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Isolation and Identification of *Serratia marcescens* from Bovine Mastitis infections in Iraq and their Susceptibility to Antibiotics

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Abstract

This study was conducted to isolate *Serratia marcescens* from bovine mastitis for the first time in veterinary medicine field in Iraq and their susceptibility against antibiotics.

One hundred and fifty samples from case of mastitic milk of cow were collected from College of University of Baghdad/College of Veterinary Medicine, University of Baghdad/College of Agriculture, Dora zone, Radhwanya zone and Abu-Ghraib zone. Cultured by selective and differential media then Gram stain were done. After purification of cultured bacteria biochemical tests were done and confirmed by API 20 E System and RapID™ ONE System kit.

Results showed that 6 (4%) samples out of 150 samples were positive for *Serratia marcescens* and it's the first time to isolate in veterinary medicine field.

The resistance of these isolates against antibiotics Gentamycin (30 mg), Imipenem (10 mg), Ciprofloxacin (5 mg), Nalidixic acid (30 mg), Ampicillin (10 mg), Cefotaxime (30 mcg), Amikacin (30 mg), Chloramphenicol (30 mg), Tetracyclin (10 mg) and Amoxicillin-Clavulanic acid (20/10 mg) were studied. The results showed that *Serratia marcescens* were resistant 100% to Ampicillin, Imipenem and Tetracyclin, 66.66% to Cefotaxime and susceptible 100% to Ciprofloxacin, 83.33% to Gentamycin, 66.66% to Chloramphenicol and 50% to Nalidixic acid. While Intermediate 83.33% to Amoxicillin-Clavulanic acid, 50% to Nalidixic acid and 33.33% to Chloramphenicol and Cefotaxime.

Keywords: *Serratia marcescens*, Bovine Mastitis, Susceptibility, Antibiotics

1. Introduction

Serratia species are one of gram negative family it is opportunistic bacteria; classified in the ethnic group Klebsiellae and the large family Enterobacteriaceae, it's also, a widespread in the environment [1].

Serratia marcescens is the most important pathogenic species of *Serratia*, rare reports have described disease consequential from infection with other type like from *Serratia odorifera*, *Serratia plymuthica*, *Serratia liquefaciens*, *Serratia rubidaea* and *Serratia fonticola* [2-6].

Some strains of *S. marcescens* are capable of producing a pigment which is called prodigiosin, it's ranges from dark red color to pale pink depending on the period of the colonies. *Serratia* are capable of thriving in diverse environments, like water, soil, and the digestive tracts of various animals, *S. marcescens* has a weakness for growth on starchy foodstuffs, where the pigmented colonies are easily misguided for drops of blood [7, 8].

Mastitis almost occurred due to the effect of infection by bacteria or mycotic pathogens, about 140 microbial species; serovars have been isolated from the mammary gland. Pathogens causing mastitis in cattle are divided into two groups the major pathogens those that cause clinical mastitis and the minor pathogens those that normally cause subclinical mastitis [9-13].

A number of special kind of *Serratia* can act as a reason of mastitis, including *S. marcescens*, *S. rubidaea* and *S. liquefaciens*. Firstly the dry period is considered a major phase of infection [14, 15] secondly the lactation phase [16-18]. In spite of, the source of the outbreak was not detected the outbreaks of *Serratia* to cause mastitis have been occurred may be due to growing of the organism in a teat dip cup and in the bedding [16-20]. On the other hand, Poor hygiene and damage to teat ends are thought to elevate the rate of mastitis [21]. Albeit *Serratia* is considered to be an environmental microorganism, transmitted by the milking machine may occur [16]. Serological typing can be used to determine whether *Serratia* isolates belong to different strains, or to a single strain, from a point source or contagious transmission.

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The clinical signs of *Serratia* mastitis characterized by discolored milk and flakes; the infections have a tendency to be chronic and it can be lasted from months to years; on the other hand the infection occurred independent of milk production or production string [14, 18, 20].

On instance outburst of *Serratia* which cause mastitis may have occurred due to contagious conduction [16], on this incident, infected cows should be separated and managed at milking time. *Serratia* as a family are challenging to mainly familiar antibiotics; so that treatment with this antibiotics is not not compulsory, in spite of the majority of cases cure spontaneously; many authors report that antibiotic therapy in clinical cases that gives less than 14% of quarters with *Serratia* infection were treated [15, 16, 18, 22, 23].

Necessitating the use of antibiotics usually kept in reserve. Therefore, this study was carried out to give current information regarding bacteria *Serratia marcescens* involved in causing mastitis and their antibiotic susceptibility profile in Iraq.

2. Materials & Methods

2.1 Milk sampling

A whole of One hundred and fifty milk samples had been collected starting from Abu- Ghraib zoon, College of Veterinary Medicine/University of Baghdad, College of Agriculture/University of Baghdad and Dora zoon, Radhwanya zone and Abu-Ghraib zone during the period of October 2015 through February 2016, from cows suffering from clinical and subclinical mastitis. The udder was washed directly with tap water to remove dirt then dry with clean towel, the teat dip in Iodine solution 1:1000 and leave to dry than the teat was dip in 70% alcohol than dry, before sample taken one or two streams of milk discarded. Milk was collected in sterile vial (test tube 10 ml). These samples were transferred to the lab. In cooled container.

2.2 Identification of *Serratia marcescens*

2.2.1 Cultural and microscopical characteristics

The shape and color of colonies observed after plating the samples on Nutrient agar, the suspected isolates were cultured on MacConkey agar, Luria agar, Xylose lysine deoxycholate agar by streaking method. Raffinose, arabinose, peptone broth and sugar fermentation were similarly used to check identification. Then Gram stain were done in using light microscope to differentiate the bacteria is either Gram negative or Gram positive bacteria. After purification of cultured bacteria biochemical tests, API 20 E System and RapID™ ONE System kit were done to complete the diagnosis of *Serratia marcescens*.

2.3 Antibiotics susceptibility test

To study the susceptibility of the isolates against ten different antibiotics Gentamycin (30mg), Imipenem (10 mg), Ciprofloxacin (5mg), Nalidixic acid (30mg), Ampicillin (10mg), Cefotaxime (30mcg), Amikacin (30mg), Chloramphenicol (30mg), Tetracycline (10mg) and Amoxicillin-Clavulanic acid (20/10mg). The trial was accomplished using a disc diffusion method on Mueller – Hinton Agar according to [24], the results were logged as

susceptible or resistant by the measurement of Diameter of the inhibition zone in millimeter.

3. Results and Discussion

3.1 Microscopical, cultural characteristics, biochemical and confirmatory tests

Results of bacterial isolation of 150 milk samples showed that six samples were belong to *Serratia marcescens* represented 4% from milk samples [25] showed that 4(3%) out of 120 cow affected by *Serratia marcescens* mastitis and these outcomes in contract with the present study.

The results of samples culturing showed different morphological characteristics of bacteria on different media, after incubation at 37 °C for 24hours. On MacConkey agar colonies were appeared are lactose fermentor and appear red colonies duo to the ability of *Serratia marcescens* to produce pigment as showed in Fig. (1) These results in agree with [26]. Isolated bacteria were appeared under light microscopic lenseas gram negative rods.

The Biochemical identification of *Serratia marcescens* showed that bacteria were Gram –ve, Rod, Catalase positive, oxidase negative, lactose non- fermenter, motile, Indole negative, citrate utilization positive, TSI y/y, DNase positive and Urease negative as shown in table (1) as [27]. To confirm the diagnosis, Api 20 E system and Rap ID™ ONE System were used.

The results of antibiotics susceptibility test showed that *Serratia marcescens* were resistant 100% to Ampicillin, Imipenem and Tetracyclin, 66.66% to Cefotaxime and susceptible 100% to Ciprofloxacin, 83.33% to Gentamycin, 66.66% to Chloramphenicol and 50% to Nalidixic acid. While Intermediate 83.33% to Amoxicillin-Clavulanic acid, 50% to Nalidixic acid and 33.33% to Chloramphenicol and Cefotaxime. As shown in Table (1), Fig. (2).

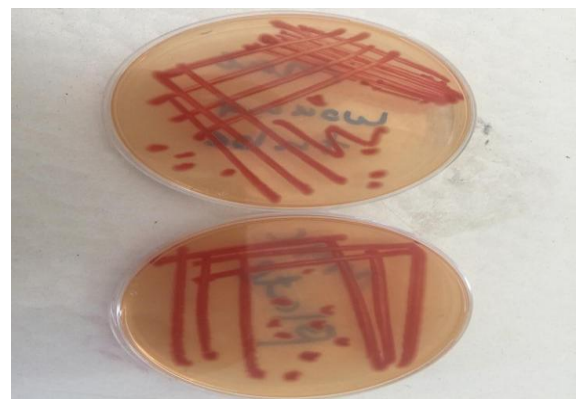


Fig 1: Colonies of *Serratia marcescens* on Luria agar showed the ability of bacteria to produce pigment.

[28] Showed that *Serratia Spp.* was highly resistant to Cephalosporin and the Polymyxin B, and various proportions were also resistant to other antibiotics including Kanamycin, but all of the isolates were susceptible to Gentamicin. The appearance of resistance to kanamycin and nalidixic acid among endemic strains was confirmed and these results in agreement with the present study.

Table 1: Results of sensitivity test of, 6 isolates of *Serratia marcescens* against 10 of antibiotics

Antibiotic	Sensitive		Intermediate		Resistant	
	Number	%	Number	%	Number	%
Amikacin(30mg)	2	33.33	4	66.66	0	0
Tetracyclin(10mg)	0	0	0	0	6	100

Imipenem (10mg)	0	0	0	0	6	100
Gentamicin (10mg)	5	83.33	1	16.66	0	0
Nalidixic acid (30mg)	3	50	3	50	0	0
Ciprofloxacin (10mg)	6	100	0	0	0	0
Amoxycillin-Clavulanic acid (20/10mg)	0	0	5	83.33	1	16.66
Chloramphenicol (30mg)	4	66.66	2	33.33	0	0
Cefotaxime (10mg)	0	0	2	33.33	4	66.66
Ampicillin(10mg)	0	0	0	0	6	100

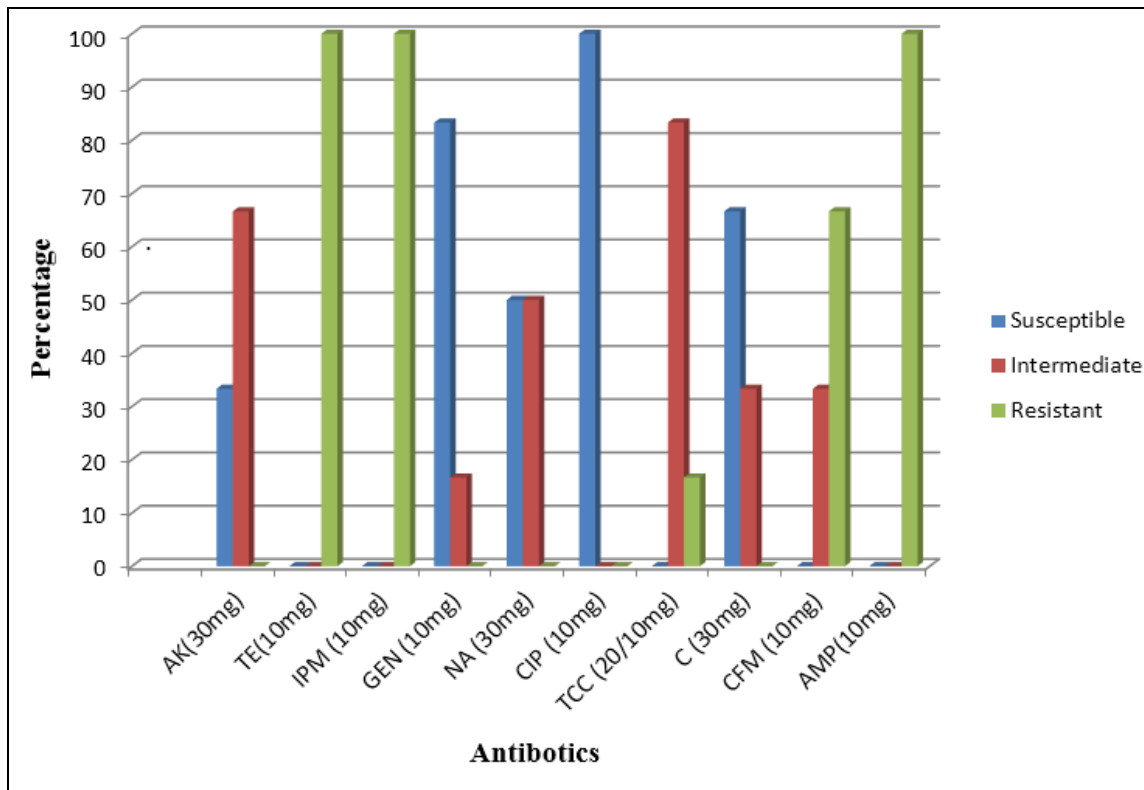


Fig 2: Results of Antibiotic Susceptibility Test of All *Serratia marcescens* Isolated from Cow

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