Microbiology of the external ear canal in six African elephants (*Loxodonta africana*)

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Samples collected from both external ear canals of six adult female African elephants *(Loxodonta africana)* were cultured for fungi, yeasts and aerobic and anaerobic bacteria. All the samples produced heavy growths of several aerobic bacteria, but anaerobic bacteria were rare and no fungi or yeasts were isolated. The most common bacterium isolated was *Staphylococcus epidermidis*, which was cultured from 11 of the 12 ears. *Acinetobacter calcoaceticus lwoffi*, α-haemolytic *Streptococcus* and *Corynebacterium* species, and *Aeromonas caviae* were all isolated from at least six of the 12 ears.

THERE is little information about diseases of the ear canal in elephants (Suedmeyer 2006). When collecting microbiological samples from clinically affected animals, it is important to differentiate between pathogenic and commensal bacteria (Kowalski 1988). Most mammals carry commensal bacteria, fungi and yeasts that are rarely pathogenic and can protect the ear from colonisation by pathogenic organisms. Commensal bacteria can also induce an immune response and stimulate antibody formation. The microbial flora of the human ear is affected by the person's age and their history of antibiotic treatment (Van Eldere 2000); 99 per cent of the organisms identified in healthy human ear canals are Gram-positive and include staphylococci and coryneforms (Stroman and others 2001).

The normal aural flora has been characterised in several domestic species including gerbils, horses and dogs (Thompson and others 1981, Sargent and others 2006, Aoki-Komori and others 2007). The only ungulate for which comprehensive microbiological information is available is the horse. Sargent and others (2006) found that most cultures of the proximal ear canal in horses are sterile and when bacteria are present they consist primarily of *Corynebacterium* species and *Staphylococcus intermedius*. In both human beings and horses the presence of *Staphylococcus* and *Corynebacterium* species may be due to the colonisation of the external ear canal by the normal skin flora.

The external ear canal is rarely examined during routine examinations of elephants. The external auditory meatus can be completely closed by voluntary muscular contraction and the positioning of the pinna (Suedmeyer 2006), preventing its full examination in a conscious animal. When a visual examination is not possible, a microbiological examination can help to determine the health of the external ear. The goal of this study was to characterise the normal aerobic, anaerobic and fungal flora in the ears of six healthy captive African elephants (*Loxodonta africana*), so that pathogenic organisms might be identified more easily.

Materials and methods

Samples were collected from six healthy female African elephants housed at the Kansas City Zoo. They were sampled annually during routine examinations and had been trained to allow the samples to be collected without being restrained. A sterile culture swab (Copan Diagnostics) was inserted aseptically into the external canal of each ear. Care was taken to avoid the pinna or external auditory meatus. The swabs were cultured for aerobic and anaerobic bacteria, and fungi and yeasts, and the bacteria were tested for antimicrobial susceptibility. The samples were inoculated on to 5 per cent sheep trypticase soy agar (SBA), MacConkey's agar, prereduced SBA, thioglycollate broth, and Sabouraud's and Mycocel medium (Remel). The aerobic plates (SBA and MacConkey's agar) were read after 24 to 48 hours of incubation at 35°C. The anaerobic plates were read after 72 hours of incubation at 35°C in anaerobic jars (BBL-GasPak Jars; Becton Dickinson). The fungal and yeast cultures were given a final reading after 30 days of incubation at 25°C. The Gram-positive and anaerobic bacteria were identified by standard methods (Murray and others 1999). The Gram-negative organisms were identified by means of the Sensititre AP-80 Gram-negative identification system (Patten and others 1995). The antimicrobial susceptibility tests used Sensititre Microbroth dilution freeze-dried susceptibility trays (Trek Diagnostic Systems) in accordance with the standards of the National Committee for Clinical Laboratory Standards (2003). The quality control isolates used in the study were as follows: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATTC 27853 and *Enterococcus faecalis* ATCC 29212.

Results

Four of the elephants had each ear cultured three times and two had each ear cultured twice. One had its left ear cultured a fourth time, while under anaesthesia, when its right ear was not accessible. Five of the elephants were healthy at the time of the evaluations, but one was being evaluated for a molar dental plate sequestrum (Suedmeyer and

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TABLE 1: Frequency of isolation of different bacterial species from the ears of six African elephants (*Loxodonta africana*) from which a total of 33 samples were taken

Bacteria	Times isolated	Number of Elephants	Ears
Staphylococcus epidermidis	20	6	11
Acinetobacter calcoaceticus lwoffi	12	5	8
α-haemolytic *Streptococcus*	10	5	7
Corynebacterium species	6	5	6
Aeromonas caviae	10	4	6
Moraxella osloensis	6	4	5
Klebsiella pneumoniae	5	4	5
Enterococcus species	5	3	4
Aeromonas sobria	4	3	4
Leclercia adecarboxy	4	3	4
Escherichia coli	4	3	3
Enterobacter sakazakii	3	3	3
Escherichia hermanii	3	3	3
Pseudomonas mendocina	3	3	3

others 2007) during one sample collection. The samples were collected with no obvious discomfort to the animals.

From the six elephants, 33 samples were collected. They all produced heavy growths of several aerobic bacteria but none had growths of fungi or yeasts. One sample produced a light growth of *Clostridium* species, but no other anaerobic bacteria were isolated. A total of 42 bacteria were isolated, and each sample yielded between three and six isolates. Seven samples contained two distinct populations of the same bacterium (*Staphylococcus epidermidis* in two samples, *Acinetobacter calcoaceticus lwoffi* in two samples, *Aeromonas sobria* in one sample and *Aeromonas caviae* in one sample) with different antimicrobial susceptibilities.

The most common bacterium isolated was *S epidermidis*, which was cultured from 11 of the 12 ears. *A calcoaceticus lwoffi*, α-haemolytic *Streptococcus* and *Corynebacterium* species, and *A caviae* were all isolated from at least six of the ears. Four species of *Pseudomonas* were isolated. *Pseudomonas mendocina* was found in three of the elephants, *P aeruginosa* was found in two, and *Pseudomonas alcaligenes*, *Pseudomonas luteola* and *Pseudomonas* species were each found in one. The prevalence of the most commonly isolated bacteria is shown in Table 1. The antimicrobial susceptibilities of the 10 most common isolates are shown in Table 2.

Discussion

The results suggest that the normal external ear canal of African elephants can be colonised by a wide variety of aerobic bacteria. The most common isolate was *S epidermidis*, which was found in at least one ear of each elephant. However, anaerobic bacteria were rare and no fungi or yeasts were detected. In contrast, it has been reported that *Malassezia pachydermitis* may be part of the normal otic flora of Asian elephants (*Elaphus maximus*) (Kuttin and Müller 1994).

The bacterial flora of these healthy African elephants differs slightly from the flora in horses and human beings. In one large study of human beings, Gram-positive aerobes constituted 96 per cent of the bacterial isolates from the external ear, with staphylococci being the most common bacterial type. Gram-negative aerobes, including *Pseudomonas* and *Acinetobacter* species, were rare. In horses, 43 per cent of the cultures collected by Sargent and others (2006) were sterile, whereas in this study all the elephants had heavy growths of several organisms. This difference may be due to a true species difference in bacterial load, although some of the organisms isolated in this study may have been due to contamination by the flora of the external ear or skin. The elephants were not chemically restrained and the collection of the samples was not as straightforward as in domestic horses, increasing the likelihood of contamination.

TABLE 2: Antimicrobial resistance patterns of the most common bacteria cultured from the ears of six female African elephants (*Loxodonta africana*), expressed as the percentages that were susceptible (S), intermediately susceptible (I) and resistant (R)

	Antibiotic agent																							
	Amikacin			Amoxicillin/ clavulanic acid			Ampicillin			Cefazolin			Ceftiofur			Chloramphenicol			Erythromycin			Gentamicin		
Bacteria	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Staphylococcus epidermidis	100			77	5	18	64		36	73		27	77		23	100			91		9	82		18
Acinetobacter calcoaceticus lwoffi	100			82	18		82		18	36	27	36	91		9	91		9	64	36		100		
α-haemolytic *Streptococcus*	64	36		100			100			91	9		82	9	9	91	9		27	64	9	100		
Corynebacterium species	100			100			50	50		83		17	100			100			100			100		
Aeromonas caviae	100			60	40		20		80	10	10	80	60	40		90	10				100	100		
Moraxella osloensis	100			100			100			80	20		100			100			100			100		
Klebsiella pneumoniae	100			100					100	100			100			80		20	20		80	80		20
Enterococcus species	50	50		100			100			100			100			100			100			100		
Aeromonas sobria	100			40	60				100	60	20	20	80		20	100			20		80	100		
Leclercia adecarboxy	100			75	25		75		25	75		25	75	25		100					100	100		
Escherichia coli	100			100			100			67	33		100			100					100	100		
Enterobacter sakazakii	100					100			100			100	100			100					100	100		
Escherichia hermanii	100			100					100	100			100			100					100	100		
Pseudomonas mendocina	100					100			100			100		50	50	50	50				100	100		

	Imipenem			Penicillin			Rifampin			Tetracycline			Ticarcillin/ clavulanic acid			Ticarcillin			Trimethoprim/ sulfamethoxazole		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Staphylococcus epidermidis	82		18	68		32	100			95	5		77		23	68		32	100		
Acinetobacter calcoaceticus lwoffi	100			45	27	27	82	18		100			91	9		82	9	9	100		
α-haemolytic *Streptococcus*	100			82	9	9	100			91		9	100			100			100		
Corynebacterium species	100			83	17		100			100			100			100			83		17
Aeromonas caviae	100					100	10	30	60	80	20		60	30	10	30	20	50	70		30
Moraxella osloensis	100			100			100			100			100			100			100		
Klebsiella pneumoniae	100					100			100	80		20	100					100	80		20
Enterococcus species	100			100			100			100			100			100			50		50
Aeromonas sobria	100					100	40	40	20	100			60	40			20	80	100		
Leclercia adecarboxy	100					100			100	100			75	25		50	25	25	75		25
Escherichia coli	100				33	67	33		67	67		33	100			100			100		
Enterobacter sakazakii	100					100		67	33	100		100	100				100	100	100		
Escherichia hermanii	100					100			100	100			100					100	100		
Pseudomonas mendocina	100					100			100	50		50	100			100			50		50

Two features of the ears of elephants that may affect their normal flora and prevalence of otitis are the ability of elephants to close the external auditory meatus completely and the presence of thick mucoid fluid in the normal ear. The humid environment of the ear of an elephant may favour the growth of certain bacteria, such as *Pseudomonas* species, in the absence of disease. No reports of disease of the external ear canal in elephants could be found, possibly owing partly to the difficulty in making a complete otic examination.

The prevalence of *Pseudomonas* species was surprisingly high. The species is not a normal part of the endogenous flora of the ear canals of horses (Sargent and others 2006) or dogs, but it is often found in dogs with otitis externa (Sharma and Rhoades 1975). The clinical significance of *Pseudomonas* in these elephants is not known, but they may be part of the normal flora, or due to a subclinical infection or contamination. Given the large numbers of other bacteria present, it is possible that the heavy growth of non-pathogenic bacteria may limit the impact of potentially pathogenic *Pseudomonas*, *Moraxella*, *Escherichia* and *Enterococcus* species that were also isolated frequently. These bacteria are often considered to be contaminants in domestic animals and positive cultures should be viewed critically before any treatment is applied.

The bacteria isolated had different degrees of resistance to common antimicrobials, but more than 50 per cent of the most common bacteria were resistant to penicillin and erythromycin. Resistance to imipenem, gentamicin and amikacin was rare. Most of the isolates of *S epidermidis*, the most common bacterium isolated, were susceptible to ampicillin, rifampin, trimethoprim/sulfamethoxazole and tetracycline, all of which are commonly used to treat elephants. This is clinically significant, because systemic antibiotic therapy for unrelated diseases could alter the microbial populations in the ear, damaging the normal flora and possibly allowing pathogenic bacteria to flourish (McCarthy and Kelly 1982, Van Eldere 2000). The six elephants had been treated with various antibiotics during their lives and the antimicrobial susceptibility patterns identified may not be applicable to other groups of elephants with different treatment histories.

The results suggest that clinicians should expect heavy growths of several aerobic bacteria from microbial cultures of the external ear canal of healthy African elephants. However, the antimicrobial susceptibility profiles of such normal bacterial flora may vary between institutions and individual animals. Elephants are expected to have mixed bacterial populations and any monoculture should be considered abnormal. These six elephants commonly carried Gram-positive and Gram-negative aerobic bacteria, unlike human beings or horses, which typically carry only Gram-positive organisms. Anaerobic organisms were rare and no fungi or yeasts were isolated; they should therefore be considered abnormal.

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