Original Research

Antimicrobial Resistance Profile of Isolates from Calves with Respiratory Affections

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Rec. Date:	Nov 01, 2018 04:54
Accept Date:	Apr 02, 2019 17:44
DOI	10.5455/ijlr.20181101045445

Abstract

Calves are indispensable assets of the poor dairy farmers as they play an important role in determining the profitability of the dairy farms. Calf hood diseases are considered to be cause of economic losses in the livestock sector, respiratory affections being the major one. The present work was conducted to monitor the antimicrobial resistance against the isolates obtained from the upper respiratory tract of bovine calves in Palampur, Himachal Pradesh. Nasal swabs were collected from 46 clinically sick calves showing respiratory signs. The samples were processed for bacteriological studies to check for microbial growth colonies. Disc diffusion method was used to study the drug sensitivity pattern using standard protocol. Ciprofloxacin was found to be the most sensitive antibiotic followed by Gentamicin. The most resistant of all antibiotics was Cloxacillin. Thus, the present study was conducted so that better prophylactic measures and treatment could be met accordingly to further sustain the animal life.

Key words: Calf, Culture Sensitivity Test, Himachal Pradesh, Respiratory Affections

How to cite: Pradhan, S., & Gupta, V. (2019). Antimicrobial Resistance Profile of Isolates from Calves with Respiratory Affections. International Journal of Livestock Research, 9(10), 37-43. doi: 10.5455/ijlr.20181101045445

Introduction

Livestock is the major source of livelihood of the poor marginal Indian farmers mostly residing in rural areas. Successful rearing of the young calves exclusively determines the profitability of the dairy farms and the farmers as the calves are their indispensable assets. Radostits (2001) have roughly estimated that a calf mortality of 20% may reduce net profit by 38%. The total number of cattle in India as per 2012 census is 190.90 million contributing around 37.28% to the total livestock population. According to the 18th livestock census (2007) the total bovine population in Himachal Pradesh (HP) was 3.03 million, which is approximately 1% of India's bovine population. Highest mortality has been recorded in calves (21.53%) followed by young stocks (9.35%) and adults (4.73%) (Chaudhary *et al.,* 2013). Calf mortality and

morbidity has been mostly attributed to respiratory affections and digestive disorders (Prasad *et al*., 2004; Mishra *et al*., 2015). Diseases of the respiratory system are some of the leading causes of morbidity and mortality in preweaned dairy calves (NAHMS, 2007) and weaned heifers (Poulsen and Mcguirk, 2009). This leads to economic loss not only due to loss of present value of the calf but also due to loss of genetic potential for herd improvement (Wells *et al*., 1996). Respiratory affections have been known to increase by 34% in the last 20 years with 21% neonatal mortality (NAHMS, 2007).

Pneumonia is one of the major respiratory affections taking a heavy toll on the life of the calves during their first few months of life (Svensson *et al*., 2006; Ramakrishna *et al.,* 2008, Gulliksen *et al*., 2009a). Calfhood diseases have the potential to reduce daily gains and affect the age at first calving (Virtala *et al.,* 1996). Calf pneumonia is a multifactorial disease, involving interplay of infectious agents such as viruses, bacteria, mycoplasma and parasites, managerial errors, stressors and host susceptibility. Bacterial pathogens commonly isolated from the calves dying of pneumonia are *Pasteurella multocida, Mannheimia haemolytica, Arcanobacterium pyogenes, Escherichia coli,* α and β haemolytic *Streptococcae* (Trigo *et al*., 1982; Taoudi *et al*., 1983; Svensson *et al*., 2006; Singh *et al.,* 2009). Resistance of these pathogens to antimicrobial agents has become a major issue in therapeutics where the animal succumbs to a disease at an early age. Antimicrobial resistance is a significantly growing problem in the world today due to inevitable and natural process of over administering and over-utilizing antimicrobial drugs. Therefore, selection of an appropriate antibiotic is very crucial for the treatment of animals. In current scenario, a regular monitoring of the antimicrobial resistance against the isolates obtained from respiratory tract could be of great significance. Thus, the present study was conducted so that better prophylactic measures and treatment could be met accordingly to further sustain the animal life.

Material and Methods

Collection of Samples

Nasal swabs were collected from 46 clinically sick calves' upto six months of age showing respiratory signs with nasal discharge and dull and depressed demeanour from the University farm of CSKHPAU and surrounding districts of Palampur, Himachal Pradesh. External nares were cleaned by mopping with spirit swabs to clean the discharges before the swab was passed through nares, penetrated deep into the meatus and rotated firmly and smoothly in a circular fashion against the mucosa (Barnum *et al.,* 1969).

Sample Processing

The swabs were taken to the laboratory for further processing for bacteriological studies within 1-2 hours of collection. The primary isolation was done on blood agar by streaking the swab over the blood agar

 DOI 10.5455/ijlr.20181101045445

plates. This was carried out under the laminar flow and incubated aerobically at 37°C for 16-24 hours to check for any pure microbial growth colonies.

Culture Sensitivity Test

Disc diffusion method was used to study the drug sensitivity pattern of different isolates (Cruickshank *et al*., 1975). Four to five colonies of the isolate were transferred to 5 ml of nutrient broth and incubated at 37°C for 24 hours. Subsequently, sterile cotton swab was dipped into the broth and evenly mopped onto the entire agar surface of Mueller Hinton Agar plate. The antimicrobial discs were placed equidistant with 30 mm minimum center to center distance. These plates were incubated at 37°C for 24 hours and results were recorded according to the size of zone of growth inhibition by referring to a standard chart. The following antimicrobials were used (Hi-Media Laboratories Pvt. Ltd. Mumbai India) (Table 1).

Table 1: List of antimicrobial discs used for Culture sensitivity test

Name of Antibiotic	Concentration of Antibiotic
Ampicillin (AMP)	25 mcg
Amoxycillin /Sulbactum(AMS)	30/15 mcg
Gentamicin (GEN)	50 mcg
Ciprofloxacin (CIP)	30 mcg
Erythromycin (E)	10 mcg
Oxytetracycline (O)	30 mcg
Cloxacillin (COX)	30 mcg
Ofloxacillin (OF)	2 mcg

Results and Discussion

The present study was carried out to determine the antimicrobial resistance of 46 isolates obtained from the upper respiratory tract of clinically sick calves with respiratory affections. Out of the total 46 cases, two showed mixed infection. Therefore, a total of 48 bacterial isolates were obtained. Hence the bacteria isolated from the clinically sick animals were *E. coli* (45.83%), *Streptococcus.* sp (27.08%), *Staphylococcus.* sp (14.58%), *Citrobacter*. sp (12.5%). Ciprofloxacin (50%) was found to be the most sensitive antibiotic followed by Gentamicin (30%). The most resistant of all antibiotics was Cloxacillin (65.21%) (Fig. 1, 2 & 3) (Table 2).

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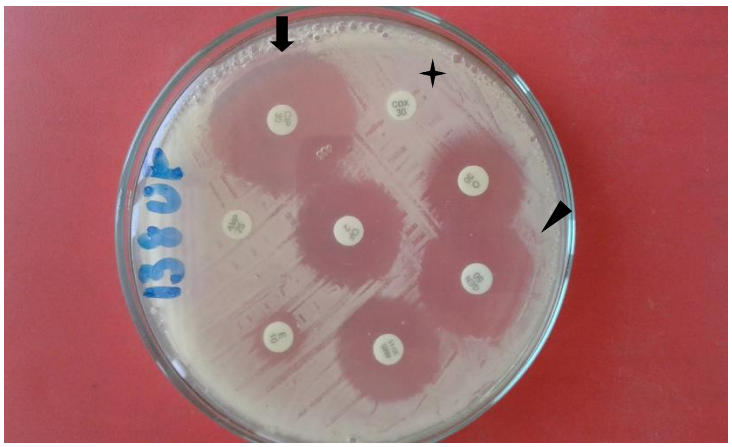

Fig. 1: Ciprofloxacin (arrow) and Gentamicin (arrowhead) showing highest sensitivity while Cloxacillin (star) is resistant

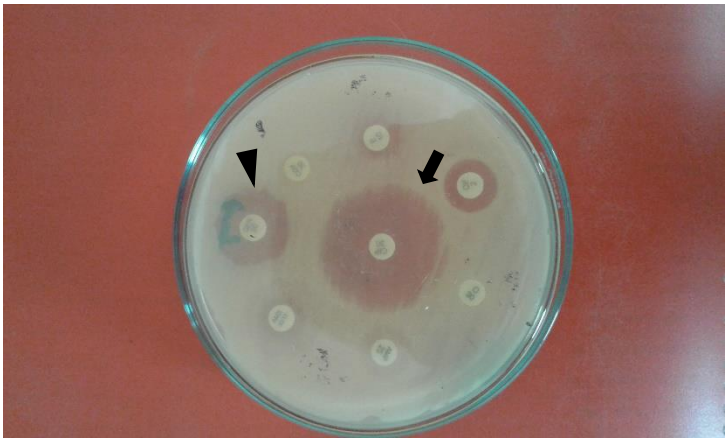

Fig. 2: Ciprofloxacin (arrow) showing highest sensitivity pattern while the others are resistant. Gentamicin (arrowhead) showing mild sensitivity

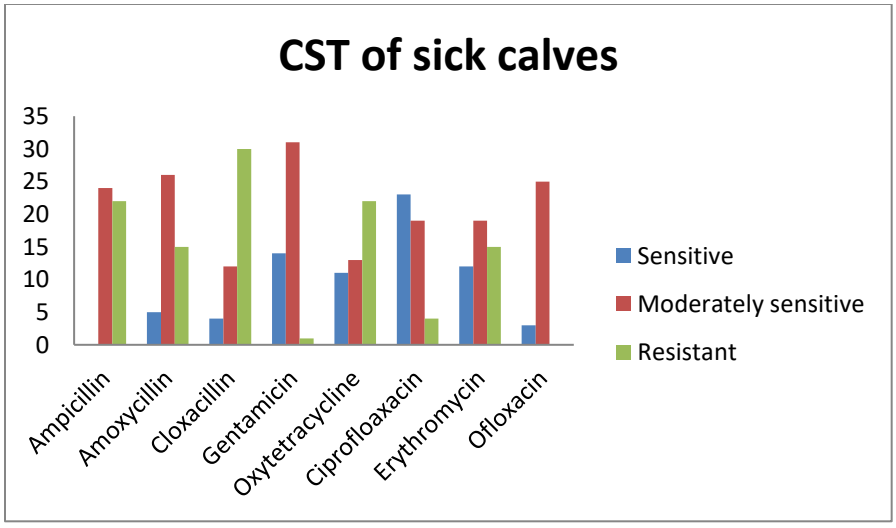

Fig. 3: Graphical presentation of sensitivity patterns of different antibiotics

DOI 10.5455/ijlr.20181101045445

Table 2: Antimicrobial resistance against the isolates from the upper respiratory tract

Antibiotic (n=8)	Sensitive	Moderately Sensitive	Resistant
Ampicillin (AMP)	0	24 (52.17%)	22 (47.82%)
Amoxycillin /Sulbactum(AMS)	5 (10.86%)	26 (56.52%)	15 (32.60%)
Cloxacillin (COX)	4 (8.69%)	12 (26%)	30 (65.21%)
Gentamicin (GEN)	14 (30.43%)	31 (67.4%)	1 (2.17%)
Ciprofloxacin (CIP)	23 (50%)	19 (41.3%)	4 (8.69%)
Erythromycin (E)	12 (26.08%)	19 (41.3%)	15 (32.6%)
Oxytetracycline (O)	11 (23.91%)	13 (28.26%)	22 (47.82%)
Ofloxacillin (OF)	3 (6.52%)	25 (54.34%)	18 (39.13%)

In current scenario, a regular monitoring of the antimicrobial resistance against the isolates obtained from respiratory tract could be of great significance to provide better prophylactic measures and treatment. Reports of the previous treatment studies were not very similar to the present study. Verma *et al.* (1995) reported 60% recovery rates with Ampicillin which was contrary to the present study that showed 52.17% moderate sensitivity and 47.82% resistance towards ampicillin. The development of resistance could be possible due to the inappropriate and indiscriminate usage of antimicrobial agents which is an emerging threat to the global public, animals and environment. There is a hike in the number of infections caused by antibiotic-resistant bacteria. The use and misuse of antibiotics in the livestock sector as growth promoters or as nonspecific means of infection prevention and treatment has boosted antibiotic consumption and resistance among bacteria in the animal habitat (Economou and Gousia, 2015). Resistance also emerges from the subsequent transfer of resistance genes and bacteria among animals and animal products and the environment (McEwen and Fedorka-Cray, 2002). Sen and Albay (2003) and Wadhwa *et al*. (2006) reported 92% and 91% recovery rates with Amoxicillin and Clavulanic acid combination and Enrofloxacin respectively. However, in present study, Amoxicillin/Sulbactum showed 10.86% full sensitivity, 56.52% moderate sensitivity and 32.60% resistance. In a study conducted in Romania it was reported that the most effective antibiotic was the cefquinome followed by penicillin G and amoxicillin with clavulanic acid (Nadas *et al.*, 2016). In India, a pneumonic calf was treated with Amoxicillin and Clavulanic acid combination at the rate of 2.5mg per kg body intramuscularly twice a day for five days along with some supportive therapy (Veena and Sumathi, 2011). However, the present study was not consistent with the work of the previous authors. In a study, oxytetracyclines were considered the first choice, macrolides the second and cephalosporins the last choice (Constable *et al*., 2008) whereas in the present study oxytetracycline showed more resistance (47.82%). Low level of resistance was also shown by erythromycin (32.6%). The lowest resistance levels were shown by gentamicin and ciprofloxacin, 2.17% and 8.69% respectively.

DOI 10.5455/ijlr.20181101045445

An appropriate antibiotic therapy is warranted, once the clinical signs of respiratory diseases are manifested by a calf. The treatment is mostly directed against the bacterial pathogens as the drugs are not so effective in eliminating the viral infections (Apley and Faijt, 1998). As there is a wide range of aetiologic agents involved in the genesis of bacterial pneumonia, it is wise to consider the spectrum of possible causes while choosing the antimicrobial drugs (Mosier, 1997). Proper timing and dosage of the drug administration is very important in making the bacterial pathogens susceptible to the drugs.

Conclusion

The present study revealed that Ciprofloxacin was most sensitive followed by gentamicin. As there is always a chance of mixed bacterial infection it is necessary to use a broad-spectrum antibiotic for quick recovery. However, drawing conclusion from the present study Ciprofloxacin could be used as an emergency therapeutic antibiotic for respiratory infections in calves keeping the timing and dosage in mind.

Acknowledgements

The authors express their gratitude to Mrs. Shweta, Mr. Krishan, Mr. Som Raj and the Department of Pathology and Microbiology DGCN COVAS CSKHPAU, Palampur who took care of all the logistics necessary for the study. This work was supported by grants from the All India Network Programme on Neonatal Mortality in Farm Animals AINP-NM, ICAR.

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DOI 10.5455/ijlr.20181101045445