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Prevalence of antimicrobial resistance from bacterial culture and susceptibility records from horse samples in South Africa

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ABSTRACT

The continuous increase in prevalence of antimicrobial resistant bacteria presents a significant public health problem and is an indicator that antimicrobial prudent usage guidelines are not being followed, especially in developing countries. Despite trends being available from numerous countries, there is little published for South Africa.

This study was aimed at estimating the prevalence and trends of antimicrobial resistance from bacterial isolates from equine clinical samples submitted for culture and susceptibility testing to the veterinary bacteriology laboratory of the University of Pretoria. The study covered a period of seven years from 2007.

A total of 1505 bacterial isolates were included in this study comprising isolates from 2007 (n = 447); 2008 (n = 285); 2009 (n = 258); 2010 (n = 102); 2011 (n = 89); 2012 (n = 248) and 2013 (n = 76). For this study, multiple drug resistance was above 50% for all the isolates. The Cochran-Armitage test showed evidence of a significantly increasing trend in prevalence of resistance to several antimicrobial agents, including amikacin (*E. coli*, Staphylococcus), AMX/AMP (Corynebacteria, Lactobacillus and Salmonella), chloramphenicol (Enterococcus, *E. coli*, Pseudomonas, Streptococcus, Staphylococcus and Salmonella), enrofloxacin (*E. coli*, Staphylococcus, Salmonella and Pseudomonas) and gentamicin (Salmonella, Staphylococcus). The data obtained from this study is relevant to equine practitioners, as it helps enhance the body of veterinary knowledge pertaining to antimicrobial resistance in common equine pathogens in South Africa.

1. Introduction

Bacterial culture and antimicrobial susceptibility testing records are a valuable and inexpensive source of information that can be used to determine the prevalence and trends of antimicrobial resistance (AMR) to different antimicrobials (Toombs-Ruane et al., 2015). The results from such trends can provide guidance as to which antimicrobials are appropriate for use in a particular area as susceptibility profiles vary from anatomical area to area (Walker, 2006; Bowen, 2013). When a horse with a suspected bacterial infection is presented to a veterinarian, the ideal thing to do will be to obtain appropriate and correctly collected samples for culture and antimicrobial susceptibility testing, and make therapeutic decisions based on the laboratory results (Johns and Adams, 2015). However, this is not practical in most ambulatory and hospital clinical situations; for example in life-threatening bacterial infections where waiting for laboratory results could potentially affect the clinical outcome, as well as the long term performance, of the animal (Hughes et al., 2013; Johns and Adams, 2015).

Faced with the above challenges, most veterinarians will resort to empirical antimicrobial prescribing based on anticipated bacterial isolates and susceptibility patterns (Hughes et al., 2013); knowledge gained from past experience or what they learned from their clinical years at school (Chipangura et al., 2017). Since empirical antimicrobial prescribing is common practice in South Africa (Chipangura et al., 2017), trends in AMR from recent clinical cases need to be available to guide empirical selection practice, especially if sensitivity testing is not possible. On-going monitoring of resistance is vital to ensure that empirical antimicrobial therapy is evidence-based and current (Bowen, 2013; Toombs-Ruane et al., 2015). Antimicrobial resistant trends when relevant for a particular area, will allow veterinarians to make informed decisions regarding appropriate antimicrobial choice awaiting results from culture and susceptibility testing (Johns and Adams, 2015). Since continuous antimicrobial usage is considered the biggest driver for AMR development, identifying AMR trends can be used in making decisions to limit the use of particular antimicrobials thereby minimising the progression of resistance (Aucoin, 2007; Bowen, 2013).

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Reports of AMR trends from bacteria isolated from horses in South Africa are rarely available, but may be the most accurate method of assessing anticipated antimicrobial efficacy. The current study reports AMR patterns with the objective of determining the prevalence and trends in equine antimicrobial resistance, based on records from the veterinary bacteriology laboratory, University of Pretoria.

2. Methodology

In order to meet the objectives, the study analysed past records from equine samples submitted to the veterinary bacteriology laboratory, University of Pretoria. The samples were submitted for bacterial culture and susceptibility testing. The study covered a period of seven years from 2007. In addition to supporting the University of Pretoria's veterinary academic and referral hospital, the laboratory services mainly the veterinary practices in the Gauteng province. Bacterial culture and antimicrobial susceptibility testing was performed by experienced technicians supervised by a veterinary microbiologist. The laboratory used disc diffusion assay to determine antimicrobial susceptibility of the isolates. Each isolate was tested for susceptibility using a panel of standardised discs to the following antimicrobials: amikacin, amoxicillin/ampicillin (AMX/AMP), ceftiofur, chloramphenicol, doxycycline/oxytetracycline (DOX/TET), enrofloxacin, gentamicin, penicillin G and trimethoprim-sulphamethoxazole (SXT). Susceptibility against carbenicillin, ceftazidime, imipenem and piperacillin was specifically tested in isolates that were resistant to all the antimicrobials mentioned before. Data was collected from all available equine records and captured onto Microsoft Access then records were placed in tables.

2.1. Statistical analysis

The resistance data was organized into a contingency table with one row for each time period and two columns for resistance and susceptibility. Prevalence of resistance was calculated as the number of bacterial isolates of the same genus found to be resistant to at least one antimicrobial, expressed as a percentage of the total number of isolates for a given time period. The editing of data was then performed using Microsoft Excel and evaluated by descriptive statistics. Statistical analysis was performed using SPSS software. The chi-square ($\chi 2$) test was undertaken to test for significant changes in antimicrobial resistance among each bacterial genus over the period and temporal trends in the prevalence of antimicrobial resistance investigated for each antimicrobial agent using the Cochran Armitage trend test. For these analyses, p-values < 0.05 were considered significant.

3. Results

The isolates were tested for susceptibility against the commonly used antimicrobials and in some cases not all were tested. For the purposes of this study, isolates with intermediate susceptibility were classified as resistant and multiple drug resistance (MDR) was reported as resistance to three or more antimicrobials. Oxytetracycline and doxycycline resistance were combined in this study as a general indication of tetracycline resistance. In a similar manner, resistance to amoxicillin and ampicillin, were grouped due to the similarity between the molecules.

A total of 1505 bacterial isolates from 907 samples (2007 = 278; 2008 = 169; 2009 = 144; 2010 = 52; 2011 = 61; 2012 = 143; 2013 = 60) were included in this study comprising isolates from 2007 (n = 447); 2008 (n = 285); 2009 (n = 258); 2010 (n = 102); 2011 (n = 89); 2012 (n = 248) and 2013 (n = 76). Of these the majority were from clinical cases, with the exception of 14 isolates recovered post-mortem (organ aggregated samples). The isolates were collected from various organ systems, and are listed by the region of collection as specified on the laboratory submission form (Table 1). The laboratory did not log the records of samples, for which no growth was observed.

Table 1
Number of isolates per sample type received per year for culture and susceptibility testing.

Sample	2007	2008	2009	2010	2011	2012	2013	Total
Abdominal swab	15	–	5	2	–	–	1	23
Abscess	25	10	9	3	3	4	–	54
Aspirate	9	2	1	3	1	–	–	16
Bronchiolar-alveolar lavage	18	11	6	3	2	5	2	47
Blood	5	–	8	6	2	11	2	34
Bone	5	4	1	–	–	–	3	13
Brain	1	–	–	2	1	–	–	4
Clitoral swab	14	–	–	–	–	–	–	14
External Ear	–	–	–	–	–	1	–	1
Eye swab	1	–	6	–	3	–	–	10
Faeces	20	6	1	1	9	14	11	62
Gastric fluid	1	4	6	–	4	–	2	17
Guttural pouch	18	6	6	4	6	3	–	43
Intestine swab/biopsy	3	1	–	2	1	–	–	7
Joint aspirate/swab	3	6	7	9	1	4	–	30
Kidney	–	–	–	–	1	–	–	1
Laryngeal swab	2	–	–	–	–	–	–	2
Liver	1	–	–	–	–	1	–	2
Milk	–	–	1	–	–	–	–	1
Organ pool	1	1	1	8	–	3	–	14
Placenta/Umbilical cord	16	1	4	1	2	–	3	27
Semen/scrotal aspirate/ spermatic cord	3	1	1	1	1	2	–	9
Swab	14	2	1	12	5	73	–	107
Tooth	–	–	–	–	–	4	–	4
Trans-tracheal aspirate	60	51	21	8	6	28	5	179
Nasal swab	65	34	60	12	10	64	34	279
Uterine/endometrial swab	128	136	100	9	3	2	6	384
Wound/skin biopsy/ swab	19	9	13	16	28	29	7	121
Total	**447**	**285**	**258**	**102**	**89**	**248**	**76**	**1505**

The most common source of bacterial isolates was from endometrial swabs (25.5%), upper respiratory (nasal swabs; 18.5%) and lower respiratory (trans-tracheal aspirates and bronchiolar-alveolar lavage; 21.7%). With all samples aggregated, the most commonly identified organism were Streptococci (19.0%), *E. coli* (16.0%) and Staphylococcus (15.0%).

When looking at resistance of the various bacterial microorganisms cultured to the various antimicrobial agents tested (Table 3), the penicillin group of drugs, had over 50% resistance against most of the isolates while that for enrofloxacin, chloramphenicol, gentamicin averaged 35%. This study also looked at resistance to the various antimicrobials by sample type (Table 4). The lowest resistance for the most common sample types was as follows; uterine/endometrial swab (chloramphenicol 31.6%; imipenem 25.0%), URT/nasal swab (chloramphenicol 21.7%, enrofloxacin 28.2%), TTA (chloramphenicol 23.1%; enrofloxacin 25.4%; imipenem 15.4%) and wound (chloramphenicol 37.8%; imipenem 23.8%; ceftiofur 39.8%).

Prevalence of antimicrobial resistance was reported for the following isolates; Acinetobacter, Actinobacillus, Aeromonas, Bacillus, Citrobacter, Corynebacteria, *E. coli*, Enterobacter, Enterococcus, Klebsiella, Lactobacillus, Micrococcus, Pasteurella, Proteus, Providencia, Pseudomonas, Rhodococcus, Salmonella, Serratia, Staphylococcus and Streptococcus (Table 2). Resistance data for each bacterial genus was aggregated for analysis, as the trend was difficult to evaluate from year to year due to differing number of samples submitted for evaluation. Multiple drug resistance was above 50% for all the isolates (Fig. 1). The Cochran-Armitage test showed evidence of a significantly increasing trend in prevalence of resistance to several antimicrobial agents, including amikacin (*E. coli*, Staphylococcus),