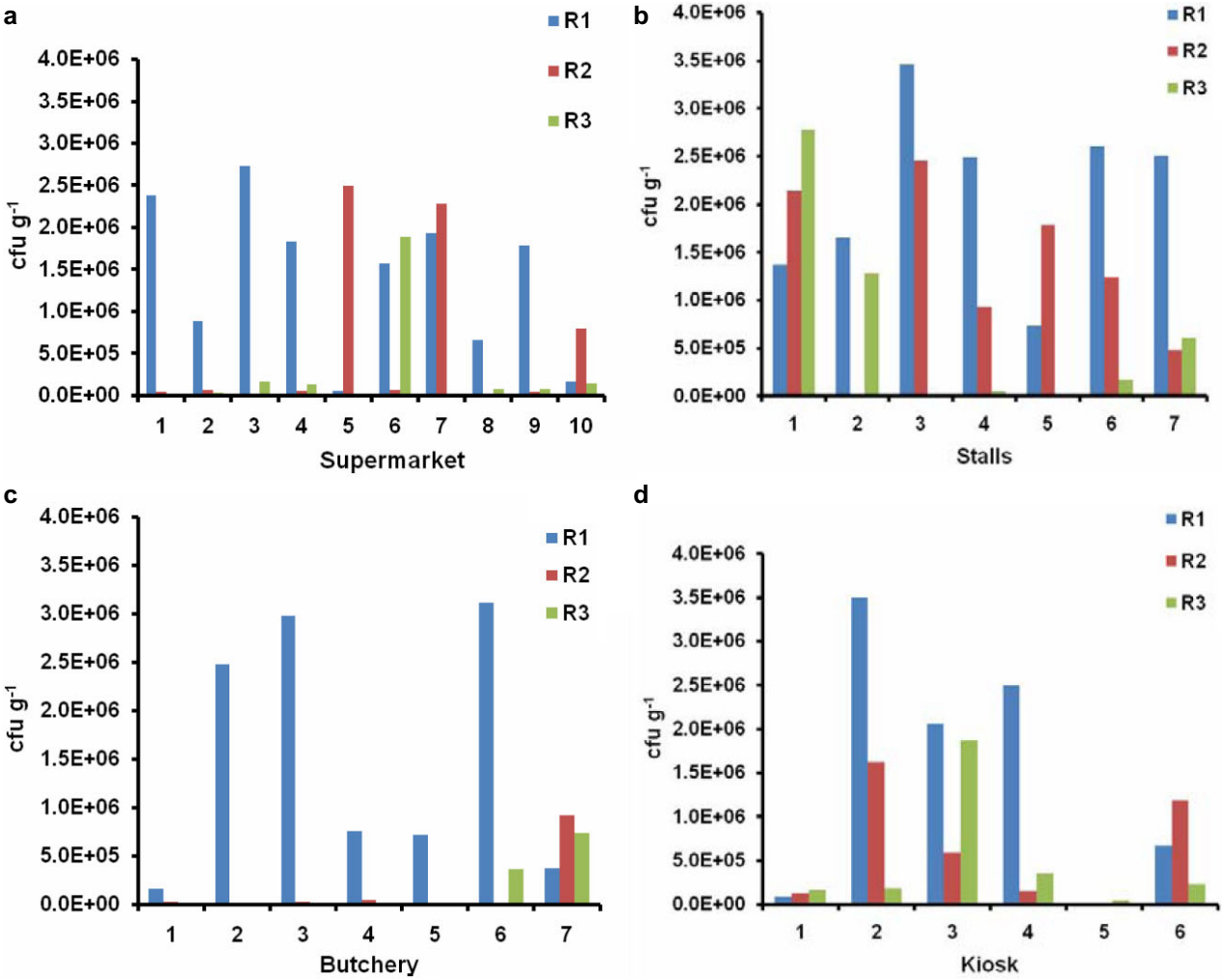

FIG. 1. A COMPARISON OF TOTAL VIABLE COUNTS OBTAINED FROM BILTONG FROM SAMPLED OUTLETS OBTAINED OVER THREE ROUNDS
R, round; cfu, colony forming units.

decreasing order from rounds 1–3. The highest recorded values in all supermarkets were 2.7×10^6 in round 1 and 1.9×10^6 cfu/g in round 3. Even though the latter was the highest recorded value during round 3, the results show a decrease in TVC from rounds 1 to 3 (Fig. 1a). Supermarkets 5, 7 and 10 were the only exceptions with high TVC counts in round 2 (Fig. 1).

In round 1, the stalls recorded the highest mean value of 2.1×10^6 cfu/g, and this decreased in rounds 2 and 3 to 1.3×10^6 and 7.0×10^5 cfu/g, respectively (Fig. 1b). The maximum mean value for all butcheries was 1.5×10^6 cfu/g, and this value was recorded in round 1 (Fig. 1b). Butcheries 2, 3 and 6 had high counts when compared with other butcheries, with butchery 6 recording the highest count of 3.1×10^6 cfu/g (Fig. 1c). The last group, the kiosks, had a high mean value of 1.5×10^6 cfu/g and a maximum value of 3.5×10^6 cfu/g, both recorded in round 1 (Fig. 1d). Kiosks 2, 3 and 4 had the highest counts when compared with other kiosks during round 1, with kiosk 2 recording the highest count at 3.5 10^6 cfu/g (Fig. 1d).

In general, all chosen outlets recorded the highest mean counts during round 1, but the counts decreased during rounds 2 and 3. Stalls and kiosks had the highest recorded mean counts, followed by supermarkets and butcheries, which showed the lowest counts of all the groups.

S. aureus. The mean values for *Staphylococcus* counts detected were 5.74×10^5, 6.0×10^5, 1.26×10^6 and 1.01×10^5 cfu/g for supermarkets, butcheries, kiosks and stalls, respectively (Fig. 2a–d). These counts were unacceptably high and indicative of suboptimal hygiene management at all outlets. Overall, the highest recorded counts were

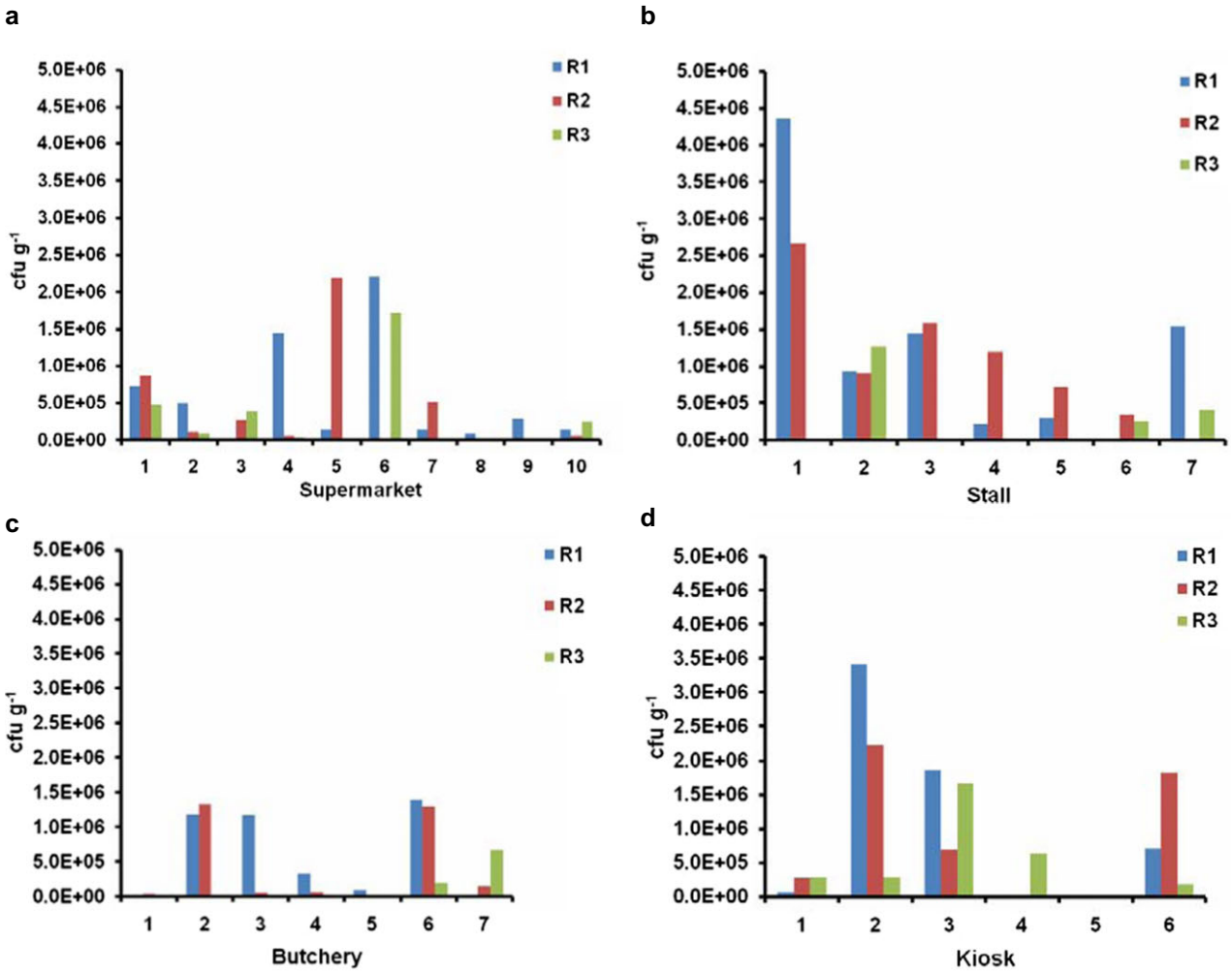

FIG. 2. A COMPARISON OF *STAPHYLOCOCCUS AUREUS* COUNTS OBTAINED FROM BILTONG FROM SAMPLED OUTLETS RECORDED OVER THREE ROUNDS
R, round; cfu, colony forming units.

observed at stalls and kiosks. Stall 1 had high counts of 4.36×10^6 and 2.67×10^6 cfu/g during the first and second rounds, respectively (Fig. 2b); this was also observed at kiosk 2 (Fig. 2d) with counts of 3.41×10^6 and 2.24×10^6 cfu/g, respectively. High counts were at supermarkets 5 and 6, with supermarket 5 at 2.19×10^6 cfu/g during round 2 and this decreased in round 3. Supermarket 6 had high counts of 2.21×10^6 and 1.72×10^6 cfu/g in rounds 1 and 3, respectively (Fig. 2a). Generally, butcheries had the lowest counts when compared with all the outlets sampled.

B. cereus. *Bacillus cereus* quantification of the four sampled outlets over three rounds is presented in Fig. 3a–d. The supermarkets recorded maximum counts ranging from 9.1×10^5 to 3.2×10^5 cfu/g, with the mean values between 1.4×10^5 and 2.4×10^6 cfu/g (Fig. 3a). Meanwhile, the stalls' counts reached a maximum of 2.7×10^6 cfu/g and mean counts of 1.2×10^6 cfu/g (Fig. 3b). The lowest counts were observed in the samples collected from the butcheries, which recorded a maximum value of 1.4×10^6 cfu/g and a mean value of 5.7×10^5 cfu/g (Fig. 3c). Samples collected from the kiosk were also analyzed and counts ranging from 2.7×10^6 to 3.5×10^5 cfu/g (Fig. 3d) were documented in all three rounds. In general, kiosks (Fig. 3c) and stalls (Fig. 3d) had the highest *Bacillus* counts of all the groups.

Antibiotic Susceptibility Test

In the current study, 90 strains of *S. aureus* were present in all biltong samples investigated. The rapid latex agglutination test confirmed that the 90 strains were indeed *S. aureus*. All strains isolated from biltong were analyzed for antimicrobial

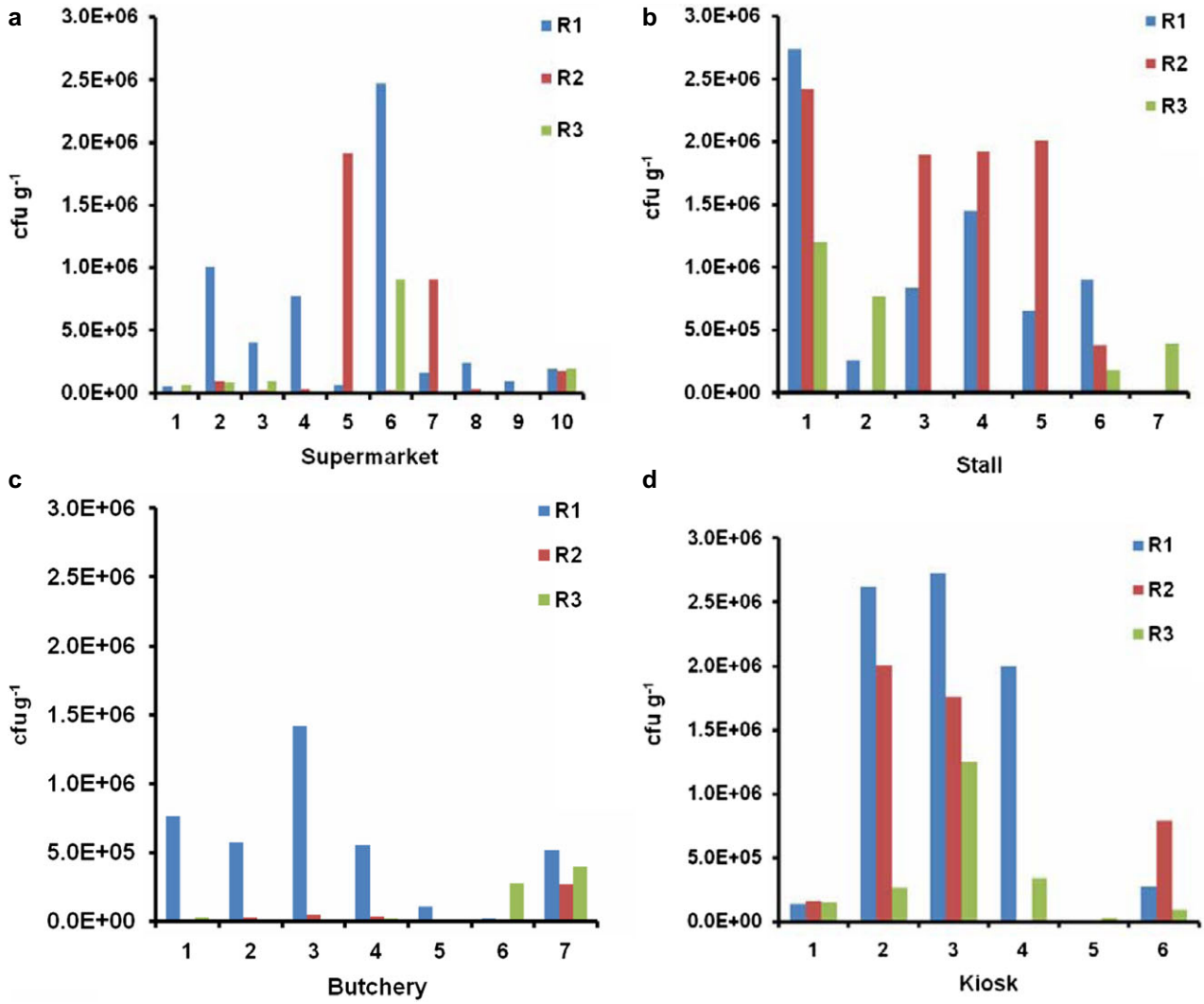

FIG. 3. A COMPARISON OF *BACILLUS CEREUS* COUNTS OBTAINED FROM *BILTONG* FROM SAMPLED OUTLETS RECORDED AFTER THREE ROUNDS
R, round; cfu, colony forming units.

TABLE 1. SUSCEPTIBILITY PROFILES OF *STAPHYLOCOCCUS AUREUS* (n = 90) STRAINS FROM BILTONG

Antibiotic	Susceptible (%)	Intermediate (%)	Resistance (%)
Penicillin	53	47	0
Oxacillin	50	47	3
Methicillin	93	7	0
Nalidixic acid	0	0	100
Tetracycline	83	0	17
Lincomycin	40	20	40
Chloramphenicol	37	10	53
Bacitracin	65	30	5
Oxytetracycline	74	20	6

resistance on selected antibiotics and are presented in Table 1. Fifty percent of the isolates were susceptible to penicillin, with 47% intermediately resistant. While 93% of the isolates were susceptible to methicillin, and 83% were susceptible to tetracycline and penicillin, respectively. The susceptibility of isolates to oxacillin was 50%, with 47% intermediate and 3% resistant. *S. aureus* was 100% resistant to nalidixic acid. The reason for resistance of *S. aureus* to nalidixic acid is because this antimicrobial agent is not active against gram-positive cocci as compared with others above.

AR was also determined for *B. cereus*, an important food poisoning organism partly because of its widespread occurrence in nature. *B. cereus* can be transmitted to food products when contaminated ingredients, such as spices, are used in the

TABLE 2. THE SUSCEPTIBILITY PROFILES OF *BACILLUS CEREUS* ($n = 90$) STRAINS ISOLATED FROM BILTONG

Antibiotic	Susceptible (%)	Intermediate (%)	Resistance (%)
Penicillin	63	33	4
Oxacillin	63	33	4
Methicillin	97	0	3
Nalidixic acid	3	0	97
Tetracycline	97	1	2
Lincomycin	57	29	14
Chloramphenicol	49	21	30
Bacitracin	58	25	17
Oxytetracycline	80	15	5

processing of biltong. In this study, 90 strains of *B. cereus* were present on all biltong samples collected. Antimicrobial resistance patterns for *B. cereus* found on biltong samples are presented in Table 2. Sixty-three percent of the isolates were susceptible, 33% intermediately resistant and 4% resistant to penicillin and oxacillin, respectively. Ninety-seven percent of the isolates were susceptible to methicillin and tetracycline. However, 97% of the isolates were resistant to nalidixic acid and only 3% were susceptible. Similar results were observed by Rusul and Yaacob (1995), who found that the majority of *B. cereus* isolates were susceptible to antimicrobials used therapeutically in humans.

DISCUSSION

TVCs

The quality and safety of a finished meat product is highly dependent on the raw materials used and the conditions under which it is processed. TVCs are normally used to evaluate the impact of time–temperature history or slaughter sanitation conditions. If counts are very high or if they vary widely among samples from different lots or within the same lot, inadequate microbiological control during harvesting, transport and processing may be an explanation.

TVCs observed in the present study were lower than those reported by Wolter *et al.* (2000), where the average counts were $>10^7$ cfu/g, and these were higher than those reported by Rahman *et al.* (2005), where the counts reached 5.95×10^6 cfu/g. The mean TVCs were unacceptably high and indicative of suboptimal hygiene management at the retail outlets. The purpose of salting and drying the meat product such as biltong is to inhibit or restrict the microbial growth: the counts of TVC recorded in the current study, however, might be indicative of deterioration of hygiene standards of the local retail outlets (Nortjé *et al.* 1999). Yet it was encouraging to observe that high counts were mainly encountered in round 1, which could mean people at these outlets had changed their hygiene habits over the sampling period.

S. aureus. The presence of *S. aureus* in foods commonly indicates contamination that may be directly introduced into the food by food handlers, the processing environment and/or postprocess contamination (Gundogan *et al.* 2005). Food poisoning is one of the common causes of foodborne illness due to the ubiquitous nature of *S. aureus* and the ability of many strains to synthesize enterotoxins (Kérouanton *et al.* 2007). A relatively high number of staphylococcal strains are known to be resistant to quite a number of antimicrobials commonly used in the therapeutic protocols of many human and animal infections (Normanno *et al.* 2007).

Staphylococci counts in this study were higher than expected. This could possibly be due to handling by humans (who are primary reservoirs of *S. aureus*) during slicing of biltong before merchandising. Samples in this study exceeded the proposed maximum limit of 100 cfu/g for *S. aureus* in raw meat and processed meats proposed by the South African Department of Health (Republic of South Africa 2000). Furthermore, the majority of the samples exceeded the proposed infective dose for *S. aureus*, which is 10^5 cfu/g (Republic of South Africa 2000; Nel *et al.* 2004; Shale *et al.* 2005). An infective dose for *S. aureus* is commonly defined as the dose at which illness may result after consumption of food.

B. cereus. The spore-forming ability of *Bacillus* spp. allows its survival during food processing treatments; and the spores germinate if the food is left at room or refrigeration temperature. During the preparation of biltong, different marinating techniques and recipes are employed, and *Bacillus* spp. have been isolated from various spices that are used as ingredients in the marinating process (Banerjee and Sakar 2004; Iurlina *et al.* 2006). The incidence of psychrotrophic *B. cereus* in IMM such as biltong and ready-to-serve convenience foods is very high and is a public concern as *B. cereus* grows and causes infection due to unhygienic handling of food. This is because some strains of these organisms are capable of producing toxins as well as spoiling dried and ready-to-eat food products (Rusul and Yaacob 1995; Byrne *et al.* 2006; Martinez *et al.* 2006).

According to Banerjee and Sarkar (2003), there have been numerous reports of *B. cereus* foodborne illness, and for these outbreaks, the level of *B. cereus* in the food was found to be 10^6–10^9 cfu/g. The highest count reached in the current study falls within these values. There is very limited information on food poisoning caused by *B. cereus* in South Africa. Moreover, there are only a few documented reports of this problem possibly because *B. cereus* causes self-limiting symptoms, where affected persons may recover within 24 h and do not see the need to report the matter. Furthermore, it is important to note that many foodborne illnesses are sporadic and may not be counted as part of an outbreak. Factors such as lack of consumer awareness, inadequate disease surveillance by the local and national public health departments, and the varying incubation periods might be the reason why foodborne ill-

nesses that might be caused by *B. cereus* are not reported. With the modern fast-paced lifestyle, there is an increase in demand for convenient, ready-to-eat foods such as biltong. If this is coupled with the increased numbers of immunocompromised individuals, this may increase the frequency and severity of illnesses caused by organisms such as *B. cereus* (Mahakarnchanakul and Beuchat 1999; Gandhi and Chikindas 2007).

All the samples had counts above the South African Department of Health national guideline for *B. cereus* (10^3 cfu/g). Furthermore, although counts from few samples were below 10^5 cfu/g, there is a cause for concern as the majority of counts were above the *B. cereus* national infective guideline of 10^5, which means that the possibility of food poisoning exists (Republic of South Africa 2000; Nel *et al*. 2004).

Antibiotic Susceptibility Test

S. aureus and *B. cereus* were 100% and 97%, respectively, resistant to nalidixic acid. According to a study done by Duffy *et al*. (1999), AR of retail isolates may be attributed to a number of possible sources, including intrinsic resistance to certain antibiotics, possible transfer of AR among species and the use of subtherapeutic doses of antibiotics in animal fodder to improve animal productivity. In a study conducted in Italy by Zanelli *et al*. (2002) on the resistance of isolates from human nasal carriages, 9.52% *S. aureus* isolates were resistant to gentamycin, while 13.2% of *S. aureus* isolated clinical samples were also resistant. A high level of resistance was also found against other drugs. The findings in the current study are worrying because the presence of AR genes in animals and food raises an even greater concern as AR is carried by mobile genetic elements such as plasmids, transposons and chromosomal cassettes, which can promote intra- and interspecific and even intergeneric transfer (Garofalo *et al*. 2007).

In conclusion, results obtained from this study revealed that most samples from the chosen biltong outlets had counts that exceeded the acceptable microbiological quality safety limits. The presence of selected microorganisms on biltong even after it had gone through the drying and salting process indicated that microorganisms continued to proliferate, and this shows a need for improving the general hygiene of the processing environment together with that of food handlers. These results suggest that the final product of biltong is not as safe as many may perceive it to be. With the local regulations stipulating that the infective dose of both *Staphylococci* and *B. cereus* is 10^5 cfu/g, this is of great concern because some samples exceeded this limit.

In the current study, it was found that the majority of kiosks and stalls where biltong was sold had questionable hygiene maintenance. Poor hygiene and sanitation practices prevailing in the kiosks and stalls as well as in the shops encouraged microbial contamination, survival and growth. Routine and proper cleaning of all relative equipment, surfaces and display cabinets is mandatory. Furthermore, it is imperative that biltong be stored under proper conditions of humidity in order to prevent contamination and for overall shelf stability. Butcheries had the lowest counts in this study when compared with other outlets, meaning that butcheries had better hygiene standards than other outlets.

The second part of this study indicated the presence of nalidixic acid-resistant *S. aureus* and *B. aureus* strains on biltong, which presents a risk of infection and transmission of resistance to other bacteria. While the use of antibiotics is essential in the treatment of bacterial infection, their indiscriminate use can have adverse consequences by promoting the selection and prevalence of drug-resistant microbial populations. The incidence of resistant bacteria on biltong is a major public threat as these organisms have been isolated from a wide range of foodstuffs consumed by human beings. The relevance of information obtained in the current study on the resistance of bacteria to antibiotics is to appreciate the magnitude of the problem and establish baselines for action should a need arise.

Furthermore, these results indicate that a possibility exists that organisms found to be present may be vectors that spread resistance to antibiotics in humans. The spread of resistant pathogens and genes can have serious implications for the treatment of human infections because many of the antibiotics used on animals are either identical to or related to drugs used in human medicine (Tollefson and Karp 2004). Even though there is information available on which antibiotics to use for a specific purpose in animals, there seems to be no control of the flow of a particular antibiotic into farm animal production.

To address the problem of cross-contamination and recontamination of the product in all kiosks and stalls, the management should focus their energy on implementing and applying general preventative measures such as the prerequisite programs to hazard analysis critical control point and good manufacturing practices in order to reduce or eliminate contamination of both the raw and the final product.

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