



E-ISSN: 2320-7078

P-ISSN: 2349-6800

www.entomoljournal.com

JEZS 2020; 8(2): 287-289

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Received: 04-01-2020

Accepted: 08-02-2020

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A retrospective observational study of prevalence of clinical conditions with special reference to antimicrobial resistance pattern of coliform mastitis in cows and otitis in canines

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Abstract

A retrospective study for a period from January 2017- December 2018 was conducted by analysing the clinical samples of animals affected due to various disease condition like mastitis, wounds, otitis, reproductive infections and respiratory affections in and around the Orathanadu region, Thanjavur District of Tamilnadu. The study revealed isolation of microorganisms such as mastitis (*E.coli*- 181 isolates), *Staphylococcus* spp. (149 isolates from otitis externa of canines), *Klebsiella* spp. (43 isolates from wounds), *Pseudomonas* spp –(18 isolates from reproductive tract infections) and *Pasteurella* spp.(13 isolates from respiratory tract infections). Owing to the abundancy of coliform mastitis cases in bovines and otitis cases in canines, the antibiotic resistance pattern of *Escherichia coli* for 181 samples from clinical cases of mastitis and *Staphylococcus* spp. from 149 samples were estimated with 16 number of antibiotics and Nalidixic acid showed a more higher resistance (92.8%) and Penicillin showed highest resistance (91.7%), Streptomycin (87.8%) and Trimethoprim-Sulfamethoxazole (78.4%) in mastitis cases caused by *E.coli* and Tetracycline showed highest resistance(93.28%), Trimethoprim-Sulfamethoxazole (91.9%) and Streptomycin. Hence this study would provide a judicial insight of appropriate antibiotic use and their association with antibiotic resistance in this agrarian Orathanadu region of Tamil Nadu and the multiple drug resistance will help to rationalise the antibiotics for public and field veterinary practitioners.

Keywords: Antimicrobial resistance-e.coli-mastitis-staphylococcus spp.-otitis-canines

1. Introduction

Coliform mastitis presently leads to emergence of resistance due to indiscriminate and prolonged use of antibiotics causing pathological changes in udder parenchyma and in absence of antibiogram is a major hurdle in the characterized by physical, chemical and micro biocontrol of mastitis alongwith gross changes in milk ^[10]. The Indian Dairy Industry suffers a total from various states with an whooping monetary loss of over Rs.6000 crore per year ^[6]. Otitis externa is one of the most common and multifactorial disorders accounting for up to 10 to 20% of consultations in canine practice ^[8]. Moreover Excessive trauma to the ear canal resulting from exuberant ear cleaning or trauma from instruments used in the ear canal may allow bacterial colonization ^[2]. The data regarding antimicrobial susceptibility pattern and certain important infection of mastitis and otitis is lacking in this region. Hence this study was aimed to isolate and identify the important pathogenic microbes with special reference to mastitis caused by *E.coli* in cows and *Otitis externa* caused by *Staphylococcus* spp. in canines and their antimicrobial susceptibility pattern over a period of two years from the clinical cases.

2. Materials and Methods

The prevalence of microorganisms causing mastitis (201), wound infections (69), respiratory ailments (29), otitis cases (163) and reproductive tract infections (29) were studied from the clinical cases received from Veterinary Clinical Complex ,Veterinary College and Research Institute, Orathanadu during January 2017-December 2018. Among these, the two important conditions in cows and canines such as mastitis suspected samples (201 nos) and otitis externa cases suspected (163 nos) from canines were subjected to biochemical characterization along with ABST during the study.

2.1 Bacteriological examination

The isolation of various pathogenic microorganisms were carried out [9]. The collected swabs were primarily incubated in Brain Heart Infusion Broth (BHI). The etiological agents such as *E.coli*, *Staphylococcus aureus*, *Streptococcus spp*, *Pseudomonas aeruginosa* and *Pasteurella spp*. were isolated and identified based on staining and biochemical characters. The bacterial pure culture of *E. coli* (from Mac Conkey agar) and *Staphylococcus* from MSA were swabbed on MHA and

spread for even distribution of bacteria. The plates were incubated for 24h and the zone of inhibition for each antibiotic was noted and compared with standard discs. A total of sixteen antibiotic discs were tested viz., Oxytetracycline (30µg), Enrofloxacin (10µg), Gentamicin (10µg), Penicillin(30µg), Ceftriaxone (30µg), Cefotaxime (30µg), Ceftriaxone/Tazobactam (20/10µg).The Multidrug resistance strain was designated for the resistance causing atleast 3 different classes of antimicrobials [12].

Table 1: Microbial Isolates from various clinical affections retrieved during the period of January 2017- December 2018 in Orathanadu region

Organisms isolated	Affections	Number of isolates
<i>Escherichia coli</i>	Mastitis	154/193
<i>Staphylococcus aureus</i>	Otitis	130/156
<i>Klebsiella spp</i>	Wounds	43/69
<i>Pseudomonas spp</i>	Reproductive tract infections	18/29
<i>Pasteurella spp</i>	Respiratory tract infections	13/29

The specific bacteria causing mastitis and otitis externa were presented in accordance to the recommendations for primary isolation and identification by Staining and biochemical tests and the ABST conducted for the analysis of resistance to atleast 1antimicrobial dug among the three antimicrobial classes were categorized [7].

3. Results

The antibacterial resistance pattern from mastitis was more than of otitis cases which were significantly higher ($P > 10^{-5}$). Out of 16 antimicrobial employed for mastitis and otitis cases, none were resistant and 87% of *E.coli* and *Staphylococcal* isolates showed resistance to one antibiotic. The resistance was seen on a higher rate (68.2%) with drugs like Oxytetracycline, Nalidixic acid, Sulphamethoxazole,

Sulpha trimethoprim and Ampicillin. On an overall, 79.8% (380/476) of the isolates showed resistance to at least three antimicrobials. The current findings elucidate that extensive, indiscriminate use of streptomycin for treating mastitis might have led to the development of high resistance and repeated therapeutic use and indiscriminate usage of antibiotics results in the development of antibiotic resistance. In recent times, both clinical and sub-clinical mastitis are treated with new higher antibiotics and hence the emergence of antibacterial resistance seen in this study with Ceftriaxone/Tazobactam. As antibiotic sensitivity test plays a key role in successful treatment of bovine mastitis, the maximum and consistent intermediate sensitivity was recorded against Gentamicin (50.8%), Enrofloxacin (43.09%) followed by Ciprofloxacin (44.7%) throughout the study period of two years.

Table 2: Multidrug resistance pattern among Coliform mastitis and *Staphylococcal* infections assessment by ABST isolated from bovine mastitis and otitis (canines) in Orathanadu region

S. No	Name of antibiotic	Mastitis caused by <i>E. coli</i> (%)			Staphylococcal infection (%) (Otitis)		
		R	I	S	R	I	S
1	Gentamicin	33 (18.28)	92(50.8)	56(30.9)	14(9.39)	36(24.16)	99(66.4)
2	Enrofloxacin	28(15.46)	78(43.09)	75(41.4)	19(12.75)	28(18.8)	102(68.4)
3	Ciprofloxacin	62(34.2)	81(44.7)	38(21.0)	17(11.5)	83(55.8)	49(32.9)
4	Tetracycline	163(9.0)	2(1.1)	16(8.8)	139(93.28)	3(2.0)	7(4.7)
5	Chlromphenicol	39(21.50)	93(51.3)	49(27.0)	35(23.4)	97(65.1)	17(11.4)
6	Ampicillin	46(25.4)	8(44.1)	127(70.1)	133(89.2)	4(2.6)	12(8.0)
7	Sulphamethoxazole	142(78.4)	15(8.20)	24(13.29)	135(90.6)	6(4.02)	7(46.9)
8	Trimethoprim/Sulphamethoxole	155(85.6)	16(8.8)	10(5.5)	137(91.9)	4(2.7)	8(5.36)
9	Amoxicillin/Clavulnic acid	14(7.7)	28(15.4)	149(82.3)	5(3.30)	6(4.02)	138(92.6)
10	Streptomycin	159(87.8)	16(8.8)	6(3.3)	134(89.9)	6(4.0)	9(6.0)
11	Penicillin	166(91.7)	9(4.9)	6(3.3)	127(85.2)	12(8.0)	10(6.7)
12	Cefatoxime	26(14.3)	6(3.3)	149(82.3)	21(14.0)	9(6.0)	119(79.8)
13	Amikacin	9(4.9)	6(3.3)	166(91.7)	8(5.3)	10(6.7)	131(87.9)
14	Ceftriaxone	20(11.0)	9(4.9)	152(83.9)	18(12.0)	15(10.0)	116(77.8)
15	Ceftriaxone-Tazobactam	3(1.65)	31(17.1)	147(81.2)	41(27.5)	10(6.7)	98(65.7)
16	Nalidixic acid	168(92.8)	8(4.4)	5(2.7)	127(85.2)	20(13.4)	2(1.3)

4. Discussion

The purpose of this study clarifies the selection of antibiotics for the veterinarians as retrospective antimicrobial analysis will surely help for empirical antimicrobial therapy discussed previously [4]. The staphylococcal isolates were found to have an increased resistance trend for Sulpha-Trimethoxazole, Penicillin and Tetracycline as they were commonly used for systemic treatments of dairy cattle over a period of time. Our findings support the evidence of findings of previous works carried out where the antimicrobial resistance in mastitis

causing pathogens is quite uncommon as of that of lesser evidence of increasing trends [1].

The prevalence of drug resistant *E. coli* isolates of mastitis in Tamilnadu was studied and reported that in MRSA mastitis, treated with amoxicillin+sulbactam and enrofloxacin showed 50 % clinical recovery and lower clinical recovery compared to *E. coli* and *S. aureus* mastitis might be due to multi-drug resistant i.e resistance to 3 or more of antimicrobials. Tamil Nadu [3].

5. Conclusion

In the present retrospective study, the clinical samples of animals affected due to various disease conditions like mastitis, wounds, otitis, reproductive infections and respiratory affections revealed presence of *E. coli* (181 nos), *Staphylococcus aureus*, (149 nos), *Pseudomonas aeruginosa* (18 nos) and *Pasteurella* spp. (13 nos) among which the antibiotic resistance pattern of *Escherichia coli* for 181 samples from clinical cases of mastitis and *Staphylococcus* spp. from 149 samples estimated with 16 number of antibiotics revealed Nalidixic acid with a more higher resistance (92.8%) and Penicillin showed highest resistance (91.7%) followed by Streptomycin (87.8%) and Trimethoprim-Sulfamethoxazole (78.4%) in mastitis cases caused by *E. coli* and Tetracycline showed highest resistance (93.28%) followed by Trimethoprim-Sulfamethoxazole (91.9%) and Streptomycin. The pathogenic *Escherichia coli* and *Staphylococcus* species and its use in animals is considered the most important factor which would promote the emergence, selection and dissemination of antimicrobial-resistant microorganisms in near future.

6. Acknowledgement

The author expresses his gratitude to the Dean, VCRI, and Orathanadu for providing facilities to complete the work in a successful manner.

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