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Antibiograms of faecal *Escherichia coli* and *Enterococci* species isolated from pastoralist cattle in the interface areas of the Kafue basin in Zambia - short communication

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ABSTRACT

Antimicrobial resistance to a panel of ten agents was determined by the disc diffusion technique for 83 *Escherichia coli* isolates, 29 *Enterococcus faecium* isolates and 62 *Enterococcus faecalis* isolates from faecal samples of apparently healthy pastoral cattle in the wildlife/livestock interface areas. Of all the *E. coli* isolates, 8% were diarrhoeagenic *E. coli* strains, 6% were enteropathogenic *E. coli* strains and 2% were enterotoxigenic *E. coli* strains. A high frequency of *E. coli* resistance to penicillin, erythromycin, cotrimoxazole and nitrofurantoin was observed. *Enterococci* showed the highest percentage of resistance to gentamycin, amoxycillin, ampicillin and tetracycline. None of the *E. coli* strains and *Enterococci* strains was resistant to tetracycline and vancomycin respectively. The results of this study underscore the presence of an animal reservoir of antibiotic resistant microorganisms that have the potential to enter the food chain.

Key words: *E. coli*, *Enterococci*, antibiogram, pastoral cattle, Zambia

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Introduction

The increasing use of antimicrobial agents in both human medicine and animal agriculture has resulted in many pathogens developing drug resistance. Certain organisms like, *Escherichia coli* and *Enterococci* play an important role in many existing surveillance systems for antibiotic resistance in the bacterial flora of livestock and food of animal origin (RIOU et al., 1985; WRIGHT et al., 1999). But recently a decline in the effectiveness of antibiotics has raised concerns in veterinary and human medicine (PHILIPPA and TRNOBRANSK, 1998; MATEU and MARTIN, 2001; STEINKE et al., 2001; CATRY et al., 2003).

In Zambia, studies on antibiotic resistance are very rare and the few that may be present are biased towards bacteria from livestock in the commercial sector (NGOMA et al., 1993; HANG'OMBE et al., 1999) where antimicrobial usage is wide spread. No information could be found on pastoralist entities, yet such information could help to describe the extent of resistant strains contamination in the environment. This study was therefore designed to collect antibiotic usage data from pastoral cattle. The study was conducted in the Kafue basin where cattle owners are semi nomads and their cattle graze on the plains during the dry season where they mix with wild animals such as Kafue lechwe (*Kobus leche kafuensis*), buffalos (*Syncerus caffer*) and zebra (*Equus burchelli*) and are moved back to higher grounds during the rainy season. The aim of the present paper was therefore to evaluate the level of intestinal bacteria resistance to antimicrobial agents in pastoral cattle especially *E. coli* and *Enterococci*.

Materials and methods

Prior to collection of faecal samples, a pilot study was carried out in the form of a questionnaire distributed to pastoral cattle owners in the wildlife/livestock interface areas of the Kafue basin to assess the common diseases experienced among the pastoral cattle and the kind of antimicrobial agents commonly used. The Kafue basin forms one of the biggest wetlands in Africa and provides the widest interface between pastoral cattle and wildlife.

Eighty-three faecal samples were collected at random from apparently healthy pastoral cattle and belonging to different independent owners living in the Kafue basin interface areas. Fresh faecal samples from individual cattle were collected with a sterile wooden applicator. All samples were stored at 4 ^{0}C till they were processed in the laboratory.

The samples were analyzed within 48 hrs of collection. Bacteriological counts were carried out as detailed by KOLLER and JELINEK (1976). Briefly, samples (0.1 g) were suspended in 0.9 mL of sterilized normal saline and the suspension was thoroughly mixed. The suspension (0.1 mL) from the appropriate dilution was surface plated on a

duplicate Desoxycholate Hydrogen Sulfide Lactose Agar plate (Nissui, Tokyo, Japan) and *Enterococcus faecalis* (EF) medium plate (Nissui) for determination of *E. coli* and *Enterococci* count respectively. Desoxycholate Hydrogen Sulfide Lactose agar plate cultures were incubated at 37 ^{0}C for 24 hrs and EF plate cultures at 37 ^{0}C for 48 hrs. Suspect *E. coli* isolates were cultured on Triple Sugar Iron (Mast, Merseyside, U.K) and Lysine, Indole, Motility medium (Nissui) for preliminary identification. Confirmation of *E. coli* isolates was carried out using the BBL Enterotube II biochemical identification kit (Becton Dickinson, Maryland, USA). All confirmed *E. coli* isolates were cultured on CHROM agar O157 medium (Rambach, Paris, France) and incubated at 37 ^{0}C for 24 hrs to identify enterohermorrhagic *E. coli* strains. Colonies appearing purple on CHROM agar medium were considered pathogenic and were subjected to *E. coli* polyvalent serotyping. Serological grouping of *E. coli* isolates was performed by agglutination test using an available kit (Denka, Tokyo, Japan). With the use of polyvalent antiserum, the agglutination test was performed on bacterial isolates according to the manufacturer's procedure. Suspected *Enterococci* isolates were identified by the conventional method utilizing colony appearance on EF agar plates. Pink and yellow colonies on EF medium were considered *E. faecalis* and *E. faecium* respectively. Antibiograms of *E. coli* and *Enterococci* isolates under investigation were determined according to BAUER et al. (1966). Commercial antimicrobial paper discs (Oxoid, Hampshire, England) were used.

Results

Analysis of the questionnaire. A total of 47 households that practiced pastoral farming responded to our questionnaire. All the respondents indicated that they used tetracycline to treat their animals through an intra muscular injection and were satisfied with the efficacy of tetracycline in the treatment of tick borne and bacterial diseases in animals. Procaine penicillin was another drug used by the respondents.

Table 1. Bacterial counts and isolates isolated from faecal samples of pastoral cattle in Zambia

Type of organisms	Approximate range cfu/g[a]	Total number of isolates
Escherichia coli	2.0×10^2-1.3×10^6	83
Enterococcus faecium	5.2×10^2-1.3×10^6	29
Enterococcus faecalis	5.2×10^2-1.3×10^6	62

[a]The approximate range of bacterial counts is from the lowest to the highest count of 83 samples

Bacterial isolates analysis and their drug resistance pattern. A total of 174 isolates were evaluated. Of these 83 were *E. coli*, 29 *E. faecium* and 62 *E. faecalis*. In total

Enterococci spp. isolates were more than *E. coli* isolates. Counts of *E. coli, E. faecium* and *E. faecalis* isolates (cfu/g) in the individual samples were done (Table 1). Higher counts of *Enterococci* spp. were observed too. Out of all the *E. coli* isolates, seven (8.43%) were O typeable. Five (6.02%) were enteropathogenic *E. coli* strains (EPEC) and 2 (2.41%) were enterotoxigenic *E. coli* (ETEC) strains (Table 2).

Table 2. Serotyping of 83 *E. coli* isolates from pastoral cattle

Type of *E. coli*	Number typed	%
EPEC	5	6.02
ETEC	2	2.41
O typable	7	8.43
Untypable	69	83.1

Table 3. Antibiogram and prevalence of resistance of *E. coli* and *Enterococci* spp. isolates from pastoral cattle in Zambia

Antibiotic agent	*E. coli*		*E. faecium*		*E. faecalis*	
	Nº of resistant isolates	(%) resistant isolates	Nº of resistant isolates	(%) resistant isolates	Nº of resistant isolates	(%) resistant isolates
Erythromycin	69	83.1	21	72.4	35	56.5
Gentamycin	2	2.41	28	96.6	53	85.5
Ampicillin	7	8.43	15	51.7	47	75.8
Amoxycillin	12	14.5	19	65.5	39	62.9
Cotrimoxazole	59	71.1	26	89.7	53	85.5
Penicillin	80	96.4	23	79.3	50	80.6
Nitrofurantoin	19	22.9	19	65.5	31	50.0
Tetracycline	3	3.60	15	51.7	26	41.9

E. coli isolates showed a pattern of resistance to penicillin 96.4%, followed by erythromycin 83.1%, cotrimoxazole 71.1% and nitrofurantoin 22.9%. Resistance was also displayed, but to a lesser extent to amoxycillin 14.5%, ampicillin 8.43%, tetracycline 3.60% and gentamycin 2.41% (Table 3). The percentage of resistant strains of *E. faecium* and *E. faecalis* respectively were most frequently observed to gentamycin 96.6% and 85.5%, cotrimoxazole 89.7% and 85.5%, penicillin 79.3% and 80.6%, erythromycin 72.4% and 56.5%, amoxycillin 65.5% and 62.9%, nitrofurantoin 65.5% and 50.0%, tetracycline 51.7% and 41.9% and ampicillin 51.7% and 75.8% (Table 3). None of the

E. coli and *Enterococci* strains was resistant to tetracycline and vancomycin respectively. All *E. coli* and *E. faecium* strains were resistant to one or more antibiotics. Only one isolate of the *E. faecalis* strain was found to be susceptible to all antimicrobial agents under investigation. There was no antibiotic to which all bacterial isolates were found fully resistant.

Discussion

Our investigation proceeded on the information obtained from the questionnaire study. It was assumed that the widespread use of tetracycline could have probably induced a form of antibacterial resistance to intestinal bacterial flora of pastoral cattle. On the contrary, this was not the case. Some workers reported that selective pressure exerted by the use of tetracycline antibiotic as a growth promoter in food animals appears to have created large reservoirs of transferable antibiotic resistance in the ecosystem, for which a transfer of relevant resistant determinants to bacterial pathogens of humans was demonstrated (WITTE, 2000).

A high prevalence of resistance was observed in *E. coli* isolates to penicillin, erythromycin and cotrimoxazole, where as *Enterococci* isolates were resistant to gentamycin, cotrimoxazole and penicillin. In Denmark, the occurrence of erythromycin resistance among *E. faecium* and *E. faecalis* isolates from pigs was almost 90% (AERESTRUP et al., 2001). The study revealed that *E. coli* isolates were completely susceptible to tetracycline (100%) while moderate resistance was seen to 38% *E. faecium* isolates and 30% *E. faecalis* isolates. This observation seems consistent with our results despite differences in animal species. Our findings are also in line with other workers who have reported acquired high-level resistance of *Enterococci* to several classes of antibiotics, particularly to aminoglycosides and penicillin (IREGBU et al., 2002). The emergence of bacteria resistant to antibiotics following the use of antimicrobial agents is relatively well documented (AERESTRUP, 1999). Resident enteric micro flora normally considered non pathogenic may serve as a reservoir of antibiotic resistance for both animals and humans (JACQUELINE et al., 1982). We wish to submit that the high level of antimicrobial resistance reported in the present study may not be clinically important in animals, because the antimicrobial agents involved are rarely used in pastoral cattle. But the antimicrobial agents under investigation are considered important in the human health care services in Zambia where they are dispensed for various illnesses, as they are relatively cheap and affordable. They are also available over the counter, where they may be acquired without a prescription, promoting unrestricted drug use, as observed by SCHORLING et al. (1991) regarding use of antibiotics in developing countries. The use of cotrimoxazole prophylaxis in patients living with HIV/AIDS is well documented as it decreases the risk of opportunistic infections (WOOD, 2002).

This study suggests that there is a significant reservoir of antibiotic resistant *E. coli* and *Enterococci* among pastoral cattle. These microorganisms may form a possible source of antibiotic resistance, which could pose a public health hazard.

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SAŽETAK

Osjetljivost 83 izolata vrste *Escherichia coli*, 29 izolata vrste *Enterococcus faecium* i 62 izolata vrste *Enterococcus faecalis* izdvojenih iz uzoraka izmeta klinički zdravih goveda u području dodira domaćih i divljih životinja, određena je difuzijskim postupkom prema deset antimikrobnih pripravaka. Od ukupno izdvojenih, 8% sojeva bakterije *E. coli* bilo je dijarejogeno, 6% enteropatogeno i 2% enterotoksigeno. Ustanovljena je česta otpornost vrste *E. coli* prema penicilinu, eritromicinu, kotrimoksazolu i nitrofurantoinu. Enterokoki su u najvećem postotku bili otporni prema gentamicinu, amoksicilinu, ampicilinu i tetraciklinu. Nijedan od izolata *E. coli* nije bio otporan prema tetraciklinu, a nijedan od enterokoka nije bio otporan prema vankomicinu. Rezultati istraživanja upućuju na postojanje životinjskoga rezervoara bakterija otpornih na antibiotike za koje postoji mogućnost da se prenesu u ljudski prehrambeni lanac.

Ključne riječi: *E. coli*, *Enterococci*, antibiogram, govedo, Zambija