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Research Note

Prevalence and Antibiotic Resistance Profiles of *Escherichia coli* O157:H7 in Beef Products from Retail Outlets in Gaborone, Botswana

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ABSTRACT

Four hundred meat samples (134 meat cubes, 133 minced meat, 133 fresh sausages) were collected from 15 supermarkets and butcheries in Gaborone, Botswana, between the summer months of October 2002 and March 2003. Samples were assayed for *Escherichia coli* O157 by selective enrichment in modified *E. coli* broth containing novobiocin, followed by immunomagnetic separation and plating onto sorbitol MacConkey agar supplemented with potassium tellurite. The isolates were biochemically and serologically confirmed by API 20E and O157 antisera, respectively. The prevalence rates for *E. coli* O157 were 5.22% in meat cube samples, 3.76% in minced meat samples, and 2.26% in fresh sausages. The isolates showed single, double, and triple antibiotic resistance. Fifty-three percent of them were resistant to cephalothin. Resistance was also recorded for sulphatriad (33%), colistin sulphate (26%), streptomycin (0.7%), and tetracycline (26%). It is recommended that the cause for antibiotic resistance be investigated using a larger number of samples from cattle, especially from ranching areas of the country.

Beef is Botswana's chief revenue earner. Meat is exported to several countries including those in the European Union. Strict regulations are maintained and European Union inspectors pay regular visits to Botswana Meat Commission to ascertain conformity of Botswana's beef to standards that have been set in the European community *(15).*

While the international community enjoys export beef of good microbiological quality, locals consume meat slaughtered by private individuals or municipal abattoirs. These abattoirs may not strictly adhere to meat handling regulations. Numerous loopholes have been identified in the existing laws, acts, and regulations that place the consumer at a risk due to foodborne infections such as those from emerging pathogens like *Escherichia coli* O157:H7 *(15).*

E. coli O157:H7 was first recognized as a foodborne pathogen in America *(28)* after an outbreak of hemorrhagic colitis following the ingestion of undercooked hamburgers at a fast-food restaurant chain *(27).* Since its discovery, *E. coli* O157:H7 has become an important public health problem in both the developed and the developing world *(12).*

E. coli O157:H7 illnesses have so far been reported mainly in the developed countries. It is only beginning to be included routinely in surveys done in developing countries. It has been estimated that the enterohemorrhagic *E. coli* is responsible for the death of up to a million infants per year in Africa, South America, and Asia *(34).*

In Botswana, diarrheal diseases in children under the age of five are the second most common cause of death after acute respiratory infections *(31).* However, apart from *Salmonella*, *Shigella*, and rotavirus *(20, 26, 31),* no documentation on *E. coli* O157 exists in Botswana. This paper reports on the prevalence of *E. coli* O157 in beef and beef products in supermarkets and butcheries and the antibiotic resistance profiles of the isolated strains.

MATERIALS AND METHODS

Meat samples. A total of 400 fresh meat samples, including meat cubes ($n = 134$, ca. 2 cm^2), minced meat ($n = 133$), and fresh sausages ($n = 133$), each weighing between 400 and 700 g, were collected between the summer months of October and March, 2002 to 2003. All the samples were collected randomly from 15 supermarkets and butcheries around Gaborone, the capital city of Botswana.

Sampling was mainly done in the morning. The fresh meat samples were placed in an insulated cooler box containing ice blocks and were immediately transported to the laboratory for analyses (30 to 45 min). Samples were analyzed within 1 h after collection.

Enrichment and isolation. A 25-g portion of meat sample was obtained aseptically and added to 225 ml of modified *E. coli* (Oxoid, Basingstoke, UK) broth containing novobiocin (20 μg/ml). It was then homogenized in the stomacher (Seward, London, England) for 1 min. The samples were incubated for 18 h at 42°C for *E. coli* enrichment. After incubation of the samples for 18 h at 42°C, immunomagnetic separation was applied following manufacturer's instruction *(21).*

Presumptive identification of *E. coli* O157. Presumptive identification was done using eosin methylene blue agar (CM69;

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TABLE 1. *Prevalence of* E. coli *O157 in meat sold in Gaborone*

Type of meat	Number of samples	Prevalence rate (%)	95% CI[a]
Cubes	134	5.22	3.26, 7.18
Minced	133	3.76	1.80, 5.75
Sausages	133	2.26	0.30, 4.22
Total	400	3.75	1.79, 5.71

[a] CI, confidence interval.

Oxoid). Colorless or pale pink colonies from sorbitol Mac Conkey agar were subcultured onto eosin methylene blue agar and incubated at 37°C for 18 to 24 h. Presumptive *E. coli* O157 colonies appear blue-black with a greenish metallic sheen on eosin methylene blue agar plates. The colonies were then subcultured onto nutrient agar (CM3; Oxoid) and incubated at 37°C for 18 to 24 h, and their purity was checked. These pure cultures were maintained at 4°C for further biochemical and serological tests.

Biochemical and serological tests. Biochemical confirmation of the presumptive *E. coli* O157 cultures was done using the API 20E system (bioMérieux, Marcy l'Etoile, France) as per the manufacturer's instructions. The confirmed isolates were serotyped with *E. coli* O157 antisera (Mast Diagnostics, Bootle, UK) following the manufacturer's instructions.

Antibiotic sensitivity testing. The Bauer-Kirby standard procedure *(3)* was used to test sensitivity to the following antibiotics: ampicillin (AM, 10 μg), cephalothin (KF, 5 μg), colistin sulphate (CO, 25 μg), gentamicin (GM, 10 μg), streptomycin (S, 10 μg), sulphatriad (ST, 200 μg), tetracycline (T, 25 μg), and cotrimoxazole (TS, 25 μg). The inhibition zone was measured and interpreted as susceptible (equal to or greater than 3 mm), intermediate (greater than 2 mm but smaller than 3 mm), or resistant (2 mm or less) *(8)*.

RESULTS

Overall, *E. coli* O157 was isolated from 15 out of 400 meat samples, representing 3.75%. Of these, 1.75% (7 of 400) was from meat cubes, 0.75% (3 of 400) from sausages, and 1.25% (5 of 400) from minced meat. The prevalence rate was higher in meat cubes, 5.22% (7 of 134); followed by minced meat, 3.76% (5 of 133); and lastly sausages, 2.26% (3 of 133) (Table 1). All the isolates were susceptible to ampicillin, gentamicin, and cotrimoxazole. On the other hand, 53% of the isolates showed resistance to cephalothin. This was followed by resistance to sulphatriad (33%), colistin sulphate (26%), tetracycline (26%), and streptomycin (0.7%). The isolates also showed single or double and triple resistances to the antibiotics used in the test (Table 2). Six of them were resistant to one antibiotic (single resistance), and eight isolates showed resistance to two or three antibiotics (multiple resistance). On the other hand, one isolate was susceptible to all the antibiotics.

DISCUSSION

Reports indicate that *E. coli* O157:H7 is the third most common cause of bacterial foodborne diarrheal cases worldwide after *Salmonella* and *Campylobacter* species *(5)*. The isolation of *E. coli* O157:H7, however, from foods such as meat is problematic because the bacterium is likely to be present in low numbers, may be sublethally injured, and is usually accompanied by large populations of competing microflora *(6)*. Studies that have used insensitive procedures have either failed or detected reduced numbers of the pathogen in meat products. The immunomagnetic separation is more sensitive than the traditional procedures of isolating *E. coli* O157:H7 *(21, 33)*.

TABLE 2. *Antibiotic resistance profiles of* E. coli *O157 isolates from meat*

Meat type	Code of isolates	Resistance antibiograms
Cubes	1	KF
	3	KF, ST
	4	T, ST
	6	KF, S
	10	CO
	14	KF, ST
	15	KF
Sausages	2	KF, ST, T
	7	KF, CO
	9	KF, ST, T
Minced	5	None
	8	KF
	11	CO, T
	12	CO
	13	KF

It is difficult to directly compare these results with previously published studies because of the use of different isolation procedures. However, reports of surveys conducted in other countries concerning the level of *E. coli* O157:H7 in meats and meat products are similar to the results of the present study. In Argentina, *E. coli* O157:H7 was detected in ground beef at a rate of 3.8% *(6)*. In another study in North America, *E. coli* O157:H7 was recovered from 3.7% of ground beef samples obtained from retail outlets *(10)*.

Season of epidemiological data collection has a marked influence on the results. Maule *(24)* showed that cattle carry more *E. coli* O157 in the summer. The data on cattle carriage of *E. coli* O157 also correlate with the seasonal variation in the incidence of human diseases *(17)*. The reason for this correlation is unknown, but it may be related to temperature abuse during warmer seasons *(11)*. Our study was conducted in summer, but we did not consider the role of seasonality in influencing the carriage of *E. coli* O157. Younger animals tend to carry *E. coli* O157 more frequently than older ones *(25)*. Information on age of the slaughtered animals in Botswana was not available.

The results indicated that the prevalence level in meat cubes was 5.22%. This was higher than in other meat products (Table 1). Contamination of meat carcasses with intestinal contents during slaughter could be responsible for the initial contamination *(29)*. Reported levels of *E. coli* O157 in the feces of dairy cattle in the United States have ranged from 5.8 to 19.0% *(9, 32)*, and data from other countries also fall within this range. Other sources of contamination of carcasses could include feces; hide contact; aerosols;

contact with workers' hands, gloves, and other equipments; and accidental spillage of body fluids during skinning and evisceration *(4).* The meat cubes are usually made from various cuts, for example, legs and brisket. The neck, brisket, and legs easily get contaminated *(4).* This may be the reason why there was a higher prevalence of *E. coli* O157 in meat cube samples.

Microbiological surveys of butchery premises and products have found *E. coli* O157:H7 and other members of the *Enterobacteriaceae* from chopping surfaces, a weighing balance pan, and meat slicer blades. Meat cubes will pick up contaminating microorganisms as they contact work surfaces and process equipment in the factory or slaughterhouse *(24). E. coli* O157:H7 have been shown to become entrapped in the porous surface of the wooden cutting boards and to remain viable on these materials for more than 12 h *(1).* Observation from some of the butcheries where sampling was done shows that the same cutting blades and weighing pans are used in cutting and weighing different meat types (tripe and steak).

Minced meat and sausages had a prevalence level of 3.76 and 2.26%, respectively. Other reported prevalences of *E. coli* O157:H7 in minced meat range from 1.3 to 4% *(6, 13, 18, 19, 30).* Sources of *E. coli* O157 in the minced meat and sausages could possibly be from contamination of the meat directly or indirectly from contaminated grinders and cutting surfaces. Grinding of meat is known to mix the bacteria into the meat *(29).*

Many of our existing antibacterial agents are under threat from the widespread emergence of bacterial resistance *(7).* Though early surveys of antibiotic resistance showed that *E. coli* O157:H7 isolates were sensitive to antibiotics, recent studies have revealed a trend toward increased resistance to antibiotics *(11).* The dynamics of antibiotic resistance depend on many aspects including the environment and the antibiotic itself *(16).* The problem of resistance appears greatest in those countries with a high level of antibiotic usage and little restriction of sale *(2).* In Botswana, cattle are kept in ranches where use of antibiotics to cure animal illnesses is minimal or none at all and one would expect low levels of resistance. In this study, however, 53% of the isolates were resistant to cephalothin, 33% were resistant to sulphatriad, 26% were resistant to colistin sulphate and tetracycline, and 0.7% were resistant to streptomycin. Source of resistance of the *E. coli* O157:H7 isolates is not known. Kadavy et al. *(19)* investigated the antibiotic resistance profiles of microorganisms inhabiting a natural system where there is no antibiotic stress. The data were not different from those of microorganisms living in antibiotic stress environments. Thus in nature there may be more than one way to become resistant to antibiotics *(22, 23, 29).*

The isolates in the study showed multiple antibiotic resistances. Galland et al. *(14)* have also found that 33% of the *E. coli* O157 isolates from healthy antibiotic free cattle were resistant to one or more antibiotics. One likely explanation for this resistance is the relative ease with which genetic elements, such as plasmids, transposons, and integrons that may confer resistance to numerous antibiotics, are exchanged among promiscuous bacteria *(29).*

The implementation of hazard analysis critical control point system at the abattoir, butcheries, and even households could reduce the risk from *E. coli* O157 significantly. The findings of this research also suggest future studies in Botswana be conducted on meat safety focusing on prevalence of *E. coli* O157:H7 on cattle farms to determine cattle carriage rate, age of carriage, cattle feeds, seasonal factors, and antibiotic resistance.

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