Isolation of enteric bacterial pathogens from raw mince meat in Mafikeng, North-West Province, South Africa

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Abstract: A large proportion of the world's population rely on meat as a source of food. Enteric bacteria species can cause infections in humans when undercooked meat products are consumed. The aim of the study was to isolate enteric bacteria species from raw mince meat obtained from some supermarkets and butcheries in Mafikeng. Ten raw mince meat samples were collected from these two types of meat retail shops in the Mafikeng area in the North-West Province, South Africa. The makeup of these included; 6 meat samples from butcheries and 4 from supermarkets. The samples were analyzed for the presence of enteric bacteria using SSA and XLD agar. A total of 150 isolates that satisfied the preliminary biochemical tests (oxidase, citrate utilization and TSI tests) were further confirmed using API 20E assay and 96 were positively identified as enteric bacteria species. The proportion of enteric bacteria was higher in samples obtained from butcheries (B1 and B3 with 86.7%, respectively) than in the supermarkets (43.3% to 53.3%). The most frequently identified species were *Serratia odorifera* (17.3%), *Escherichia coli* (10.0%), *Klebsiella oxytoca* (6.7%) and *Enterobacter aerogenes* (6.0%). Enteric bacteria species were isolated and positively identified in all meat sample collected from the different sampling sites in Mafikeng. Although most of the species identified are pathogenic to humans, some have strains that are known to cause foodborne outbreaks even in countries with proper public health facilities. It is therefore recommended that effective food safety education and training of personnel that handle food at retail points will help in reducing the effect of these pathogens on humans.

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1. Introduction

Enteric bacteria are facultative aerobic, gram-negative, non-spore forming rods that belong to the family *Enterobacteriaceae* (Brenner, 1984). This family is subdivided into a number of genera that include *Escherichia, Shigella, Salmonella, Citrobacter, Klebsiella, Enterobacter, Erwinia, Serratia, Hafnia, Edwardsiella, Proteus, Providencia, Morganella, Yersinia* (Blood and Curtis, 1995). This classification it is primarily based on relatedness on biochemical characteristics, genetic and antigenic properties and pathogenicity (Blood and Curtis, 1995).

The human gut is the natural habitat for these bacteria community and these bacteria species participate in metabolic activities that salvage energy and absorbable nutrients protect the colonized host against invasion by alien microbes, and important trophic effects on intestinal epithelia and on immune structure and function (Guarner, 2006). They thus play an essential role in the development and homeostasis of the immune system (Guarner, 2006). On the other hand, although most enteric bacteria were previously considered to be harmless, there is evidence implicating them in certain pathological conditions and thus cause health problems including multisystem organ failure, colon cancer, diarrhea, enteritis, urinary tract infections and inflammatory bowel diseases in humans (Cormican et al., 1998; Goldstein, 2000; Dromigny et al., 2002; Guenther et al., 2010).

Diseases usually result through the production of enterotoxins of which two major types known as endotoxins and exotoxins have been identified (Tornadijo et al., 2001). Moreover, there are other virulence factors that usually contribute to their pathogenicity (Albert et al., 1992; Frankel et al., 1994; Ridell et al., 1994; Tschäpe et al., 1995, Lai et al., 2000; Mirold et al., 2000). The capsular polysaccharide, adhesion factors, polysaccharide chains and surface antigens have been reported as putative virulence factors that facilitate attachment of bacteria to host cell wall (Albert et al., 1992; Frankel et al., 1994; Ridell et al., 1994; Tschäpe et al., 1995).

The mode of transmission of these pathogens to humans is through the faecal – oral route hence a number of outbreaks have resulted from the consumption of contaminated food and/or

water (Borch and Arinder, 2002; Gudbjornsdottir et al., 2004). Consequently, food products such as red meat, poultry, sea foods, diary products and vegetables have been implicated in the transmission of these pathogens to humans (Choma et al., 2000; Guerra et al., 2001, Humphrey and Jorgensen, 2006; Zalalem et al., 2007). These pathogens may contamination meat if proper hygiene practices are not implemented during the slaughtering, processing and packaging of meat (Dickson and Anderson, 1992; Juneja and Sofos, 2001, Grandin, 2006; Stopforth and Sofos, 2006; Sofos, 2008). This therefore, amplifies the need to constantly monitor the levels of these pathogens in food products so as to reduce the number of unnoticed infections that are caused on consumers. Moreover, infections caused by these pathogens are usually more severe in the elderly people and immunocompromised individuals (Sofos, 2008). In a country like South Africa where the incidence of HIV/AIDS is high the impact of such surveys cannot be overemphasized.

2. Material and Methods

Area of the study and sampling sites.

This research was conducted in Mafikeng in the North-West Province South Africa. Meat samples were collected from 2 supermarkets and 3 butcheries located within the Mafikeng area.

Sample collection and analysis

Ten meat samples were purchased from these randomly selected supermarkets and butcheries in the Mafikeng locality. The makeup of these included; 6 meat samples from butcheries and 4 from supermarkets. The samples were placed in plastic sample collection bags and transported on ice to the laboratory for analysis. On arrival in the laboratory approximately 5g of the meat sample was washed in 5ml of 2% buffered peptone water (Biolab, Merck Diagnostics, South Africa). Aliquots of 100µl were spread plated on Salmonella-Shigella agar (SSA) (Biolab, Merck Diagnostics, South Africa) and Xylose Lysine Deoxycholate agar (XLD) plates (Biolab, Merck Diagnostics, South Africa). This was basically to increase the chances of isolating different types of enteric bacteria. The plates were incubated aerobically at 37°C for 24 hours. Suspected colonies were purified by sub-culturing on SSA and XLD agar. The plates were incubated aerobically at 37°C for 24 hours. Pure isolates were retained for presumptive identification and confirmatory biochemical tests.

Gram Staining

Presumptive isolates on SSA and XLD agar plates were subjected to the Gram stain reaction using standard methods (Cruikshank et al., 1975). Gram negative rods were retained for further identification using conventional microbiological methods.

Primary biochemical identification tests

Oxidase Test

This test was performed using the Oxidase Test reagent™ (PL. 390) as recommended by the manufacturer, Mast Diagnostics, Neston, Wirral, U.K. The oxidase is based on the principle that tetramethyl-p-phenylenediamine is oxidized by bacterial cytochrome in the presence of atmospheric oxygen to form purple coloured compound. In performing the test, a pure isolated colony was picked up using a sterile wire loop and placed on a Whatmans filter paper. A drop of the Oxidase™ reagent was added to make a smear. After 30 seconds the formation of a purple or blue indicated an oxidase positive result and vice versa.

Triple Sugar Iron (TSI) Test

A well isolated pure colony was stab inoculated into the butt and streaked on the surface of the slant on the TSI agar (Biolab, Merck Diagnostics, South Africa) using a sterile wire loop. The agar was incubated at 37°C for 24 hours. The results were recorded based on fermentation (change of colour of the medium from red to yellow) on the butt and slant, hydrogen sulphide gas production and gas production (Forbes and Weissfeld, 1998).

Simmons Citrate Utilization Test

A well isolated pure colony was streaked on the slant of Simmons citrate agar (Fluka, Biochemika) that was contained in 10ml McCartney bottles using a sterile wire loop. The media were incubated at 37°C for 24 hours. After incubation a change in colour from green to blue was recorded as a positive reaction and vice versa.

Secondary biochemical identification test

API 20E Test

API 20E is a standardized system that is used for identification of bacteria belonging to the family *Enterobacteriaceae* and other non-fastidious Gram-negative bacteria. The test was performed as recommended by the manufacturer (Bio-Mérieux, France). Indices were calculated based on the metabolic pattern of the isolates and the identities of the isolates were confirmed using API web software.

3. Result and Interpretation

Gram Staining and Primary Biochemical Test

Ten meat samples were purchased from five different sampling sites (2 Supermarkets and 3 Butcheries), in duplicates. Forty-five (45) presumptive bacteria isolates from each sample were

randomly selected and screened using preliminary identification tests. A total of 450 isolates were screened using the Gram staining, oxidase test, Citrate utilization test and the TSI test. The number of isolates that were either positive or negative for the tests are shown in Table 3.1. As shown in Table 3.1 all the isolates were Gram negative and oxidase negative. These were satisfactorily considered as enteric bacteria and were subjected to further testing.

Table 3.1: Results from primary biochemical tests (Gram staining, Citrate utilization, Oxidase Test and Triple Sugar Iron Test).

SS	Biochemical Tests								
	GS	CU		OT	TSI				
	-	-	+	-	SF (+)	H_2S (+)	H_2S (-)	Gas (+)	Gas (-)
SM 1	90	32	28	90	60	11	49	33	27
B 1	90	58	2	90	60	13	47	20	40
SM 2	90	60	0	90	60	20	40	32	28
B 2	90	41	19	90	60	15	45	25	35
B 3	90	40	20	90	60	19	41	15	45
Total	450	231	69	450	300	78	222	125	175

Confirmation of the identities of the isolates using the Analytical Profile Index (API) 20E

All the 450 isolates satisfied the preliminary biochemical identification tests for enteric bacteria. However, 150 (30 isolates per sample) representative isolates were randomly selected and their identities were further confirmed using API 20E (BioMériux, France). The number of the different bacteria species that were identified are shown in Table 4.2. As shown in Table 4.2, the most predominant species in sample 1 that was obtained from the supermarket were *Serratia odorifera, Klebsiella oxytoca, Enterobacter cloacae* and *Enterobacter aerogenes*. However, in sample 2 from another supermarket the predominant species were *Serratia odorifera, Salmonella arizonae, Klebsiella oxycota*. The predominant species for samples obtained from butcheries were *Enterobacter aerogenes*, *Citrobacter freundii*, and *Escherichia coli* for samples B1, B2 and B3, respectively. A cause for concern was the fact that pathogen enteric bacteria was isolated from samples obtained from both supermarkets and butcheries. Although, these isolates were not identified at strain level, they could belong to some of those that are the leading cause of food borne infections worldwide. This therefore amplifies the need to implement proper hygiene practices when handling and processing meat.

Discussion

According to the International Meat Secretariat Newsletter, (November 30, 2005) it is reported that as the standard of living improves, meat consumption also increases. These increases in meat demands is said to be due to increased urbanization, higher disposable income, and the human desire for a greater variety in their diets (Sofos, 2008). Therefore, the safety of meat has been in the forefront society concerns in recent years and evidence exists that the challenges of meat safety will continue in the future (Sofos, 2008). Consequently it is very important to implement proper hygiene and safety procedures not only during slaughter but also when handling and processing meat.

The main objective of the study was to isolate and identify enteric bacterial pathogens from raw mince meat samples that were bought from some randomly selected supermarkets and butcheries in Mafikeng, North- West province, South Africa. The supermarkets and butcheries selected for this study are amongst those that serve a large number of individuals per day in the area. Although, some studies have been conducted globally (WHO, 1997; Paton and Paton 1998; Müller et al., 2001), only one study has been done the Mafikeng area in particular and the North West Province in general (Ateba and Mbewe, 2010). This amplifies the importance of such studies as these do not only determine the prevalence of pathogens in meat but also health risks associated with the consumption of improperly cooked contaminated meat.

Isolation and identification of enteric pathogens was achieved using conventional microbiology methods which include both primary (Gram staining, oxidase, citrate utilization and Triple Sugar Iron tests) and secondary biochemical identification tests (API 20 E). Four hundred and fifty (450) isolates were screened and all the isolates were Gram negative and Oxidase negative which satisfied the identification characteristics for *Enterobacteriacea.* Of the 150 presumptive isolates that were screened by API 20E analysis, 96 were positively identified as enteric bacterial species. Although some of these species identified were non-pathogenic, a cause for concern was the detection of species that are potentially pathogenic to humans. Of the nineteen species identified 16 have been reported to cause diseases in humans if ingested in improperly cooked contaminated food and/or water (Alballaa et al., 1992; Hoppe et al., 1993; Maraki et al., 1994; Oh and Tay, 1995; Funke and Rosner, 1995; Tschäpe et al., 1995; FAO/WHO, 2004).

Table 4.1: Preliminary results from primary biochemical tests (Gram staining, Citrate utilization, Oxidase Test and Triple Sugar Iron Test).

Enteric bacteria identified	**Sampling Site**					**Total N[b]=150**
	SM1 (%) N[a]=30	B1 (%) N[a]=30	SM2 (%) N[a]=30	B2 (%) N[a]=30	B3 (%) N[a]=30	
*Klebsiella oxytoca**	3	3	2	2	0	10 (6.7%)
*Serratia odorifera**	5	2	5	2	2	26 (17.3%)
*Enterobacter aerogenes**	2	6	1	0	0	9 (6.0%)
*Enterobacter cloacae**	3	1	0	1	1	6 (4.0%)
*Enterobacter sakazakii**	1	0	1	0	2	4 (2.7%)
*Serratia marcenscens**	1	2	1	0	2	6 (4.0%)
*Citrobacter freundii**	0	0	0	3	1	4 (2.7%)
*Escherichia coli**	0	5	0	1	9	15 (10.0%)
*Hafnia alvei**	0	1	0	2	3	6 (4.0%)
*Proteus vulgaris**	0	0	0	1	0	1 (0.7%)
*Salmonella arizonae**	0	1	2	0	1	4 (2.7%)
*Kluyvera spp**	0	2	0	0	0	2 (1.3%)
*Klebsiella ornithinolytica**	0	2	0	0	0	2 (1.3%)
*Serratia liquefaciens***	1	0	0	0	0	1 (0.7%)
*Citrobacter youngae***	0	0	0	2	0	2 (1.3%)
*Citrobacter braakii***	0	1	1	2	2	1 (0.7%)
*Escherichia vulneris**	0	0	0	0	1	1 (0.7%)
*Salmonella choleraesuis**	0	0	0	0	1	1 (0.7%)
*Enterobacter cancerogenus**	0	0	0	0	1	1 (0.7%)
TOTAL	**16 (53.3%)**	**26 (86.7%)**	**13 (43.3%)**	**15 (50.0%)**	**26 (86.7%)**	**96 (64.0%)**
No. of species that were not able to be identified using the API 20E	14 (46.7%)	4 (13.3%)	17 (56.7%)	15 (50.0%)	4 (13.3%)	54 (36.0%)

The most frequently identified bacteria species in all the samples was *Serratia odorifera* 26 (17.3%), *Escherichia coli* 15 (10.0%), *Klebsiella oxytoca* 10 (6.7%) and *Enterobacter aerogenes* 9 (6.0%), respectively, and all of them are pathogenic to humans (Alballaa et al., 1992; Hoppe et al., 1993; Maraki et al., 1994; Oh and Tay, 1995; Funke and Rosner, 1995; Tschäpe et al., 1995; FAO/WHO, 2004). These bacteria species have been found to cause diseases such as diarrhea, sepsis, meningitis,

systemic infections, necrotizing enterocolitis, bacteraemia, urinary tract infection, gastroenteritis, surgical wound infections in humans (Alballaa et al., 1992; Hoppe et al., 1993; Maraki et al., 1994; Oh and Tay, 1995; Funke and Rosner, 1995; Tschäpe et al., 1995; FAO/WHO, 2004). These infections are considered to be opportunistic and would pose severe challenges in immunocompromised individuals (Albert et al., 1992; Frankel et al., 1994; Ridell et al., 1994, 1995; Tschäpe., 1995; Sofos, 2008). Based on the aforementioned, it is very important to determine the level of contamination with these pathogens considering the high incidence of HIV/AIDS in the country. Moreover, the enteric bacteria species identified in mince meat that was assessed does not only indicate the ability of the food products to transmit these pathogens to humans if consumed raw or improperly cooked but also increases the possibility of causing food spoilage if not properly refrigerated (Piette et al., 2000; Gram et al., 2002). Food spoilage results in undesirable changes which usually affects the quality of the food product (Piette et al., 2000; Gram et al., 2002). It is therefore important to ensure that all safety measures are put in place to facilitate rapid and reliable identification of these enteric bacteria species in food substances so as to reduce the burden on humans (Mustapha, 2004).

Of the samples that were screened, pathogenic bacteria species were higher in samples obtained from butcheries than in supermarkets. This basically reflects the level of hygiene that is practiced in the two different types of settings as identified during the collection of samples.

Conclusion

In conclusion, enteric bacteria species were isolated and positively identified in all meat samples collected from the different sampling sites in Mafikeng. Both pathogenic and non-pathogenic enteric bacteria species were isolated in this study. The identification of pathogenic enteric bacteria species such as *Enterobacter sakazakii, Serratia mercescence, Klebsiella oxycota*, in any food product indicates that proper hygiene practices should be implemented during slaughter, processing and handling of these food products. Moreover, it has been proven that if the hygiene standards implemented during these processes are compromised, there is bound to be contamination (Dickson and Anderson, 1992). The presences of these bacteria on meat pose a health risk on consumers especially those that may be immune-compromised, the elderly and young children if not properly prepared (Sofos, 2008).

This therefore, calls for intervention and collaborations by food safety regulatory authorities, industries, scientific community and public health agencies to monitor the safety procedures that are implemented during the slaughtering, processing, packaging and transportation of food products as these affect the microbiological quality of the meat delivered at sale points (Edwards, 1995; Lambooij and Mulder, 1995; Stolle and Hiepe, 1995; Bacon et al., 2000; Sofos, 2008).

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