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Susceptibility pattern of aerobic bacteria isolated from septicemic cattle in Adama, Ethiopia

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From November, 2011 to March, 2012 a total of 586 cattle were examined for febrile conditions. From these animals, 62 (10.6) animals were found to be febrile. So the study was conducted on these animals with the objective of identifying and carrying out antibacterial susceptibility testing on the isolates. From the total of 62 blood cultures processed, only 37 cultures gave bacteria while the remaining 25 cultures were discarded because of absence of visible bacterial growth. From the total isolates, *Staphylococcus aureus* were predominant by accounting 11 (29.7%) of the total isolate followed by other *Staphylococcus* species and *Pasteurella multocida* which constitutes 21.6 and 18.9% of the total population, respectively. On the other hand *Salmonella* and *Streptococcus* species were also isolated in considerable amount being encountered in 16.2 and 13.6% of the total organisms, respectively. Furthermore, antibacterial sensitivity test was performed on the isolates by the discs amoxicillin, ampicillin, gentamicin penicillin-G, chloramphenicol, oxy tetracycline polymixine B, streptomycin and ciprofloxacin. From these drugs chloramphenicol was the most effective drug being active on most of the isolates. This drug was effective on 97.3% of the total isolates followed by ciprofloxacin, gentamicin, and ampicillin were effective on 89.2, 83.8 and 81.1% of the isolates, respectively.

Key words: Adama, cattle, septicaemia, febrile, *Staphylococcus* susceptibility test.

INTRODUCTION

Septicaemia is a condition in which toxic bacteria invade the bloodstream. It is very serious because the organisms and their toxins produced become widely distributed throughout the tissues, and every organ is affected by them (Edward, 2005). Bacteraemia is different from septicaemia in that bacteraemia is not accompanied by sepsis or septic shock. The difference between septicaemia and bacteraemia is of one degree. In bacteraemia, bacteria are present in the bloodstream for only transitory periods and do not produce clinical signs; for example, a clinically unimportant bacteraemia probably occurs frequently after rectal examination or other manipulations in which mucosa are disturbed. In

septicaemia, the pathogen is present throughout the course of the disease and is directly responsible for initiation of the disease process (Radostitis et al., 2010).

Resistance to antimicrobial agents has resulted in morbidity and mortality from treatment failures and increased health care costs. Although defining the precise public health risk and estimating the increase in costs is not a simple undertaking, there is little doubt that emergent antibiotic resistance is a serious global problem. With the introduction of a variety of antimicrobials, it became necessary to perform the antimicrobial susceptibility test as a routine (Lalitha, 2004).

In developing countries, like Ethiopia, there is no enough

data on what type of micro-organisms causing septicaemia and which regime of antimicrobials should be re-commended for each type of microbes. The aim of this study was to detect bacterial pathogen responsible for septicaemia in bovine species in Adama area and determination of their antibiotic response.

MATERIALS AND METHODS

Description of the study area

This study was conducted in Adama. Adama city is located in Oromia National Regional State, East Shewa Zone at a distance of 100 km from Addis Ababa. Its geographical location is 8° 44' North Latitude and 39° 04' East Longitude.

Study animals

The study was conducted on bovine species of all age groups that come to adama animal health clinic from different areas of Adama and its surroundings.

Study design

A purposive sampling was used during the study period conducted from November, 2011 to March, 2012 by collecting data on events associated with septicaemia in bovines that come to adama open-air veterinary clinic. Clinically sick animals were considered as positive for septicaemia based on rise in body temperature and general febrile conditions purposively.

Sample size determination

The sample size for the study was determined by Thrusfied (1995), taking into account 95% confidence interval, desired accuracy level of 5 and 50% expected prevalence of septicaemia in bovine species. Eventhough by this formula 384 animals were enough, the sample size was increased to 586 in order to increase its accuracy.

Sample collection

Clinically septicemic animals were identified by clinical signs of septicaemia primarily by measuring whether there is rise in body temperature or not. In addition to this, animals were clinically examined for the presence of general depression, shivering, rapid breathing (tachypnoea) or rapid heart rate (tachycardia). Blood sample was collected from clinically sick animals by using heparinised tube aseptically. The collected blood sample was immediately transported for further bacteriological and biochemical tests to Addis Ababa university microbiology laboratory found in Debrezeit.

Cultural procedures

Isolation and identification of bacterial species from collected blood sample were conducted according to standard methods recommended by Quinn et al. (1994). Blood samples collected from cattle were brought to microbiology laboratory and they were enriched in brain heart infusion in order to follow if there is a possible bacterial growth. They were incubated for a maximum of one week and checked every 24 h for any visible change indicative of bacterial growth. If there was a sign of turbidity the sample was further propagated to blood and MacConkey agar. The growth of typical colonies on blood agar, and MacConkey agar was characterized. On blood agar, the presence or absence of haemolysis, the types of haemolysis and general appearance of the colonies (colour, shape, size, consistency etc.) were examined. On MacConkey agar, the colonies were examined for the presence or absence of growth; general appearance and ability to ferment lactose were recorded. To get a single purified and isolated colony, each colony having a unique character on primary culture media was taken and subculture on nutrient agar. Further identification of bacterial isolates to the species level was made by conducting appropriate biochemical test based on the genera of bacteria isolated on primary tests.

Further species identification and differentiation of *Staphylococcus* was made by colonial morphology from gram stain smear, catalase test, tube coagulation test using rabbit plasma, growth and fermentation on Mannitol salt agar. On the other hand, *streptococcal* identification and differentiation was made at genus level by colonial morphology on blood agar, gram stain catalase test, and OF tests. Gram negative bacteria were differentiated by Gram stain, Giemsa stain colonial morphology and fermentation on MacConkey agar, motility test, indole, Citrate utilization test, MR-VP test Triple sugar iron agar, and carbohydrate fermentation and hydrogen sulphide gas production tests.

Antimicrobial sensitivity test

The Kirby-Bauer Plate agar disc diffusion method was used to test *in vitro* antimicrobial sensitivity test of each isolated species of bacterial pathogens. For most bacterial pathogens Mueller Hinton agar medium was used except *streptococcus* for which 7 to 10% sheep whole blood was added to Mueller Hinton agar. The depth of the test medium in 90 mm petiridish was nearly 4 mm, which obtained by pouring 25 to 30 ml of prepared medium. All procedures were performed according to (CLSI, 2012).

Data analysis and management

Data collected was managed and prepared on Microsoft Excel Sheet, coded and analyzed using IBM SPSS19 software. Descriptive statistics and chi-square test were used for analysis. Statistical significance for association was checked at $p \geq 0.05$.

RESULTS

Bacteriological examination

In this study a total of 586 animals were examined for septicaemia during the study period (from November 2011 to March 2012). From these animals only 62 (10.6%) were found to be septicemic, hence only 62 blood samples were taken for bacteriological examination. Among these samples cultured, only 37 cultures gave bacterial results while others discarded because of absence of visible bacterial growth on brain heart infusion. The isolated bacteria were 24 gram positive and 13 gram negatives. All the bacterial belong to four genera namely, *Staphylococcus* species, *Streptococcus* species *Pasteurella* spp. and *Salmonella*

Table 1. The distribution of septicaemia with age, breed and sex**.**

Parameter		Positive for bacteria	Negative for bacteria	Total
Breed	Local	29 (46.8)	16 (25.8)	45 (72.5)
	Cross	8 (12.9)	9 (14.5)	17 (27.4)
	Total	37 (59.7)	25 (40.3)	62 (100)
Age	Adult	22 (35.5)	15 (24.2)	37 (59.7)
	Young	15 (24.2)	10 (16.1)	25 (40.3)
	Total	37 (59.7)	25 (40.3)	62 (100)
Sex	Male	21 (33.8)	17 (27.4)	38 (61.3)
	Female	16 (25.9)	8 (12.9)	24 (38.7)
	Total	37 (59.7)	25 (40.3)	62 (100)

spp. The frequency of the isolates was as follows; *Staphylococcus aureus* 11 (29.7%), other *Staphylococcus* species 8 (21.6%), *Pasteurella multocida* 7(18.9%), *Salmonella* species 6(16.2%) and *Streptococcus* species 5 (13.6%) (Table 1). Among the isolates *Staphylococcus aureus* isolates predominate by accounting 29.7% of all the isolates followed by *Staphylococcus* species. These species were 21.6% of all the isolates. The other species isolated were *Pasteurella multocida*, *Salmonella* species and *Streptococcus* species which account 18.9, 16.2 and 13.6%, respectively.

Sensitivity test result

In this study *S. aureus* showed 100% susceptibility to chloramphenicol, followed by gentamicin and ciprofloxacin which had 90.9% efficacy on these isolates. *S. aureus* species found to be highly resistant to penicillin G with the finding of 0% susceptible organism. In addition these organisms were less susceptible to the routinely used drug, oxy tetracycline with susceptibility result of 63.6%. *Staphylococcus* species other than *Staphylococcus aureus* showed a great susceptibility to most of the drugs used. Ampicillin, streptomycin, Chloramphenicol, erythromycin and polymixine B were 100% effective on these isolates. Oxy tetracycline and penicillin G showed relatively the lower result by accounting 75%efficacy. On the other hand *P. multocida* were fully susceptible to penicillin G, streptomycin and ciprofloxacin followed by Chloramphenicol Erythrtromicin, ampicillin and gentamicin with susceptibility pattern of 85, 85.5, 57.1, and 57.1%, respectively.

Furthermore, *Salmonella* species showed a high degree of susceptibility to Chloramphenicol and ciprofloxacin by 100%. These organisms were resistant to penicillin-G and to oxy tetracycline. *Streptococcus* species were fully susceptible to all drugs used except ciprofloxacin and oxy tetracycline with susceptibility

pattern of 80 and 60%, respectively. From all tested drugs chloramphenicol showed the highest activity on all isolates being effective on 97.3% of the isolates followed by ciprofloxacin, gentamicin, and ampicillin were effective on 89.2, 83.8 and 81.1% of the isolates, respectively. From all drugs used penicillin showed the least result by accounting an overall effectiveness of 48.6%.

DISCUSSION

This study showed that high rate of positive case of bacteria with fever indicative of septicaemia (59.7%) which was reported to be higher side than Shiferaw et al. (2009) who reported 20.6% of isolation rate in Debrezeit. This difference may come from the time elapse and agro ecological difference between the two studies. This study finding agrees with the work of (Demissie, 2011) who reported 60% isolation rate of bacteria from septicemic cattle in Sebeta Veterinary Clinic. Septicaemia is a disease complex that have various etiologist. That is why all septicemic animals were not positive for bacteria. Other septicemic conditions may rise from infectious agents other than bacteria, Radostitis et al. (2010) discussed that viruses, fungus and some protozoa can cause septicaemia. The isolated bacteria belong to four genera, namely *Staphylococcus* which accounts for 51.3%, *Pasteurella* 18.9%, *Salmonella* (16.2%) and *Streptococcus* species (13.6%). Among the isolates, *Staphylococcus* species were found to be the leading cause of septicaemia which agrees with the finding of (Demissie, 2011). *S. aureus* was found to be predominant in the study population. This may be due to wide spread of thorny plants like acacia in the study area which can causes skin damage for entrance of organisms to the circulatory system. The second most dominant isolate was found to be *Pasteurella multocida* this account for 18.9% of the total isolates which is supported by the work of (Demissie, 2011). This bacterium is mostly associated with stress causing agents like over working

and poor body conditions, animals in this study area are subjected to hard work and they are in poor body condition hence, highly significant in causing septicaemia this idea is supported by (Rodestitis et.al., 2010). Rodestitis et al. (2010) also discussed that pasteurellosis is mostly significant in young aged from 6 months to 2 years old. The other isolates were found to be *Salmonella* species and *Streptococcus* species which accounts for 16.2 and 13.5%, respectively. Even though *in vitro* susceptibility pattern does not ideally represent the real treatment, it is mandatory to perform sensitivity test before prescribing drug regime. In this particular study, isolated bacteria were subjected to *in vitro* susceptibility test by referring all the recommend routine procedures (CLSI, 2012).

From all tested drugs, chloramphenicol showed the highest activity on all isolates being effective on 97.3% of the isolates because the drug have wide spectrum activity on both gram negative and gram positive bacteria. The findings were supported by (Quinn et al., 1994) who discussed the effectiveness of the drug to various microorganisms including chlamydia and rekettissia. Numerically this drug was 100% effective on *Staphylococcus* species, *Salmonella* and *Streptococcus* species and 85.5% effective on *P. multocida* species. Following chloramphenicol, other drugs also showed relatively a high degree of effectiveness on the isolates. Ciprofloxacin, gentamicin, and ampicillin were effective on 89.2, 83.8, and 81.1% of the isolates, respectively. In this particular study, the potential drug penicillin G showed the least efficacy on the bacterial isolates. This may come from the fact that most predominant isolates were *S. aureus* (29.7%) which are resistant to penicillin because of their pencillinase activity. This idea is supported by (Quinn et al., 1994). On the other hand, *Salmonella* species which constitutes 16.6% of all the isolates were also resistant to penicillin.

Robert (2006) discussed that in some gram negative bacteria plasmid mediated resistance is common because their enzymes are constitutively expressed and cause high level of resistance. Eventhough penicillin was found to be less effective on these organisms it showed great deal of effectiveness on *P. multocida*, *Streptococcus* species and other *Staphylococcus* species 100, 100, 75%, respectively. On the other hand, the most widely used drug, oxytetracycline have showed potential that was indicative that organisms are getting resistant to these drugs. This may be due to extensive use of the drug for all febrile cases in the study area. Generally oxytetracycline was effective on 85.5% of *P. multocida*, 75% of other *Staphylococcus* species, 63.6% of *S. aureus* 60% of *Streptococcus* species and 0% of *Salmonella* species. Among all the isolates streptococcus species showed a high degree of susceptibility to all drugs used. Numerically, 100% susceptibility to ampicillin streptomycin, gentamicin, chloramphenicol penicillin G and ciprofloxacin; 80% to polymixine B and 60% to oxytetracycline.

CONCLUSION AND RECOMMENDATIONS

The study showed that bacterial cause of septicaemia is highly prevalent in the study area. From the total animals examined 59.7% septicemic cases were due to bacteria. *S. aureus* followed by other *Staphylococcus* species and *P. multocida* were dominant which constitutes 29.7, 21.6 and 18.9%, respectively. Moreover *Salmonella* and *Streptococcus* species were also predominant in considerable amount with 16.2 and 13.6% of the total organisms, respectively. The anti-bacterial sensitivity test result revealed that, chloramphenicol was the most effective drug on much of the isolates. Based on the conclusion, the following recommendations are forwarded:

1. Chloramphenicol is the best drug for treating bacterial cause of septicaemia.
2. Some organisms are getting resistance to the routinely used drug oxytetracycline. Therefore administering this drug for every febrile case has to be reviewed in every veterinary clinic.
3. Since there is no full information concerning bacterial and other cause of septicaemia in Ethiopia, it is better to conduct researches on this area. National and Regional laboratories have to think over it.

Conflict of interest

Author has none to declare.

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