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Role of *Pseudomonas Aeruginosae* in Bovine Mastitis in Albania

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Abstract:

Mastitis is defined as inflammatory condition of mammary gland. A range of chemical, physical and biological factors are involve, however, bacteria play a significant role in prevalence of mastitis. In this study, we present the frequency of Pseudomonas aeruginosae isolated from milking caws in Albania. During the study period 2014-2017 frequency of bovine mastitis due by P. aeruginosa shows an average frequency of 3.2%, ranged from 1.7-5%. In addition, results of antibiotic susceptibility test indicate that isolates weremost susceptible to Enrofloxacin and Florfenicol. Interestingly, 77.7% (ranged from 25% -93.75%) of strains were resistant to all antibiotics in use. This result explained that successful treatment rate is low, and indicate that P.aeruginosae strains isolated from bovine mastitios are resistance to large number of antibiotics and is an emergent issue of veterinary services.

Key words: Mastitis, *Pseudomonas aeruginosae,* susceptibility test, caw, Albania

INTRODUCTION

Mastitis is the most prevalent and costly infectious disease in dairy cattle occurring throughout the world. It is of particular concern for farmers in developing countries like Albania. Costs due to mastitis include reduced milk production, condemnation

of milk due to antibiotic residues, veterinary costs, culling of chronically infected cows and occasional deaths [Radostis *et al*, 2007]. Moreover, mastitis has a serious zoonotic potential associated with shedding of bacteria and their toxins in the milk [M.Daly *et al* 1999].

Mastitis is caused by a wide spectrum of pathogens and, epidemiologically categorized in to contagious and environmental mastitis [Ojenjiy *et al* 1991]. Contagious pathogens are those for which udders of infected cows serve as the major reservoir of infection. They spread from cow to cow, primarily during milking, and tend to result in chronic sub-clinical infections with flare-ups of clinical episodes. Contagious pathogens include: *Staphylococcus aureus, Streptococcus agalactiae*, *Mycoplasma* spp. and *Corynebacterium bovis* [Jovan 1978]. On the other hand, environmental mastitis can be defined broadly as those intra-mammary infections caused by pathogens whose primary reservoir is the environment in which the cow lives [Benekov *et al.* 1996]. Environmental pathogens include *E. coli*, *Klebsiella* spp., *Strept. dysgalactiae* and *Strept. Uberis*and the majority of infections caused by these pathogens are clinical and of short duration [Çera 2016].

Mastitis can also be classified as either clinical or sub-clinical. Clinical mastitis is characterized by sudden onset, alterations of milk composition and appearance, decreased milk production, and the presence of the cardinal signs of inflammation in infected mammary quarters. It is readily apparent and easily detected. In contrast, no visible signs are seen either on the udder or in the milk in case of sub-clinical mastitis, but the milk production decreases and the somatic cell count increases. It is more common and has serious impact in older lactating animals than in first lactation heifers [Microbiology bulletin 1910]. Because of the lack of any overt manifestation, the diagnosis of sub-clinical mastitis is a challenge in dairy animal management and in veterinary practice [Çabeli 2006].

Mastitis in cattle and sheep associated with *P. aeruginosa* is rare and occurs usually as sporadic cases after intramammary infusion with contaminated material [Senthilingam 2017].

P. aeruginosa is the most common cause, although other *Pseudomonas* spp. can cause disease. *P. aeruginosa* produces a number of extracellular toxins; hemolysin is cytotoxic for most cells and is considered the most potent toxin produced, lecithinase (phospholipase) can destroy cell membranes, and protease degrades proteins. *P. aeruginosa* is common in the environment of cattle because of its innate ability to survive for long periods in dry and moist conditions [Kerry Y.R 1999]. Occasionally a number of animals in the herd are affected with *P. aeruginosa* mastitis; the infection usually originates in contaminated water used for washing udders. The organism has the capacity to colonize inert materials such as loops of hose and the interior surface of water heaters, so that high bacterial concentrations may be in the water left in the hose between milking. It may be an advantage in these circumstances to flush out the udder washing system before commencing each milking. Once the teats are contaminated, the entry of the organisms to the teats is facilitated by overmilking and by putting the milking cups on while the udder is still wet. Serious outbreaks in cows have also occurred in association with the use of a suspected contaminated mastitis infusion used as a dry period treatment. The cows became affected soon after calving and prolonged herd outbreaks are rare. In addition, rarely, strains of this organism are highly virulent and cause severe mastitis, generalized lesions and fatal cases [Quin *et al.* 1994] . Less commonly there is a high level of infection in a herd caused by a contaminated water supply but with no clinical cases. Reinfection is common unless the source of infection is removed. The aim of this study was to estimate role of *Pseudomonas aeruginosae* in bovine mastitis, its incidence and to evaluate the efficacy of antibacterial treatment used in Albania.

MATERIALS AND METHODS

Study area

The animals include in the study were located in middle Albania, where there are managed more than 20.000 milking cows in more than 3257 dairy farms and cattle holders.

Materials

During the study period 2014-2017, 811 milk samples form clinical cases of bovine mastitis were carefully collected and submitted to the national reference diagnostic laboratory at the Institute of Food Safety and Veterinary (ISUV), Tirana-Albania. A quantity of 16 antibiotic discs were used to perform antimicrobial susceptibility test. Specifically,used antibiotic discs were: Gentamycin, Streptomycin, Trimethoprim-sulfamethoxazole, Florfenicol, Enrofloxacin, Amoxicillin, Amikacin, Penicillin G, Oxytetracycline, Lyncospectin, Doxycycline, Amoxiclav, Erythromycin, Ciprofloxacin, Neomycin, Lincomycin.

Methods

Classical bacteriological method was used to isolate the bacteria. In order to identify the bacteria there were used: colony description, Gram staining and API 20 system. In addition, antibiotic susceptibility test was performed by using classical disk diffusion susceptibility method as was described earlier by Quinn *et al* (1994).

RESULTS AND DISCUSSION

The general microbiological results of 881 milk samples, from which *Pseudomonas aureginosae*was isolated are show in Table 1.

Year	Sample tested	Positive for *P. aureginosae*	January - March	April - June	July - September	October - December
2014	163	8	1	3	2	2
2015	175	3	0	1	2	0
2016	274	5	2	0	1	2
2017	199	10	1	6	1	2
Total	**811**	**26**	**4**	**10**	**6**	**6**

Table 1. Sample tested and positive samples according years and seasons of the year

The frequency of bovine mastitis due by *Pseudomonas aureginosae*

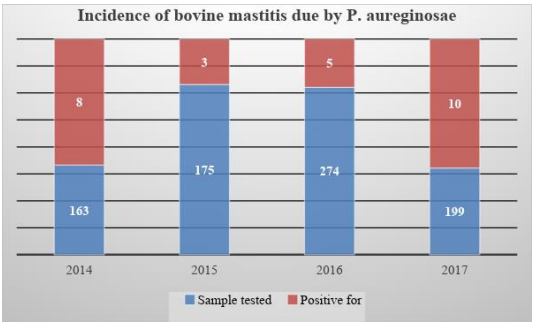

Figure 1. Incidence of bovine clinical mastitis due by *P.aureginosae* during 2014-2017

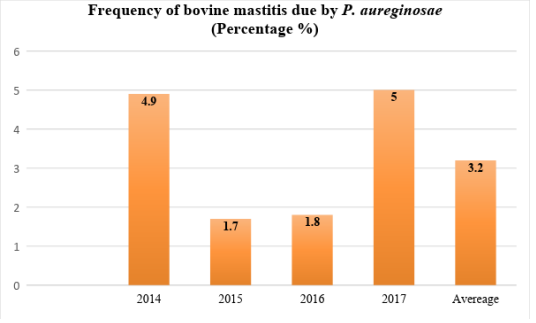

Figure 2. Frequency of bovine clinical mastitis due by *P.aureginosae* during 2014-2017

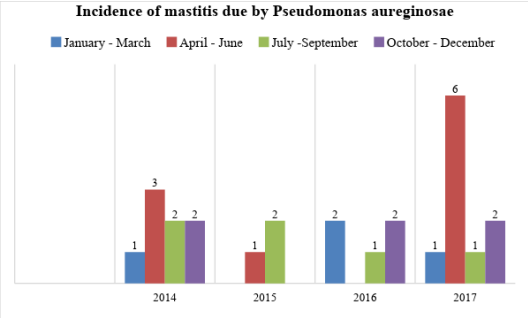

Figure 3. Incidence of bovine clinical mastitis due by *P.aureginosae*according season during 2014-2017

Results from antimicrobic susceptibility test showed that Enrofloxacin, Florfenicol and Gentamycinare the most susceptible for the isolates used in this study. Nine out of 16 isolates are resistant to a wide range of antibiotics, as shown in figure 4.

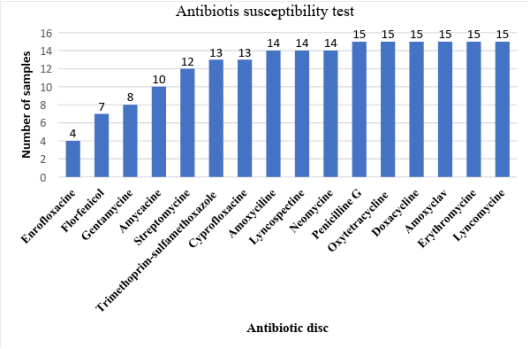

Figure 4.Number of resistant strains to specific antibiotic based on disc diffusion susceptibility test results

As a result, *Pseudomonas aureginosae* stains used in this study present resistance from a wide specter of antibiotics, specifically Amoxicillin, Amikacin, Penicillin G, Oxytetracycline, Lyncospectin, Doxacyclin, Amoxiclav, Erythromycin, Ciprofloxacin, Neomycin Lincomycin.

CONCLUSION

Results of antibiotic susceptibility test indicate that isolates were most susceptible to Enrofloxacin and Florfenicol. Interestingly, 77.7% (ranged from 25% -93.75%) of strains were resistant to all antibiotics in use. This result explained that successful treatment rate is low, and indicate that *P.aeruginosae* strains isolated from bovine mastitios are resistance to large number of antibiotics and is an emergent issue of veterinary services.

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