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Research Article

Multidrug Resistance of Common Bacterial Pathogens from Wounds and Otitis Externa in Small Animals during a 10 Year Period in Kenya

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ABSTRACT

Antimicrobial resistance is an increasing clinical challenge and a global public health concern. Emergence of resistant pathogens pose a threat to the patient due to the limitations and financial costs in managing them. Bacteriology laboratory records of clinical samples submitted from the University of Nairobi Small Animal Clinic between January 2004 and December 2013 were retrospectively reviewed with the aim to determine prevalence of common bacterial isolates from wound and otitis externa of dogs and cats and their antimicrobial susceptibility profiles

The most prevalent bacterial isolates recovered from dogs diagnosed with wounds, surgical site infections and otitis externa were: *Staphylococcus aureus* constituting 50% (140/280) and *Proteus* spp. 14% (40/280) respectively. Other less frequently recovered isolates included *Pseudomonas* spp. constituting 10% (28/280), other *Staphylococcus* spp. 8.6% (24/280), *Streptococcus* spp. 7.5% (21/280) and *E. coli* 5.4% (15/280) respectively. Resistance to antimicrobial agents was observed in the majority of the isolates in the study, with 97% (276/285) of the isolates demonstrating antimicrobial resistance to at least one drug. Antimicrobial resistance to sulphonamides (95%), potentiated sulphonamides (88%), ampicillin (67%), amoxicillin (62%) and tetracycline (56%) was relatively high for all bacterial species examined. *Staphylococcus aureus* isolates showed 95% resistance to sulfamethoxazole, 55% to ampicillin, 52% to tetracycline and 52% to amoxicillin/clavulanic acid respectively. *Pseudomonas* spp. showed the highest resistance with all (100%) isolates showing multidrug resistance (MDR) to amoxicillin, amoxicillin/clavulinic acid and sulfamethoxazole. The isolates also showed high level multidrug resistance to cotrimoxazole (93%), ampicillin (93%) and tetracyclines (80%) respectively. Low resistances to gentamicin (9%), norfloxacin (24%) and chloramphenicol (33%) were observed in all bacterial isolates.

This study confirms *Staphylococcus aureus* as the most prevalent bacterial isolate from wounds and otitis externa in small animals. *Proteus* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Streptococcus* spp. and *Escherichia coli* in descending order, were also commonly isolated. Gentamicin and norfloxacin, in that order were the most effective antimicrobial agents in the management of wound infections and otitis externa in small animals.

Key words: Antimicrobial resistance, Bacteria, Wounds, Otitis externa, Small animals

INTRODUCTION

Antimicrobial resistance is an increasing clinical threat and a global public health concern (Prescott *et al.*, 2002; Guardabassi *et al.*, 2004). Resistant pathogens pose a danger not only to the patient but may be spread in the hospital environment through healthcare workers and other routes resulting in nosocomial infections (Méan *et al.*, 2007). In companion animals, infections commonly treated with antimicrobial agents include otitis externa, urinary tract infections, wound infections and respiratory infections (Pedersen *et al.*, 2007; Windahl *et al.*, 2014). Certain infections such as canine pyoderma and some forms of otitis externa may require repeated courses of antimicrobial treatment (Guardabassi *et al.*, 2004; Pedersen *et al.*, 2007). Presently, a clinically significant number of pathogenic bacteria that cause infections in domestic animals and humans display some degree of antimicrobial resistance (Hawkey, 2008; Hunter *et al.*, 2010; Morris *et al.*, 2010). Loss of efficacy of antimicrobial substances can seriously compromise animal health and welfare.

Rational use of antimicrobial agents is necessary to control rising antimicrobial resistance of various pathogens.

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Culture and sensitivity testing is a useful procedure for determining microbes responsible for infection as well as guiding subsequent treatment (McEwen and Fedorka-Cray, 2002). However, empiric therapy is often used in treatment of infections in companion animal medicine before results of culture and sensitivity are known (Authier *et al*., 2006; Windahl *et al*., 2014). Knowledge of bacterial organisms involved in infections and their susceptibility patterns is therefore important to guide rational therapy.

The present study was undertaken to identify the most commonly isolated pathogens from privately owned dogs and cats presented to the University of Nairobi's small animal clinic with wounds and otitis externa and to determine their antimicrobial susceptibility patterns. In addition, the study aimed at prioritising the efficacy of commonly used antimicrobial agents in management of wounds and otitis externa.

MATERIALS AND METHODS

Data collection

The bacteriology laboratory records of clinical samples submitted between January 2004 and December 2013 from the University of Nairobi Small Animal Clinic were investigated. All the samples were from animals presented to the University of Nairobi's small animal clinic during the study period. The records were examined to retrieve data on microbial cultures from dogs and cats presented with otitis externa and wounds. Animal data retrieved from these records included: Date of submission, species, sex of the animal and source of the sample submitted (wound or ear)

Microbial growth

For each clinical sample submitted, the number of microbial isolates and microorganisms isolated from either wounds or ear swab were recorded. Bacteria of the Genus Staphylococcus were recorded as *Staphylococcus aureus* or broadly classified as other *Staphylococcus* spp. (for those that were coagulase negative). Bacterial species with low isolation rates of less than 1% were grouped together as other species.

Antimicrobial Susceptibility Testing (AST)

Routine disk diffusion procedures were employed in AST by the laboratory. The bacterial isolates were tested against a panel of 8 out of 16 antimicrobial agents. The antimicrobial discs used in testing were either single discs or combined, as in Multodisk® (Oxoid) and Octodics® (HI Media). The most commonly employed antimicrobial discs used in AST were, ampicillin (2µg), gentamicin (10µg), cotrimoxazole (25µg), chloramphenicol (10µg), tetracycline (10µg), potentiated amoxicillin (amoxycillin-clavulinic acid) (30µg), norfloxacin and sulfamethoxazole (25µg).The zone of inhibition (ZOI) around each antimicrobial disc was measured and the various bacteria scored by the laboratory as either being susceptible or resistant to the respective antimicrobial agent according to the following criteria:

- ZOI of 0-8mm were scored as 0 or Resistant (R),
- ZOI of 9 mm–15 mm was scored 1+ or slightly sensitive
- ZOI of 16 mm – 22 mm was scored 2+ or sensitive
- ZOI of 23 mm and above was scored 3+ or very sensitive.

Data analysis

Animal species, sex, source of the swab, number of microbial isolates, name of microorganism isolated and antimicrobial susceptibility profile of the various bacteria against the respective antibiotics were entered into a Microsoft excel worksheet and a pivot table generated to calculate the total number of samples recorded during the study period. The total number of each species, sex of the animals and source of the swabs were tallied and expressed as percentages. The total number of bacterial pathogens isolated were calculated and expressed as percentages. Antimicrobial susceptibility was expressed as either susceptible or resistance. Overall resistance for each antimicrobial agent was calculated. Percentage resistances for each bacteria were calculated for each antimicrobial agent as well as for individual bacterial isolates. Phenotypic multidrug resistance profile displayed by isolates and the frequency of multidrug resistance to different classes of antimicrobial agents was also calculated.

RESULTS

Samples

During the study period, a total of 305 samples were recorded. The samples were from 211 animals, of which 154 animals were sampled once, 36 sampled twice, 15 animals sampled 3 times, 3 animals sampled four times, 2 animals sampled 5 times and one dog sampled 12 times over the study period. The majority of samples (n=200) were submitted from dogs (over 90%) with the rest being samples from cats. Male animals accounted for 68% (143/211) compared to 27% (57/211) females. However, the sex of 11 animals was not indicated in the records. Wound samples constituted 29% (n=89) whereas ear infections constituted 71% (n=216).

Microbial growth

Growth was recorded from 280 samples, of which 278 samples were bacteria while two samples were fungal species which were neither considered nor analysed . Gram positive bacteria were 69.5% (n=194) of samples compared to gram negative bacteria which were 29.8% (n=84) of the samples. Single isolates (n= 204) were isolated more frequently than mixed isolates (n= 74). The predominant bacterial isolates recorded were *S.aureus* which consitututed 50% (140/280) and *Proteus* spp. 13.9% constituting (39/280). Other less frequently recovered isolates included *Pseudomonas* spp.constituting 10% (28/280), *Staphylococcus* spp. 8.6% (24/280), *Streptococcus* spp. 7.5% (21/280) and *E.coli* 5.4% (15/280). The percentages of different bacterial isolates recorded from either ear or wounds of animals is shown in Table 1.

S.aureus was the most common isolate, regardless of the source of the sample (Table 1). *Proteus* spp.was more frequently isolated in ear swabs (16.5%), than from wounds (6.7%). *Pseudomonas* spp. was also recorded as important pathogens in ear infections (12.6%) but found to be minor pathogens in wound infections with isolation rate of 2.7%. *E.coli* was found to be a common cause of contamination in wounds (16.2%) but did not seem to be an important cause of ear infections (1.5%) (Table 1).

Table 1: Frequency of microbial isolates from either wounds or otitis externa of dogs and cats

Isolate	Ear (Percent)	Wound (Percent)	Total	Percent
Staphylococcus spp.	126 (61.1%)	38 (51.4%)	164	58.6
- *Staphylococcus aureus*	107 (51.9%)	33 (44.6%)	140	50
- *Other Staphylococcus* spp.	19 (9.2%)	5 (6.75%)	24	8.6
Proteus spp.	34 (16.5%)	5(6.75%)	39	13.9
Pseudomonas spp.	26 (12.6%)	2 (2.7%)	28	10
Streptococcus spp.	10 (4.9%)	11 (14.9%)	21	7.5
Escherichia coli	3 (1.5%)	12 (16.2%)	15	5.4
Other spp.	7 (2.4%)	6 (8.1%)	13	4.6
Total	206	74	280	100

Table 2: Percent resistance (%) of bacterial isolates from dogs and cats against 8 antimicrobial agents

Drug	Resistant isolates	Total	% Resistant
Ampicillin	180	264	68.2
Amoxycillin	34	53	64.2
Amoxycillin/Clavulinic acid	96	169	56.8
Tetracycline	140	250	56
Sulfamethoxazole	130	135	96.3
Cotrimoxazole	222	251	88.5
Gentamicin	24	258	9.3
Norfloxacin	41	176	23.3

Table 3: Multidrug resistance profiles displayed by isolated bacterial pathogens from wound and external ear infections of dogs and cats

Bacteria	Resistance profiles	Number of isolates
Staphylococcus aureus	AMP, COT, C, TET, AMC, SXT	5
	AMP, COT	5
	AMP, COT, TET, SXT	4
	COT, SXT	4
	AMP, COT, AMC, SXT	3
	AMP, COT, TET	3
	CEF, COT	2
	COT, C, SXT	2
	COT, TET, SXT	2
	COT, AMC, SXT	2
	AMP, APC, COT, TET	2
	AMP, COT, TET, AMC	2
	AMP, TET, AMC, SXT	2
	AMP, COT, C, TET, SXT	2
	AMP, COT, TET, NOR, AMC	2
	GEN, COT, C, NOR, SXT	2
Proteus spp.	AMP, COT, C, TET, SXT, KAN, STR	2
	AMP, CEF, COT, C, TET, SXT	2
Pseudomonas spp.	AMP, COT, TET	4
	AMP, CEF, COT, TET, AMC	2
	AMP, APC, COT, C, TET, AMC, ERY	2
Escherichia coli	AMP, CEF, COT, AMC, SXT	2
	COT, SXT	2
Other *Staphylococcus* spp.	AMP, COT, AMC, SXT	2
Streptococcus spp.	AMP, TET, AMC, SXT	3

Antimicrobial resistance of bacterial isolates

Resistance to antimicrobial drugs was observed in the majority of the isolates in the study, with 98% (274/278) of the isolates demonstrating antimicrobial resistance to at least one drug. Resistances to sulphonamides (96%), potentiated sulphonamides (88%), ampicillin (68%), amoxicillin (64%) and tetracycline (56%) were relatively high for all bacterial species examined (Table 2).

Phenotypic multidrug resistance (more than two) profiles displayed by the isolates are shown in Table 3. Majority of the isolates were resistant to penicillin, sulphonamides and tetracyclines (Table 4).

Staphylococcus aureus isolates showed 95% resistance to sulfamethoxazole, 55% to ampicillin, 52% to tetracycline and 52% to amoxicillin/clavulanic acid respectively (Table 5). *Proteus* spp. isolates displayed high resistance to sulfamethoxazole (100%), ampicillin (94%), cotrimoxazole (93%) and tetracycline (69%). *Pseudomonas* spp showed the highest multidrug resistance with all (100%) isolates showing resistance to amoxicillin, amoxicillin/clavulinic acid and sulfame-thoxazole, the isolates also showed multidrug resistance to cotrimoxazole (93%), ampicillin (93%) and tetracyclines (80%) respectively (Table 5). Low resistance to gentamicin (9%), norfloxacin (24%) and chloramphenicol (33%) was observed in all bacterial isolates.

DISCUSSION

In this study, *Staphylococcus* spp. were the most common isolates from samples submitted in our laboratory accounting for over half (58.6%) of the total bacterial isolates. The study confirms the etiological and clinical importance of staphylococcal species as colonisers of skin and important causes of infection in skin of animals. The high percentage of staphylococci was expected since staphylococcal species are normally found on the skin as commensals (Weese, 2010). However, they are also opportunistic pathogens and *S.pseudintermedius* as well as *S. aureus* are leading causes of surgical site infections (SSI) in animals (Vengust *et al*., 2006; Turk *et al*., 2015).The finding in this study that *Staphylococcus aureus* was the most prevalent isolate from wounds is similar with a report from Bangladesh (Rahman *et al*., 2003). In contrast, low prevalence of *Staphylococcus aureus* from wounds was recorded with isolation rates as low as 5.8% and 12.2% for dogs and cats respectively in a study in Germany (Vincze *et al*., 2014). *Staphylococcus aureus* has been recognized as an important wound pathogen and a major cause of delayed healing and infection in wounds. The prevalence of *Staphylococcus aureus* (44.6%) isolated from wounds of dogs has not been reported in Kenya. Other microorganisms isolated from the wound swabs in this study included *E. coli*, *Streptococcus* spp. other *Staphylococcus* spp. and *Proteus* spp. This is similar to the findings by Rahman *et al*., 2003,who in their study isolated *E. Coli*, *Klebsiella* spp. and *Proteus* spp. at frequencies of 18.94%, 16.7% and 11.4% respectively. This is also in agreement with Urumova *et al*. who also found a high incidence of enterobactericiae in wounds, especially *E. coli* (28.1%). The polymicrobial growth demonstrated with 24% of the swabs yielding more than one organism in this study was consistent with other reports of similar studies conducted eslewhere (Meyers *et al*., 2008; Padhy *et al*., 2014). Colonisation in wounds is mostly polymicrobial involving different microorganisms that are potentially pathogenic (Bowler *et al*., 2001).

Table 4: Multidrug resistance profiles to different antimicrobial classes

Resistant profiles	Number of isolates	% isolates
Penicillins, sulphonamides, tetracyclines	43	15.5
Penicillins, sulphonamides	25	9
Penicillins, sulphonamides, tetracyclines, amphenicols	20	7
Penicillins, sulphonamides, tetracyclines, aminoglycosides	11	4
Penicillins, sulphonamides, tetrcyclines, cephalosporins	11	4
Penicillins, sulphonamides, aminoglycosides	10	3.6
Penicillins, sulphonamides, amphenicols	8	2.9
Penicillins, sulphonamides, tetracyclines, cephalosporins, amphenicols	7	2.5
Penicillins, sulphonamides, tetracyclines, aminoglycosides, cephalosporins	6	2.2
Penicillins, sulphonamides, teracyclines, quinolones	5	1.8
Penicillins, sulphonamides, cephalosporins	5	1.8

Table 5: Antimicrobial resistance (%) of common bacterial isolates to antimicrobial agents

Drug	S.aureus % (n=139)	Proteus % (n=40)	Pseud % (n=28)	E. coli % (n=15)	Staph % (n=24)	Strep % (n=19)
AMX	38	100	100	N/A*	74	N/A*
AmC	52	58	100	N/A*	70	40
AMP	55	94	93	78	66	54
C	15	45	61	36	29	6
GEN	11	0	3	8	0	29
NOR	28	14	14	33	20	7
TET	52	69	80	36	50	54
COT	88	97	93	93	90	63
SXT	95	100	100	90	92	100

KEY: AMP-Ampicillin; AMX- Amoxycillin; AmC- Amoxicillin/clavulanic acid; C- Chloramphenicol; GEN- Gentamicin; NOR-Norfloxacin; TET- Tetracycline; COT- Cotrimoxazole; SXT- Sulfamethoxazole. S.aureus- *Staphylococcus aureus*; Pseud-*Pseudomonas* spp.; Staph- Other *Staphylococcus* spp.; Strep- *Streptococcus*spp; E.coli- *Escherichia coli*N/A*:≤3 isolates tested for sensitivity to that antimicrobial agent

In this study, 61.1% of bacterial isolates from ear swabs of dogs with otitis externa belonged to the Genus Staphylococcus, which is comparable to other studies (Lilenbaum *et al*., 1999; Lyskova *et al*., 2007; Petrov *et al*., 2013). Malayeri *et al*., 2010 reported a high prevalence of 73.8% of *Staphylococcus* spp. in Iran. Other bacteria isolated from ear swabs in this study included *Proteus* spp., *Pseudomonas* spp. and *Streptococcus* spp. comparable to a previous study in Kenya (Mande and Kitaa, 2005) in which, *Staphylococcus aureus* (51.2%) was found to be the most prevalent isolate, with *Streptococcus* spp. (14%), *Pseudomonas* spp. (14%) and *Proteus* spp. (10%) also commonly isolated from ear swabs of dogs with otitis externa. Our findings show an increase in Staphylococci isolation from otitis externa of 59% vs 51.2% compared to a previous study in Kenya. Our study also shows that *Proteus* spp. has become a more important pathogen with a prevalence of 15.5% up from 10% compared to a study by Mande and Kitaa, 2005.

Previous studies on wounds in dogs, have found pathogens isolated to be most sensitive to potentiated sulphonamides and amoxicillin/clavulinic acid preparations (Meyers *et al*., 2008; Urumova *et al*., 2012). This observation is in total contrast with findings in this study in which comparatively higher resistance rates were observed for potentiated sulphonamides (88%) and amoxycillin/clavulinic (57%). Interestingly, Pedersen *et al*., 2007 found no resistance to amoxicillin/clavulinic acid in their study which involved bacterial isolates from clinical submissions in Denmark. Authier *et al*., 2006 even suggested that amoxicillin/clavulinic acid could be an adequate antimicrobial agent for treatment of skin infections by *Staphylococcus* spp.. However, based on the findings of this study, the use of these antimicrobials as the first line of treatment for empirical therapy could result in treatment failure. Our study demostrated that gentamicin and norfloxacin were the most effective antimicrobial agents against majority of the isolates. Gentamicin has been indicated for the treatment of staphylococcal infections (Lilenbaum *et al*., 2000), however, some investigators feel that its use should be limited to cases where initial treatment has failed (Authier *et al*., 2006).

Several studies have investigated the susceptibility of Coagulase positive *Staphylococcus* spp. (COPS) to various antimicrobials (Lilenbaum *et al*., 2000; Tejedor Junco and Martín Barrasa, 2002). In this study, 97% of the *Staphylococcus aureus* isolates were resistant to at least one drug which is in agreement with a report from Brazil by Lilenbaum *et al*., 2000 who reported that strains displayed a high level of resistance with 90.9% of the isolates in their study showing resistance to at least one drug. Our findings were in a slight contrast with those of Tejedor (Junco and Martin Barrasa 2002) who reported only 64.8% of COPS displaying resistance. In the present study, the least effective antimicrobial agents against *Staphylococcus aureus* were sulphonamides (sulfame-thoxazole), potentiated sulphonamides (cotrimoxazole), ampicillin and tetracycline. The highest level of resistance observed was for potentiated sulphonamides with a resistance rate of 95%. Lilenbaum *et al*., 2000 also reported majority of Staphylococcal isolates in Brazil to be resistant to this drug though at a slightly lower rate of 72.7%. On the other hand, a study in Denmark by Pedersen *et al*., 2007 described very low resistance of *S.intermedius* from ear isolates to potentiated sulphonamides. Most of the isolates were also found to be susceptible to amoxicillin (62%), suggesting that this drug can be used as a first line treatment prior to antimicrobial susceptibility testing.

Pseudomonas spp. and *Proteus* spp. isolates in this study displayed the highest resistance to most antimicrobial agents, which is similar to other reports (Pedersen *et al*., 2007; Malayeri *et al*., 2010). *Pseudomonas* spp. is mostly isolated in chronic cases of otitis and has been reputed for its high level resistance to most antimicrobials. The multidrug resistance (MDR) was observed to be the case in this study with 92% of *Pseudomonas* spp. isolates showing resistance to 4 or more drugs. Highest resistance was recorded for amoxicillin, amoxicillin/clavulinic and sulfamethoxazole, with all the isolates tested against these drugs showing 100% resistance. These isolates also showed significant resistance to chloramphenicol (61%) and tetracycline (80%). However, Pedersen *et al*. (Pedersen *et al*., 2007) in their study found that all the *Pseudomonas* spp. isolates were resistant to ampicillin, amoxicillin/clavulinic acid and erythromycin similar to our observation. Malayeri *et al*., 2010also concurred with our observations where all *Pseudomonas* spp. isolates in their study showed 100% resistance to ampicillin, amoxicillin/clavulinic, erythromicin, rifampin, penicillin G. Hariharan *et al*. 2006 found that *Pseudomonas* spp. isolates were also highly resistant to chloramphenicol (99%) and 98% to doxycycline which is in slight contrast to the findings in this study.

Proteus spp. was the second most frequently isolated microorganism accounting for 14% of all isolates which is a similar finding in other studies (Pedersen *et al*., 2007; Petrov *et al*., 2013). The prevalence of *Proteus* spp. was also similar to a report from the Czech republic (Lyskova *et al*., 2007). The present study found all *Proteus* spp. isolates to be resistant to amoxicillin and sulphonamides, but susceptible to gentamicin. In addition, we observed high resistance to ampicillin (94%) and cotrimoxazole (97%), while the isolates showed moderate resistance to tetracyclines (69%), amoxicillin/clavulanic (58%) and chloramphenicol (45%). Similar findings were reported by Petrov *et al*. 2013 who found all isolates to be susceptible to gentamicin. In addition they observed resistance to tetracycline (81%), chloramphenicol (74%) though at higher rates, and the isolates in their study were resistant to ampicillin. In contrast to this study, Pedersen *et al*., 2007 found all *Proteus* spp. isolates in their study to be resistant to tetracyclines and the majority of the isolates susceptible to ciprofloxacin and gentamicin which is in agreement with the findings in this study.

Conclusions

The present study confirms *Staphylococcus aureus* as the predominant bacteria in wounds and otitis externa of small animals in Kenya. The findings from the present study demonstrate that aminoglycosides and fluoroquinolones were more efficient against common bacterial isolates present in wounds and external ear infections and should be reserved the last line antibiotics in treatment of these conditions in small animals.

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