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Full Length Research Paper

Isolation and identification of aerobic, septicemic bacteria from cattle in and around Sebeta town and antimicrobial susceptibility testing

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Septicemia is one of the infectious diseases which have zoonotic and economic importance all over the world. Therefore, this study isolated and identified some of the aerobic, septicemic bacteria and then antimicrobial susceptibility test was done in the period November 2006 to May 2007 on 830 sick cattle of different age groups, which were brought to Sebeta veterinary clinic from Sebeta and its surrounding of parts Ethiopia. From these cattle, only 70 cattle found with sign of septicemia. Accordingly, the study was carried out on these 70 Septicemic cattle with objectives of isolation and identification of septicemic, aerobic bacteria and establishing of antimicrobial susceptibility test. Hence, from the study animals, whole blood samples were collected aseptically from jugular vein for bacteria culture. As a result, out of 70 blood samples, 42 (60%) cultures were found with aerobic gram positive and negative bacteria, which consists 28 (66.67%) gram positive and 14 (33.33%) gram negative bacteria. The isolated bacterial species were *Staphylococcus aureus* 5 (11.9%), other *Staphylococcus* species 14 (33.33%), *Streptococcu*s species 2 (4.8%), *Listeria monocytogenes* 3 (7.1%), other *Listeria* species 2 (4.8%), *B. anthracis* 2 (4.8%), *Escherichia coli* 4 (9.5%), *Pasteurella multocida* 9 (21.4%) and *Pseudomonas aeruginosa* 1 (2.4%). On the other hand, the *in vitro* antimicrobial susceptibility test showed that all bacterial isolates were susceptible to Novobiocin and Chloraphenenicol except *P. aeruginosa*. Generally, the bacterial isolates revealed various degree of susceptibility against gentamicin, trimethoprim-sulphamethoxazole, methicillin, polymixin B, oxytetracycline, penicillin G and ampicillin. Conversely, from all isolates, *Ps. aeruginosa* showed highest resistance to all antimicrobials except to gentamicin. Also almost all bacterial isolates were resistant to bacitracin. Of course, further study is required to elucidate the true nature of septicemic agents and rational use of antimicrobial therapy.

Key words: Septicemia, infectious diseases, and antimicrobial susceptibility test.

INTRODUCTION

Septicemia is an infectious disease accompanied by fever, toxemia, hyperthermia, rapid pulse rate, marked prostration and the presence of large numbers of infectious micro-organisms, including viruses, bacteria and protozoa in the blood stream. Pathologically the condition is characterized by petechial hemorrhages, hyperemia and edema; also degenerative changes in parenchymatous tissues (Merchant et al., 1983; Radostits et al., 2000).

The post mortem findings of septicemic animal reveal enlarged edematous or hemorrhagic lymph nodes, degenerative changes in parenchymatous organs, congestion and petechial or ecchymotic hemorrhages, spleenomegally, inadequately bleed-out carcass as a result of high fever, blood stained serous exudates in abdominal and/or thoracic cavities and anemia resulting from bone marrow depression and icterus may also be present (Herenda, 2000).

Some of the notable septicemia causing bacteria in all species of animals are anthrax, pasteurellosis, salmonellosis, leptospirosis (Radostits et al., 2000; Gyles et al., 2004). Streptococci appear to have etiological significance in septicemic infections in calves, other animal and human being (Radostits et al., 2000; Slayers and Whitt 2002; Gyles et al., 2004). *Pseudomonas aeruginosa* causes septicemia in immunocompromised

animals (Hirsh and Zee, 1999).

Escherichia coli can also be septicemia causing agent both in animal and human and it is the leading causes of infant's meningitis from human septicemia causing isolates (Slayers and Whitt, 2002).

In general, septicemia has economic and zoonotic importance. On one hand, anthrax, listeriosis, Salmonellosis, *Pseudomonas*, *E. coli* and Streptococcal infections have zoonotic importance (Aiello, 1998; Slayers and Whitt, 2002).

On the other hand, septicemic carcasses are condemned which results in economic losses (Herenda, 2000). Septicemic carcasses are unfit for human as food for two reasons: the condition may be associated with entry of pathogenic organisms in to the systematic circulation and consumption is therefore dangerous; and the congestion of the carcass as a result of pyrexia and imperfect bleeding, together with alkalinity of the meat, so impair its keeping quality as to render it unmarketable. The infective organisms in septicemic animals are numerous and many of these have zoonotic importance. Meat inspection records have shown that the flash or organs of animals slaughtered while suffering from a generalized systemic infection, especially when due to diseases of intestinal or genital tract, is the most likely to give rise to severe gastrointestinal disturbance in man (Gracey et al., 1999; Herenda, 2000).

Bacteriological examination is often required when there is general depression and pyrexia of unknown origin. This may be accompanied by general leukocytosis, particularly neurophilia but in some bacterial infections, such as pyothorax, peritonitis and pyometra, there may be leucopenia. The origin of bactremia is usually abscess or other foci of infections. Thus, when bactremia is suspected or when there is pyrexia of unknown origin, blood cultures are usually necessary (Woldehiwet, 1996).

Consequently, uncoagulated whole blood samples are collected for bacterial culture (Quinn et al., 1999). This is possible by the isolation of the causative agent from blood stream to make a positive diagnosis of septicemia (Radostits et al., 2000).

Therefore, this study was designed to isolate septicemia causing bacteria and to establish antibiogram pattern of the isolates from sick cattle which were brought to the Sebeta veterinary clinic from Sebeta town and near by rural villages.

MATERIALS AND METHODS

Sampling strategy and study design

Out of 830, 70 cattle were screened for septicemia (n = 70) in the period ranging from November 2006 to May 2007 from Sebeta veterinary clinic from Sebeta town and its surrounding rural areas of Ethiopia. The study targets were cattle of any age group with sign of septicemia, mainly based on increased body temperature (≥39.5°C). After some times of the cattle arrival to the clinic, general clinical examination was conducted and febrile cattle were selected and included in the study.

Then, whole blood samples from febrile cattle were collected from the jugular vein in to heparinized vacutainer tubes for culture by first washing, shaving and disinfecting the skin over the jugular vein with 70% alcohol and treating the vein puncture site with 2% iodine for one minute before blood collection. On average, the volume of blood taken per animal was 5 ml and these blood samples were identified by sample code, date of sampling, body temperature, age, breed and sex. After this, the blood samples were immediately transported to Sebeta National Animal Health Research Center for examination. Blood collection was carried out according to the techniques recommended by (Barrow and Feltham, 1995; Woldehiwot, 1996; Quinn et al., 1999).

Bacterial examination

Bacteriological examination of the blood samples were conducted according to standard methods recommended by Barrow and Feltham (1995), Woldehiwot (1996) and Quinn et al. (1999).

The Media used for the bacterial isolation and identification were prepared, according to instructions of the manufacturers. The procedure is described briefly as follows: The blood specimens were enriched in brain heart infusion and incubated at 37°C for up to 7 days aerobically before discarding. During this time the blood culture bottles were inspected for turbidity, which indicates the growth of bacteria.

Based on turbidity a loopeful of bacterial culture was transferred from the broth culture and streaked on to blood agar (BBL, Becton Dickinson, USA) and MacConkey agar (Oxoid, Hampshire, England) plates parallelly and incubated at 37°C for 24 to 48 h. After 24 h of incubation, colonial morphology which includes shape, color, presence or absence of hemolysis, growth on MacConkey agar and lactose fermentation was recorded. From culture positive plates the representative colonies were further streaked on to blood agar and then pure colonies were again transferred to brain heart infusion for further tests.

Pure culture isolates were subjected to tests that were used as primary identification. The primary identification of the cultural isolates used has aerobic growth on blood Agar and MacConkey agar and Giemsa and Gram stain, catalase, oxidase, motility and O-F test.

Then, the secondary biochemical tests that was used during laboratory examinations were coagulase test, indole test, urase, ONPG, methyl Red (MR), Voges-Proskuer (VP) and sugars tests like arabinose, maltose, salicin, sorbitol, sucrose, trehalose, mannitol, lactose and raffinose.

Both tests were carried out according to the techniques recommended by (Carter, 1991; Barrow and Feltham, 1995; Quinn et al., 1999). Depending on preliminary characteristics, appropriate selective media were also used for identification of the isolates.

Antimicrobial susceptibility testing

Single disk diffusion susceptibility test, also termed as Kirby-Bauer method, was utilized to determine anti-microbial sensitivity patterns of the bacterial isolates that were found from septicemic cattle (Bauer et al., 1996).

Antimicrobial drugs used for disc assay tests were ampicillin (10 µg), bacitracin (10 IU), gentamicin (30 µg), oxytetracycline (30 µg), methicillin (5 µg), novobiocin (30 µg), penicillin G (10 IU), polymixin B (300 IU), chloramphenicol (30 µg), streptomycin (10 µg) and trimethoprim-sulphamethoxazole (1.25 / 23.75 µg). The procedure for antibiogram pattern determination is described briefly as follow: Using pure colony, a bacterial suspension was made in Brain Heart infusion (Merck, Germany) and adjusted to 0.5 McFarland turbidity standards. Sterile cotton swab on a wooden applicator stick was

Table 1. Bacterial isolates from septicemic cattle admitted to Sebeta veterinary clinic.

Bacterial species	Number of bacterial isolates	Percentage (%)
Staphylococcus aureus	5	1.9
Other *Staphylococcus* species	14	33.3
Streptococcus species	2	4.8
L. monocytogenes	3	7.1
Other *Listeria* species	2	4.8
P. aeruginosa	1	2.4
P. multocida	9	21.4
B. anthracis	2	4.8
E. coli	4	9. 5
Total	42	100

used to transfer the diluted bacterial suspension to a Mueller Hinton agar plate (BBL®, Becton Dickinson, USA); excess fluid was then squeezed out by rotating the swab against the sides of the tubes.

The plates were seeded uniformly by rubbing the swab against the entire agar surface in three different planes. Within 15 min (time used to dry the inoculums) after the plates were inoculated, antimicrobial impregnated discs were applied to the surface of the inoculated plates using a sterile forceps. All the discs were gently pressed with forceps to ensure complete contact with the agar surface.

The discs were 1.5 cm away from the edges of the plates and they were 3 cm away from each other. The plates were incubated inverted aerobically for 24 h at 37°C. The zone of inhibition of the antimicrobial discs was measured by holding a ruler on the back of the plates which was illuminated with reflected light. The value determined was rounded of to the nearest whole number millimeter. The measured values were translated in to descriptive terms such as susceptible, intermediate or resistant according to the guidelines of the national committee for clinical laboratory standard (NCCLS, 1999).

Data analysis

To summarize the generated data on the rate of bacterial isolation and sensitivity patterns of bacterial isolates the descriptive statistics such as proportions was used.

RESULTS

Bacteriological examination

In this study, a total of 70 blood samples were cultured, out of which 42 (60%) samples yielded bacteria. 28 (66.7%) of the isolates were gram positive and 14 (33.33%) of the isolates were gram negative. Accordingly, the bacterial isolates belong to the genera, *Staphylococcu*s 19 (45.2%), *Streptococcus* 2 (4.8%), *Listeria* 5 (11.9%), *Pasteurella* 9 (21.4%), *Bacillus* 2 (4.8%), *Escherichia* 4 (9.5) and *Pseudomonas* 1 (2.4%). Out of these isolates, *Staphylococcus* species (45.2%) were dominant, followed by *Pasteurella multocida* (21.4 %). The over all proportion of the bacterial isolates is shown in Table 1.

Antimicrobial susceptibility testing

The causative agents of septicemia are so diverse that require antimicrobial susceptibility test before instituting of a treatment. Hence, the reason of the test was to create information about which antibiotics are effective against identified bacterial isolates. The result of this study shows all isolates of *S. aureus* were sensitive to chloramphenicol and novobiocin by 100%, followed by ampicillin, gentamicin, methicillin, trimethoprim-sulphamethoxazole and polymixin B which were effective in 80% of the isolates. Oxytetracycline and streptomycin showed lower efficacy against *S. aureus* (40%). *S. aureus* and all other isolates were also highly resistant to Bacitracin.

As that of *S. aureus*, other *staphylococcus* species, *Streptococcus species, Listeria* species, *B. anthracis* and *E. coli* were susceptible to chloramphenicol and novobiocin by 100%.

Furthermore*, Streptococcus species, Listeria* species and *B. anthracis* were susceptible to ampicillin, gentamicin, oxytetracycline, methicillin, penicillin G, polymixin B, streptomycin and trimethoprim–sulphamethoxazole by 100%. All *E. coli* isolates were mostly susceptible to Gentamicin and Trimethoprim-Sulphamethoxazole (100). Methicillin and polymixin were effective by 75%, where as Streptomycin showed a response by 50%. In other way, penicillin G and ampicillin were less effective (25%) to *E. coli*, but it was completely resistant to Oxytetracycline.

P. multocida was susceptible to ampicillin, oxytetracycline and polymixin B (100%). It was also susceptible to streptomycin and methicillin, trimethoprim-sulphamethoxazole and penicillin G by 88.9, 87.5 and 66.7% respectively, but it was resistant to Gentamicin.

Meanwhile, *P. aeruginosa* was highly resistant to all antimicrobials that chose to test except to Gentamicin and it was susceptible to gentamicin. Table 2 (a and b)

Table 2a. Antibiogram results of bacterial isolates from septicemic cattle admitted to Sebeta veterinary clinic.

Susceptibility pattern of the bacterial isolates n (%)					
Antimicrobial agents	**Potency of discs**	***S. aureus***	***Staphylococcus* species**	***Streptococcus* species**	**Listeria monocytogenes**
Ampicillin	10 µg	4(80)	14(100)	2(100)	3(100)
Bacitracin	10 IU	1(20)	6(42.9)	1(50)	1(33.33)
Chloramphenicol	30 µg	5(100)	14(100)	2(100)	3(100)
Gentamicin	30 µg	4(80)	14(100)	2(100)	3(100)
Methicillin	5 µg	4(80)	14(100)	2(100)	3(100)
Novobiocin	30 µg	5(100)	14(100)	2(100)	3(100)
Oxytetracycline	30 µg	2(40)	14(100)	2(100)	3(100)
Penicillin G	10 IU	0(0)	12(86)	2(100)	3(100)
Polymixin B	300 IU	4(80)	14(100)	2(100)	3(100)
Streptomycin	10 µg	2(40)	10(71.43)	2(100)	3(100)
Trimethoprim-Sulphamethoxazole	125 µg	4(80)	4(100)	2(100)	3(100)

Table 2b. Antibiogram results of bacterial isolates from septicemic cattle admitted to Sebeta veterinary clinic.

Susceptibility pattern of the bacterial isolates n (%)						
Antimicrobial agents	**Potency of discs**	**Listeria species**	***B. anthracis***	***E. coli***	***P. multocida***	***Pseudomonas aeruginosa***
Ampicillin	10 µg	2(100)	2(100)	1(25)	9(100)	0(0)
Bacitracin	10 IU	1(50)	1(50)	0(0)	4(44.44)	0(0)
Chloramphenicol	30 µg	2(100)	2(100)	4(100)	9(100)	0(0)
Gentamicin	30 µg	2(100)	2(100)	4(100)	4(44.4)	1(100)
Methicillin	5 µg	2(100)	2(100)	3(75)	8(88.9)	0(0)
Novobiocin	30 µg	2(100)	2(100)	4(100)	9(100)	0(0)
Oxytetracycline	30 µg	2(100)	2(100)	0(0)	9(100)	0(0)
Penicillin G	10 IU	2(100)	2(100)	1(25)	6(66.7)	0(0)
Polymixin B	300 IU	2(100)	2(100)	3(75)	9(100)	0(0)
Streptomycin	10 µg	2(100)	2(100)	2(50)	8(88.9)	0(0)
Trimethoprim-Sulphamethoxazole	125 µg	2(100)	2(100)	4(100)	7(87.5)	0(0)

shows antibiogram profile in the following manner.

DISCUSSION

Septicemia causing bacteria have not been well studied in Ethiopia. Thus, the objectives of this study were to isolate and identify aerobic, septicemic bacteria and to perform antimicrobial susceptibility test to the isolates. This study fulfills these objectives and according to this study, from 70 blood samples 42 samples (60%) were positive to bacterial culture, which is higher than findings of Ashenafi (2006). He obtained 20.83% of bacterial isolates from septicemic blood culture. This may occur due to difference in study area. The environment of study area may allow the survival and cause of a disease by favoring etiological agents than a host. This idea is supported by Thrusfield (2005) who has discussed the association of host, the agent and the environment.

In the meantime, as an outcome of this study, seven genera of aerobic, septicemic bacteria were isolated from bovine species of all age groups. The species of these isolates were *S. aureus*, other *Staphylococcus* species, *Streptococcus* species *Listeria monocytogenes,* other *Listeria* species*, Ps.aeruginosa, P. multocida, B. anthracis and E. coli*. Out of these isolates, *Staphylococcus* was of the highest proportion. This may be due to the bacteria that developed from local lesions and its existence on the skin mucous membrane of an animal. The farmers of the area have using their cattle mainly for draft power, which increases the probability of an animal to be infected by bacteria, even from normal inhabitants of the skin. The owners usually use sticks or whips for driving animals which create wounds that

facilitate easy entrance of the agents to a host. This idea is supported by Volk and Wheeler (1980) and Radostits et al., (2000). The second most predominant isolate was *P. multocida* with a proportion of 21.43%. Hirsh and Zee (1999) have discussed that the higher occurrence of *P. multocida* with tropical areas as seasonal epidemics with high morbidity and mortality. Radostits et al. *(*2000) has indicated that *E. coli* is the most common in calves during the first four days of life. According to this study, three *E. coli* were identified from young animals; but one of the isolates was isolated from adult animal of six years. Potential septicemia causing agents like *Listeria, Bacillus, Pseudomonas* and *Streptococcus* were isolated with a proportion of 11.94, 4.8, 2.4 and 4.8%, respectively. Hirsh and Zee (1999), Radostits et al. (2000), Slayers and Whitt (2002) and Gyles et al. (2004) have discussed some notable septicemic causing bacteria which strengthen the above facts.

Although higher proportion of septicemic bacteria was isolated from septicemic cattle, still all septicemic blood culture did not yield bacteria that is 40% of septicemic cattle were negative to bacterial culture. This is due to the existence of other septicemia causing micro-organisms. Carter (1991) and Radostits et al. (2000) have explained other micro-organisms causing septicemia, which include virus, protozoa, mycotic infection, rickettisa and non-spore forming anaerobes. On the other hand, sunstroke and stress causing conditions can mislead with fever and thus reduce the number of septicemic bacteria. Additionally, the farmers of Sebeta and its surroundings do not bring their cattle at early stage of a disease process, when there is high probability of isolating bacteria from blood stream. Rather they bring their animals at about 2 to 3 days even weeks later or sometimes when an animal is anticipated to death. This declines the probability of isolating septicemic bacteria.

The antimicrobial susceptibility testing showed that all bacterial isolated were susceptible to novobiocin and chloramphenicol except to *Ps. aeruginosa*. But all isolates were resistant to Bacitracin. *Pseudomonas aeruginosa* was highly resistant to all antimicrobials used in the test, except to gentamicin. Other authors, Hirsh and Zee (1999), Quinn et al. (1999) and Radostits (2000) also have described the resistance of *Ps. aeruginosa* to multiple antibiotics leaving aminoglycosides. Penicillin G, oxytetracycline and ampicillin were effective to some isolates but, they were totally resisted by other bacteria (Table 2a and b).

Thus, ineffective antibiotics should be reserved from using it to treat septicemic animals, but it is possible to use them if susceptibility testing demonstrates their efficacy.

CONCLUSIONS AND RECOMMENDATIONS

Although further study is needed to elucidate the true nature of septicemic agents and to adapt the rational use of antimicrobial therapy based on antibiogram profile test, 60% of bacteria were isolated from septicemic bovine species of different age, sex, breed and temperature ≥ 39.5°C. The isolates were *S. aureus*, other *Staphylococcu*s species, *Streptococcus* species, *L. monocytogenes*, other *Listeria* species, *B. anthracis, P. multocida, Ps. aeruginosa* and *E. coli*. Hence, this result indicates bacteria are one of the causative agents of septicemia in bovine species. The majority of the isolates were susceptible to limited antimicrobials. But Chloramphenicol and Novobiocin were effective to all isolates except to *Ps. aeruginosa*. *Ps. aeruginosa* was, among antimicrobial drug that are used in the test, only susceptible to gentamicin.

Gentamicin, trimethprim-sulphamethoxazole, methicillin, polymixin B and ampicillin showed good effect on the most isolated bacteria.

Based on above result the following points are recommended:

1. Further study is required to find out common septicemic bacteria in Ethiopia.
2. Awareness creation, to farmers to bring their sick cattle as early as possible, should be encouraged and adapted.
3. Taking of sick calves to a veterinary clinic should be habited in Ethiopia.
4. Animals that used for draft power should be handled carefully in order to reduce staphylococcal infections as well as to any other infectious agents.
5. In order to avoid drug resistance and able to select effective antibiotics, antimicrobial susceptibility testing is required.

REFERENCES

Aiello SE, Mays A (1998). The Merck Veterinary Manual, 8th ed New Jersy: white house station., pp. 2162-2167.

Ashenafi S (2006). Isolation and Identification of Septicemic Bacteria in cattle. Debre Zeit: Faculty of Veterinary Medicine, Addis Ababa, Ethiopia, DVM thesis.

Barrow GI, Feltham RKA (1995). Manual for Identification of Medical Bacteria, 3rd ed: Great Britain, The press Syndicate of University of Cambridge., pp. 3-475.

Bauer AW, Kirby WMM, Serris JC, Turek M (1966). Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol., 45: 493-496.

Carter GR (1991). Diagnostic procedures in veterinary bacteriology and mycology, 4th Ed.philadelphia: Lea and Febiger. pp. 12-47.

CSA (1998). Ethiopian Live stock Estimate, Addis Ababa, Ethiopia. I: 52.

Gracey JF, Collins DS, Huey RJ (1999). Meat hygiene, 10th ed. London: W.B. Saunders Company Ltd. pp. 451-453.

Gyles CC, Prescott JF, Songer JG, Theon CO (2004). Pathogenesis of Bacterial Isolation in animals, 3rd ed UK Blackwell Publishing. pp. 23-411.

Herenda D, Chambers PG, Ettrigui A, Viratna P, Silva TJP (2000). Manual on meat inspection for developing countries. pp. 36-246

Hirsh DC, Zee YC (1999). Veterinary Microbiology Black well Science, Inc. pp. 61-248.

Merchant IA, Packer RA (1983). Veterinary bacteriology and virology, 7th ed Delhi: the lowa state University press. p. 127.

NCCLS (1999). Performance standards for antimicrobial disc and dilution susceptibility tests for bacteria isolated from animals and

humans. Approved standard. National Committee for Clinical Laboratory Standards. Document M31-A, NCCLS, Villanova, P A
NMSA (1999). Rainfall and temperature data of Sebeta, National Metrological service Agency, Addis Ababa, Ethiopia.
Quinn PJ, Carter ME, Markey BK, Carter GR (eds) (1999). Clinical eterinary microbiology, 2nd ed. Mosby: Wolfe Publishing. pp. 21-618.
Radostits OR, Gay CC, Blood DC, Hinchcliff KW (2000). Veterinary Medicine: A textbook of the disease of cattle, sheep, goats and horses, 9th ed. London: Bailler Tindall. pp. 47-629.
Slayers AA, Whitt DD (2002). Bacterial Pathogenesis, A Molecular Approach, 2nd ed Am. Soc. Microbiol., pp. 19-495.
Thrusfield M (2005). Veterinary Clinical Studies Royal (Dick) School of Veterinary studies University of Edinburgh, 3rd ed. Black well Science ltd. pp. 75-93.
Volk WA, Wheelers MF (1980). Basic microbiology, 4th ed. Philadelphia: J. B. Lippincott Company, pp. 297.
Woldehiwot Z (1996). Manual of diagnostic veterinary microbiology. Liverpool: the University of Liverpool. pp. 9- 152.