Aerobic Bacterial Isolates in Equids and Their Antimicrobial Susceptibility Pattern

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ABSTRACT

A study was conducted to isolate bacteria from septicemic cases in equine species and conduct antimicrobial susceptibility from December 2005 to June 2006 in Debre Zeit and Nazareth areas, Ethiopia. Bacteria were isolated in 20% of the suspected septicemic cases. Seventy percent of the isolates were gram-negative and the rest gram-positive. All bacteria were isolated in pure culture. *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* were isolated with isolation rates of 45%, 30%, and 25%, respectively. In this study, it was found that most bacteria are susceptible to limited antimicrobials. The most effective drugs were polymyxin B (90.0%) followed by gentamicin (85.0%), chloramphenicol (80.0%), and kanamycin (80.0%). Neither of the isolates was susceptible to tetracycline. Statistically significant association was not observed between host risk factors (age, sex, species, and body temperature) with frequency of each bacterial isolate ($P > 0.05$). The present study is limited in duration and sample size, therefore, further investigation should be carried out to elucidate septicemia in equines adequately in the country. In addition, rational use of antimicrobial therapy should be encouraged to minimize the risk of antimicrobial resistance.

INTRODUCTION

Septicemia is the disease state compounded of toxemia, pyrexia, and the presence of large numbers of infectious micro-organisms including viruses, bacteria, and protozoa in the bloodstream.[1] The presence of petechiae in mucosae suggests septicemia. Evidence of localization in individual organs is also contributory evidence that septicemia is present or has occurred. Definitive diagnosis of septicemia, however, can be made only by isolation of the causative agent from blood.[1,2]

In Ethiopia, although clinically suspected septicemia has been a major health problem of equines,[3] there is no information on the types of bacterial isolates and their susceptibility patterns. Therapeutic measures have been practiced based on clinical signs without laboratory confirmation. The present study was, therefore, conducted to isolate

aerobic bacteria from blood and determine their susceptibility patterns from clinically suspected septicemic equine cases.

MATERIALS AND METHODS

Study Area and Animals

The study was conducted from December 2005 to June 2006 at Donkey Health and Welfare Project (DHWP) and Society for the Protection of Animals Abroad (SPANA) clinics at Debrezeit and Nazareth towns, Ethiopia. The animals included in this study were horses, donkeys, and mules presented to the clinics for various health problems. All equines irrespective of age, sex, and species were examined for evidence of septicemia during the study period. Blood samples were taken from equines who showed depression, lethargy, increased respiration, fever, inappetence, petechiae in mucosae, and conjunctivae. The sample size for each study site varied according to the availability of suspected septicemic cases. A total of 100 blood samples were taken: 60 samples were taken at Debre Zeit clinic (20 horses, 30 donkeys, and 10 mules) and 40 samples were taken from Nazareth clinic (30 horses and 10 donkeys).

Collection of Samples and Identification of Bacteria

Blood samples were collected from the jugular vein under strict aseptic conditions. The area over the site of venipuncture was shaved, cleaned thoroughly, and disinfected with 70% ethyl alcohol. Blood was taken after at least 30 seconds.[4] All blood samples were inoculated into brain-heart infusion media at a dilution of 5:1.[5] The inoculated medias were incubated aerobically at 37°C and checked for growth after 24, 48, and 72 hours to rule out slow-growing bacteria. Samples from brain-heart infusion were transferred and streaked onto blood agar and incubated for 24-48 hours at 37°C. Subcultures were made on MacConkey (Oxoid, Hampshire, England) and blood agar and incubated for 24 hours. The plates were examined for growth, morphologic features such as colony size, shape, color, and hemolytic characteristics. The smears from the cultures were gram stained and examined microscopically to determine gram reaction and cellular morphology. Primary biochemical tests used include catalase, oxidase, motility, and oxidation-fermentation (O-F) tests.[4] For secondary identification, the isolates were subjected to other biochemical or metabolic tests including: indole, methylene red, Voges-Proskauer, citrate utilization (IMVIC), urease, triple sugar iron (TSI), and growth on selective media.[4,6]

Antimicrobial Susceptibility Test

Susceptibility of bacteria to the commonly used antimicrobials was conducted according to Kirby Bauer procedure.[4-6] A distinct colony was inoculated into 5 mL of nutrient broth and incubated at 35-37°C for 5 hours. The turbidity of the microorganisms then was determined with 0.5 mL McFarland standard. The standard was prepared by adding 0.5 mL of solution A (0.048 M $BaCl_2 \cdot 2H_2O$) to 99.5 mL of solution B (0.18M H_2SO_4). Mueller-Hinton agar was prepared and dispensed on a sterile Petri dish on a level surface to a depth of 4 mm and cooled to 50°C. A sterile cotton swab on a wooden applicator stick then was used to transfer the diluted bacterial suspension to the plates; excess fluid was squeezed out by rotating the swab against the sides of the tube. The plates were seeded uniformly by rubbing the swab against the entire agar surface in 3 different planes roughly 60 degrees to each other. Fifteen minutes after inoculation (time used to dry the inoculums), the discs of antibiotics erythromycin (E) 15 µg, gentamicin (G) 10 µg, kanamycin (K) 30 µg, chloramphenicol (CAF) 30 µg, ciprofloxacin (CF) 5 µg, polymyxin B (PB) 300 units, streptomycin(s) 10 µg, tetracycline (T) 30 µg, penicillin G (P) 10 units, oxytetracycline (O) 30 µg, and ampicillin (Am) 10 µg were applied to the surface of the inoculated plates by using sterile forceps. All discs were gently pressed down on the agar surface with forceps to ensure complete contact with the agar surface. The discs were placed 1.5 cm from the edge of the plate and

3 cm apart from each other. The inverted plates were incubated aerobically for 18-24 hours at 35°C. Inhibition zone was measured in millimeters using a caliper on the under surface of the Petri dish. The end point was taken as a complete inhibition of growth as determined by naked eye.[7]

For purposes of comparison, an antimicrobial agent was subjectively determined to be highly effective against bacteria if at least 85% of the isolates demonstrated susceptibility, whereas an agent was deemed effective if 70%-84.9% of the isolates were susceptible. Antibiotics with less than 70% of the organisms susceptible were classified as ineffective.[8]

Data Analysis

Association of host risk factors with frequency of each isolated bacterial species was analyzed using STATA 7.0 software. A statistically significant association between variables was set to $P < 0.05$.

RESULTS

Out of 100 blood samples cultured, septicemia was confirmed only in 20% of the samples. All the bacteria were recovered as pure culture. Seventy percent of the isolates were gram-negative and 30% were gram-positive. *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* were isolated with isolation rate of 45%, 30%, and 25%, respectively (Table 1).

There was no significant association ($P > 0.05$) between the frequency of each bacteria species isolated and the risk factors (age, sex, species, and rectal temperature) (Table 2).

Table 3 shows the results of antimicrobial sensitivity pattern of the recovered bacteria. It was evident that most of the bacteria were susceptible to limited antimicrobials. *Escherichia coli* was found to be highly susceptible to CAF, Pb, and G, and susceptible to S and K. The effective antibiotics against *K pneumoniae* were Pb, CAF, G, and K. *Staphylococcus aureus* was susceptible to Pb, S, G, and K. Considering the overall efficacy of the antibiotics, Pb was found to be highly effective (90%) followed by G (85.0%), CAF (80%), and K (80%). All isolates were found to be resistant to T.

DISCUSSION

In the present study, bacterial septicemia was confirmed only in 20% (20/100) of the total 100 blood samples cultured. The lower frequency of isolation could be due to 1-time sampling. Some bacteria are found only intermittently in blood and require repeated sampling. It has been reported that bacterial growth from blood specimens is less frequent (29%, n = 55) compared to specimens from guttural pouches, wounds, and abscesses.[9] Gram-negative bacteria remain the most common isolates from blood of equine.[9,10] The findings in this study agree with the results of these authors where 70% of the isolates were gram-negative and 30% were gram-positive. The predominance of these gram-negative bacteria, with occasional gram-positive bacteria, suggests the use of broad-spectrum antibiotics in the practical treatment of suspected bacterial septicemia.

Escherichia coli was the most predominant bacteria recovered in the current study. Similar findings were reported in horses.[9,10]

Table 1. Bacteria Isolated From Suspected Septicemia Cases

Bacterial Isolates	Equine Species				Percentage of Isolates
	Horse (n = 50)	Donkey (n = 40)	Mule (n = 10)	Total (n = 100)	
E coli	5	4	0	9	45% (9/20)
K pneumoniae	1	3	1	5	25% (5/20)
S aureus	3	3	0	6	30% (6/20)
Total	9	10	1	20	100%

Table 2. Association of Risk Factors With Frequency of Isolated Bacteria Species

Risk Factors	N	Bacterial Isolates			Total, n (%)	X^2	*P*-Value
		S aureus, n (%)	*K pneumoniae*, n (%)	*E coli*, n (%)			
Equine species							
Horse	50	3 (50)	1 (20)	5 (55.5)	9 (45%)		
Donkey	40	3 (50)	3 (60)	4 (44.4)	10 (50%)	1.0490	0.592
Mule	10	0	1 (20)	0	1 (5%)		
Age							
<1 year	54	4 (66.6)	1 (20)	5 (55.5)	10 (50%)	3.4599	0.177
1-4 years	33	1 (16.6)	2 (40)	1 (11.1)	4 (20%)		
>4 years	13	1 (16.6)	2 (40)	3 (33.3)	6 (30%)		
Sex							
Male	65	4 (66.6)	1 (20)	7 (77.7)	12 (55%)	0.4011	0.527
Female	35	2 (33.3)	4 (80)	2 (22.2)	8 (45%)		
Body temperature							
39-39.5°C	26	1 (16.6)	1 (20)	0	2 (10%)	3.2021	0.202
39.6-40°C	40	3 (50.0)	4 (80)	5 (55.4)	12 (60%)		
>40°C	34	2 (33.3)	0	4 (44.4)	6 (30%)		

Table 3. Percentage of Bacterial Isolates Sensitive to Different Antimicrobials

Isolates	O	E	Pb	S	P	T	CAF	G	Cf	Am	K	Over-all
E coli	11.1[c] (1/9)[d]	0[c]	88.9[a] (8/9)	77.8[b] (7/9)	—	0[c]	100[a] (9/9)	88.9[a] (8/9)	55.6[c] (5/9)	0[c]	77.8[b] (7/9)	45.5
K pneumoniae	20[c] (1/5)	0[c]	100[a] (5/5)	60[c] (3/5)	—	0[c]	80[b] (4/5)	80[b] (4/5)	60[c] (3/5)	0[c]	80[b] (4/5)	43.6
S aureus	0[c]	66.6[c] (4/6)	83.3[b] (5/6)	83.3[b] (5/6)	16.7[c] (1/6)	0[c]	50[c] (3/6)	83.3[b] (5/6)	50[c] (3/6)	16.7[c] (1/6)	83.3[b] (5/6)	48.5
Overall	10.0	20.0	90.0	75.0	5.0	0	80.0	85.0	55.0	5.0	80.0	45.85

[a]Highly susceptible; [b]Susceptible; [c]Resistant; [d](number of bacterial isolates susceptible/ total number of bacterial isolates)
O = oxytetracycline; E = erythromycin; Pb = polymyxin B; S = streptomycin; P = penicillin G; T = tetracycline; CAF = chloramphenicol; G = gentamicin; Cf = ciprofloxacin; Am= ampicillin; K = kanamycin.

Twenty-five percent of the total isolates were *K pneumoniae*. This finding is consistence with previous report on horses,[9] where 23% of the total isolates from blood specimens were *K pneumoniae*. The role of *K pneumoniae* in community-acquired bacterial pneumonia is well documented in human medicine. *Klebsiella pneumoniae* primarily attack immunocompromised individuals and can be responsible for pneumonia, urinary tact infection, septicemia, and soft tissue infections.[11] This is the first study to document prevalence of *K pneumoniae* in equids in Ethiopia. *Staphylococcus aureus* (30%) was the only gram-positive bacteria recovered in this study. Although the current susceptibility test did not include methicillin, there are increasing reports of methicillin-resistant (MRSA) infection, and colonization in horses explains that MRSA can be transmitted between horses and humans.[12]

In the present study, the association of host factors (age, sex, species, and body temperature) with frequency of bacteria

species recovered was analyzed. The result revealed that there was no statistically significant association ($P > 0.05$).

There are few discrepancies between the current study and previous reports[9,13] on the antibiotic susceptibility pattern of the bacteria identified. These differences in the susceptibility pattern of *E coli*, *K pneumoniae*, and *S aureus* between the current study and previous reports might be because of their unpredictable susceptibility to particular antibiotics or class of antibiotics.[14] These differences suggest the need of antibiotic sensitivity test for these bacteria when they are considered significant. Such difference could result also from using different antibiotics in Ethiopia and where those studies were conducted, for example Pb and CAF are not used for animal treatment in Ethiopia. When we consider the overall efficacy of the antimicrobials used, the most effective drugs were Pb, G, CAF, and K. All isolates were susceptible to Pb except single isolates of *E coli* and *S aureus*. Polymixin B is bactericidal; it interacts strongly with phospholipids in bacterial cell membrane and rapidly disrupts their permeability and function.[15] Endotoxin-neutralizing activity of Pb in blood after intravenous administration in horses was evaluated and concluded that repeated IV use of 1 mg of Pb/kg at 8-hour intervals can be used for treating endotoxemia.[16] When considering the overall antimicrobial resistance pattern, 54% (0%-66.6%) of the isolates were resistant. Although E, P, and A were not effective, much of the resistance was attributed to T to which neither of the isolates was susceptible. These antibiotics are commonly used in the country. These antibiotics should be reserved to treat septicemia only after culture and susceptibility testing demonstrate efficacy.

CONCLUSION

In the present study, different bacteria were isolated from 3 species of equines. This indicates bacteria are one of the potential causes of septicemia in equines. Gram-negative bacteria were the predominant isolate. The majority of the tested isolates were susceptible to limited antimicrobial agents. Therefore, bacteria isolation and antibiotic susceptibility test should be conducted before dosing with antibiotics except for critical care patients. Polymixin B, gentamicin, chloramphenicol, and kanamycin were found to be effective whereas tetracycline, routinely used in Ethiopia, was totally ineffective against all the isolates. The current study was limited in duration and sample size. Therefore, further investigation should be carried out to elucidate equine septicemia adequately in the country. In addition, rational use of antimicrobial therapy should be encouraged to minimize the risk of antimicrobial resistance.

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