African Journal of Microbiology Research Vol. 4(16), pp. 1703-1707, 18 August, 2010
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 ©2010 Academic Journals

Full Length Research Paper

Antimicrobial susceptibility of non-sorbitol fermenting *Escherichia coli* isolated from cattle feaces and milk samples

Joshua Orungo Onono*, Erastus Kiambi Kangethe and William Okelo Ogara

Department of Public Health, Pharmacology and Toxicology, University of Nairobi, P. O. Box 29053 - 00625, Nairobi, Kenya.

Accepted 15 June, 2010

The objective was to determine the antimicrobial susceptibility of the non-sorbitol fermenting *Escherichia coli* colonies from cattle feaces and milk samples collected from Dagoretti division in Nairobi. A total of 285 feacal and 260 milk were collected from urban dairy farming households while non-dairy households provided 137 milk samples. The samples were used for culture and isolation of *E. coli* and the colonies isolated using standard microbiological methods. 23% (66) and 8.8% (23) of feacal and milk samples from urban dairy farming households had non sorbitol fermenting colonies, while 8.8% (12) of non-dairy farming household neighbours had non sorbitol fermenting colonies in milk samples. Antibiotic susceptibility patterns showed that isolates of *E. coli* were resistant to various antibiotics. There was a high percentage resistance to sulphamethoxazole in feacal samples isolates (14.4%), milk sample isolates (10%) from dairy farming household and milk sample isolates (11.7%) non-dairy households. The feacal isolates had a low resistance to ampicilin (1.4%), but the resistance in isolates from milk samples of urban dairy household (6.5%) and non-dairy household's milk samples (7.3%) were high. The other antibiotics showed varied resistance pattern with feacal isolates having a high percentage resistance to tetracyclines (6.7%) while most bacterial isolates were susceptible to gentamicin. Multiple antibiotic resistances was observed in feacal sample isolates (6.7%), dairy farming household milk isolates (4.2%) and non- dairy farming household milk isolates (7.3%). Non-sorbitol fermenting *E. coli* colonies from cattle feaces and milk samples were resistant to most of the antibiotics tested and the higher percentage resistance to sulphamethoxazole, ampicilin and tetracyclines requires further investigation to isolate, identify and compare the genes responsible for development of resistance.

Key words: Non-sorbitol fermenting, *Escherichia coli*, urban dairy households, antimicrobial susceptibility.

INTRODUCTION

Escherichia coli is the species most commonly isolated from human feacal samples and is part of the normal intestinal flora of healthy individuals (Muller et al., 2005). *E.coli* O157:H7 strain is the classical serotype linked to serious outbreaks and sporadic cases of enterohae-morrhagic diseases such as haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) (Muller et al., 2005; Mashood et al., 2006). Cattle are the main reservoir for *E. coli* O157:H7, but the bacteria also occurs in other animal species such as sheep, goats, pigs, cats, dogs, chickens and gulls (Callaway et al., 2003, 2004; Muller et al., 2005; Mashood, et al., 2006). Transmission of the pathogen to humans occurs through various routes; consumption of contaminated beef (Galland et al., 2001), drinking unpasteurised milk (Chapman et al., 1993), drinking contaminated water (Keene et al., 1994; O'Connor, 2002), eating contaminated vegetables

*Corresponding author. E-mail: joshua.orungo@uonbi.ac.ke, ononojo@yahoo.co.uk. Tel: +254 0721736989.

(Morgan et al., 1988) and direct from animals to animal keepers (Milne et al., 1999).

Although antibiotics are not recommended for treatment of *E. coli* O157:H7 infections in humans, there is evidence that bacterial isolates are resistant to some antibiotics (Aibinu et al., 2007). Smith et al. (2003, 2007) reported multidrug resistance in isolates of *E. coli* 0157 strains obtained from farm-animals and human infections in Lagos and Ogun state in Nigeria. They had collected a total of 350 fresh faecal droppings of animals (cattle, pig, chicken and sheep) and human stool comprising of diarrhoeic (150) and non diarrhoeic (50). Carl et al. (2002) reported that 361 *E. coli* O157 isolates kept at the *E. coli* Reference Centre at the Pennsylvania State University were resistant to sulphamethoxazole (26%), tetracycline (27%), cephalothin (17%) and ampicilin (13%). These isolates were recovered from samples collected from humans, cattle, swine, and food for a period spanning the years 1985 to 2000.

The extensive use of antibiotics in both human medicine and animal agriculture is suspected to have lead to a widespread dissemination of antibiotic resistant genes (Callaway et al., 2003). The development of resistance to antimicrobials is known to occur through stable genetic change heritable from generation to generation through specific mechanisms including mutation, transduction, transformation and or conjugation (Goodman et al., 1990).

This paper describes the antimicrobial susceptibility for the non-sorbitol fermenting *E. coli* isolates from cattle feaces and milk samples collected from urban dairy farming households and milk samples collected from non-dairy farming household neighbours in Dagoretti division.

MATERIALS AND METHODS

Household sampling

A total of 285 feacal and 260 milk samples were collected from dairy farming households and 137 milk samples from non-dairy households' neighbours in Dagoretti division.

Feacal samples from adult cattle and calves from each dairy household were pooled separately and transported in a cool box to the laboratory for the isolation of *E. coli.*

Milk samples were collected from both dairy and non dairy farming households in 15 ml sterile tubes and transported in cool boxes to the laboratory for isolation of *E. coli.* The households were requested to keep the milk in the usual containers they normally use for storing milk.

Isolation of *E. coli*

The pooled feacal samples were weighed and 0.2 g suspended in 2 ml of mac-Conkey broth and incubated for 2 h at 37°C. After this short pre enrichment period, a loopful of broth was streaked into sorbitol Mac-Conkey agar and incubated at 37°C for 24 h. Milk samples were vortexed and immediately a loopful of the milk streaked onto sorbitol mac-Conkey and incubated at 37°C for 24 h.

After 24 h of incubation, eight transparent/colourless colonies of non sorbitol fermenters were separately sub cultured into eosin methylene blue agar (EMB) for 24 h at 37°C. Colonies that were medium in size, raised and smooth with dark centres showing a greenish metallic sheen were subjected to biochemical tests, to confirm whether they were isolates of *E. coli.*

Biochemical tests

The colonies were subjected to indole, methyl red, voges proskauer and citrate fermentation tests according to standard microbiological procedures (Oxoid, 2005). Colonies that were confirmed by biochemical tests as *E. coli* isolates were further sub cultured on sorbitol Mac-Conkey agar to confirm their inability to ferment sorbitol by showing transparent/colourless colonies and the confirmed non sorbitol fermenters were stored in sterile 50% glycerol mixed with tryptone soy agar at 4°C.

Sensitivity testing

This was done according to Bauer - Kirby technique (1966). The confirmed non sorbitol fermenters were transferred into 4 ml of peptone water, incubated for 3 h at 37°C and turbidity adjusted to match the opacity tube containing 0.5 ml of 1% sulphuric acid. The test cultures were streaked evenly over the Muller - Hinton agar plate and the multi antibiotic discs (Abtek Biological Ltd) were applied onto the plate using sterile forceps. The plates were then incubated at 37°C for 24 h, after which then zone of inhibition for each antibiotic including the diameter of the antibiotic disc was measured using a vernier calliper in millimetres. The cut off points were then determined by using the current National Committee for Clinical Laboratory Standards (NCCLS, 2002). The antibiotics tested were those that are commonly used in treatment of livestock diseases and for prophylaxis in livestock production and they included tetracycline, gentamicin, nitrofurantoin, sulphamethoxazole, nalidixic acid and ampicilin.

Data analysis

Data was entered in microsoft access database and prevalence of non sorbitol fermenting *E. coli* in households produced using Instat + for windows version 3.036 (2006). The percentage antimicrobial resistance in samples and the confidence intervals around the proportions in households from which non sorbitol fermenting *E. coli* was isolated were calculated and in all cases a confidence level of 95% was used.

RESULTS

Prevalence of non sorbitol fermenting *E. coli*

The prevalence of non-sorbitol fermenting colonies of *E. coli* in milk samples from both urban dairy farming and non-dairy farming household was 8.8% (Table 1). While the prevalence of non-sorbitol fermenting colonies of *E. coli* in cattle feacal and dairy farming household milk samples were statistically different (feacal: 0.232, 0.184 - 0.285; milk: 0.088, 0.057- 0.130; z- value = 4.68, P- value < 0.0001). The high prevalence of non sorbitol fermenting colonies of *E. coli* in cattle feacal samples agrees with

Table 1. Prevalence of non- sorbitol fermenting *E. coli* in households.

Sample types (hhd)	Sampled hhd	Positive hhd samples	Prevalence	95% CI for prevalence
(Feaces) dairy	285	66	23.2	18.4 - 28.5
(Milk) dairy	260	23	8.8	5.7 - 13
(Milk)non-dairy	137	12	8.8	4.6 - 14.8

Key: hhd- household; CI- confidence interval.

Table 2. Percentage antimicrobial resistance in samples.

Antibiotics	Sample type in hhd	Samples with resistant colonies	% Resistance in hhd samples
	Feaces	4	1.4
Ampicilin	Milk	17	6.5
	Non-dairy milk	10	7.3
	Feaces	19	6.67
Tetracyclines	Milk	3	1.15
	Non-dairy milk	4	2.92
	Feaces	1	0.35
Nitrofurantoin	Milk	3	1.15
	Non-dairy milk	3	2.19
	Feaces	1	0.35
Nalidixic acid	Milk	1	0.38
	Non-dairy milk	0	0
	Feaces	41	14.4
Sulphamethoxazole	Milk	26	10
	Non-dairy milk	16	11.7
	Feaces	1	0.35
Gentamicin	Milk	0	0
	Non-dairy milk	0	0

Key: hhd- household.

earlier reports that cattle feaces is the main reservoir of this pathogen (Callaway et al., 2003, 2004).

Antimicrobial susceptibility of *E. coli* isolates

There was a higher percentage resistance to ampicilin by bacterial isolates in milk samples from both dairy farming households (6.5%) and non-dairy farming households (7.3%) as compared to the resistance in isolates from feacal samples (1.4%) (Table 2). The percentage resistance in feacal sample isolates (6.67%) to tetracyclines was higher when compared to milk sample isolates (1.15%) in dairy farming households. However, the percentage resistance to sulphamethoxazole by bacterial isolates from feacal samples (14.4%), milk samples from dairy farming households (10%) and milk samples from non-dairy farming households (11.7%) were high. Of the sixty six feacal samples from dairy farming households, nineteen (6.7%) had bacterial isolates with multiple resistances to ampicilin, tetracyclines and sulphamethoxazole, while a total of 21 milk samples had bacterial isolates with multiple resistance to ampicilin, tetracyclines and sulphamethoxazole. Of these 21 milk samples with multiple resistant bacterial isolates, ten (7.3%) were from milk samples from non-dairy farming households and eleven (4.2%) samples were from the dairy farming households. The difference in percentages between multiple antibiotic resistances in milk samples from non-dairy farming households and

dairy farming households was noted, but it could be explained by the fact that not all neighbouring non-dairy farming households bought milk from their immediate dairy farming households or that it could have occurred due to chance. But the difference in percentage resistance to ampicilin by isolates from feacal and milk samples needs to be investigated.

DISCUSSION

Prevalence

The prevalence of non-sorbitol fermenting *E. coli* was 23.2% in cattle feacal samples from urban dairy farming households, which is in the range of reported isolations in feaces of between 11 - 84% (Smith et al., 2003). The isolation at a prevalence of 8.8% in milk samples of both urban dairy farming and non dairy farming household's was higher than in earlier reports (Arimi et al., 2005; Nasinyama and Randolph, 2005). However, these isolates were not confirmed to be potential verocytoxin producers through serotyping. The isolation of the organism in milk of both urban dairy farming households and non-dairy farming neighbouring households was an indication that non-sorbitol fermenting *E. coli* which is a normal flora of cattle alimentary canal was contaminating the household milk. This was true for eight urban dairy farming households with both feacal and milk samples that were culture positive for non-sorbitol fermenting colonies of *E. coli* and the two urban dairy farming households and non-dairy farming neighbouring households whose milk samples were positive for the pathogen.

The presence of the bacteria in the non-dairy farming neighbour's milk is also evidence that they bought contaminated milk from their dairy farming neighbours, and are therefore at risk of infection. The organism is acid- resistant and therefore if the raw milk is used for the preparation of home made fermented milk (sour milk) without proper heat treatment, it may result in human infections (Bachrouri et al., 2002; Tsegaye and Ashenafi, 2005). These organisms when shed into the environment by animals and can contaminate water sources, cattle feeds, manure for use in crop fields and soils where they can remain viable for several months (Aloysio et al., 1999; Muller et al., 2001; Galland et al., 2001). The resistant bacteria would therefore become resident in the environment and be a source of contamination of humans' foods especially milk and water.

Antimicrobial sensitivity

There were a number of bacterial colonies that had multiple resistances to various antibiotics. A total of 19 out of 66 feacal samples had non sorbitol fermenting colonies with multiple antibiotic resistances while a total of 21 milk samples had bacterial isolates with multiple resistances to ampicilin, tetracyclines and sulphamethoxazole. The development of antimicrobial resistance by the bacteria to these drugs poses a major challenge in both human medicine and animal medicine because these drugs are commonly used in therapy of human patients and in veterinary practice. Uncontrolled usage of antibiotics in treatment of animals, incorporation in animal feeds has been suspected to account for majority increase in antibiotic resistance in human bacterial isolates (WHO, 2000; Galland et al., 2001). The developmental of resistance to antimicrobials occurs through stable genetic change heritable from generation to generation through specific mechanisms including mutation, transduction, transformation, and or conjugation (Goodman et al., 1990; Metlay et al., 2006). The shedding of the resistant bacteria into the environment by cattle may lead to a widespread dissemination of antibiotic resistant genes to the resident bacteria in the environment (Callaway et al., 2003, 2004, Muller et al., 2005; Mashood, et al., 2006).

CONCLUSION AND RECOMMENDATION

Non-sorbitol fermenting *E.coli* was shown to be present in the urban dairy farming system. The non-sorbitol fermenting *E. coli* isolated from cattle feaces and milk samples showed development of resistance to most of the antibiotics tested, but there was higher resistance to sulphamethoxazole, ampicilin and tetracyclines. The high percentage resistance in bacterial isolates to ampicilin, sulphamethoxazole and tetracycline requires further investigation to isolate, identify and compare the genes responsible for the development of resistance in bacterial isolates from milk and feacal samples.

ACKNOWLEDGEMENT

The authors acknowledge the support from IDRC for funding this work.

REFERENCES

Aibinu IE, Peters RF, Amisu KO, Adesida SA, Ojo MO, Tolu O (2007). Multidrug Resistance in *E. coli* O157 Strains and the Public Health Implication. J. Am. Sci., 3 (3): 22-33 ISSN: 1545-1003.

Aloysio MF, Vergers Beatriz E, Guth C, Rogerio MJ, Joao RCA (1999). *E. coli* (ETEC) in healthy cattle in Rio de Janeiro state, Brazil. Vet Microbiol., 70: 111-121.

Arimi SM, Koroti E, Kangethe EK, Omore AO, Mc Dermott JJ, Macharia JK, Nduhiu JG, Githua A (2005). Risk of infection with *Brucella* and *E. coli* O157:H7 associated with marketing of unpasteurised milk. Acta Tropica., 96: 1-8.

Bachrouri M, Quinto EJ, Mora TM (2002). Survival of *E. coli* O157:H7 during storage of yogurt at different temperatures. J. Food Sci., 67: 5.

Bauer AW, Kirby WMM, Sherries JC, Turck M (1966). Antibiotic susceptibility testing. *American* J. Pathol., 45: 493-496.

Callaway RT, Anderson CR, Edrington ST, Genovese JK, Bischoff MK,

Poole LT, Jung SY, Harvey BR, Nisbet JD (2004). What are we doing about *E. coli* O157:H7 in cattle? J. Anim. Sci., 82(E. suppl.): E 93- 99.
Callaway RT, Anderson CR, Elder RO, Edrington ST, Genovese JK, Bischoff MK, Poole LT, Jung SY, Harvey BR, Nisbet JD (2003). Preslaughter intervention strategies to reduce food-borne pathogens in food animals. J. Anim. Sci., 81(E. suppl.2).
Carl MS, Cuiwei Z, Chitrita D, Jocelyn T, Shaohua David GW, David D, Patrick FM, Robert DW, Jianghong M (2002). Antimicrobial Resistance of *E. coli* O157 Isolated from Humans, Cattle, Swine, and Food; J. Appl. Environ. Microbiol., 2(68): 576–581.
Chapman PA, Siddon CA, Wright DJ, Norman P, Fox J, Crick E (1993). Cattle as a possible source of verotoxigenic- producing *E. coli* O157:H7 infections in man. J. Epidemiol. infec., 111: 439-447.
Galland CJ, Hyatt RD, Crupper SS, Acheson WD (2001). Prevalence, antibiotic susceptibility, and diversity of *E. coli* O157:H7 isolates from a longitudinal study of beef cattle feedlots. Appl. Environ. Microbiol., 67: 4.
Goodman AG, Theodore WR, Alan SN, Palmer T (1990). The pharmacological basis of therapeutics: Eighth edition: Pergamon Press. pp. 1020- 1021.
Keene WE, Mcanulty JM, Hoesly FC, Williams LP, Hedberg K, Oxman Gl, Barret Tj, Pfaller MA, Fleming DW (1994). A swimming-associated outbreak of haemorrhagic colitis caused by *E. coli* 0157:H7 and *Shigella sonnei*. New Engl. J. Med., 331: 579-584.
Mashood AR, Minga U, Machungu R (2006). Current epidemiologic status of enterohaemorrhagic *E. coli* O157:H7 in Africa. Chinese Med. J., 119(3): 217-222.
Metlay PJ, Powers HJ, Dudleys NM, Christiansen K, Finch GR (2006). Antimicrobial drug resistance, regulation and research. Emerging infectious Diseases. 12: 2.
Milne L, Plom A, Strudley I, Pritchard GC, Crooks R, Hall M (1999). *E. coli* O157 incident associated with a farm open to members of the public. Commun. Dis. Public Health, 2: 22-26.
Morgan GM, Newman C, Palmer SR (1988). "First recognised community outbreak of haemorrhagic colitis due to verocytotoxin-producing *E. coli* 0157:H7 in the UK", Epidemiol. Infect. 101: 83-91.
Muller EE, Ehlers MM (2005). Biology identification of non-sorbitol fermenting bacteria isolated on *E. coli* O157 selective CT-SMAC agar. Available on website http://www.wrc.org.za. 31: 2.
Nasinyama GW, Randolph TF (2005). Provisional technical report: characterizing and assessing the benefits and risks of urban and peri urban (UPA) livestock production in Kampala City, Uganda. IDRC, PROJECT file 101801.
National Committee for Clinical Laboratory Standards (2002). Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement M100-S12. NCCLS, Wayne, PA.
O'Connor Dr (2002). Report of the Walkerton Inquiry: The Events of May 2000 and Related Issues. Part 1: A Summary. Ontario Ministry of the Attorney General. Queen's Printer for Ontario 2002.
Oxoid ltd (2005). Manual for laboratory reagents. Oxoid limited, Bassingstoke, England, pp. 2: 36-37.
Smith SI, Aboaba OO, Odeigha P, Shodipo K, Adeyeye JA, Ibrahim A, Adebiyi T, Onibokun H, Odunukwe NN (2003). Plasmid profile of *E. coli* O157:H7 from apparently healthy animals. Afr. J. Biotechnol. 2: 322-324.
Tsegaye S, Ashenafi M (2005). Fate of *E. coli* O157:H7 during the processing and storage of ergo and ayib, traditional Ethiopian dairy products. Int. J. Food Microbiol. 103: 11-21.
World Health Organization WHO (2000). Global Principles for the containment of Antimicrobial Resistance In Animals Intended For Food; Report of WHO Consultation With The Participation Of Food and Agriculture Organization of The United Nation and the Office International Des Epizooties, Geneva Switzerland 5- 9 June 2000. Department of Communicable Disease Surveillance and Response.