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Antibiotic sensitivity testing in subclinical mastitis in Gir Cows

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Abstract
The present investigation on Antibiotic Sensitivity Test of subclinical mastitis in Gir cow was carried out in Udaipur district of Mewar region of Rajasthan. Total of 188 quarter milk samples of milk from the 47 apparently healthy Gir cows were selecteted purposively to study the Antibiotic Sensitivity Test of subclinical mastitis in Gir cows. The antibiotic sensitivity by disc diffusion method using Muellar-Hinton agar the basis of Zone of inhibition. The Antibiogram of isolates revealed sensitivity of different antibiotics to the isolated bacteria was highest for amoxycillin-sulbactam (98 per cent), followed by cefuroxime and cefoperazone (96.36 per cent each). Chloramphenicol and ceftriaxone (90.90 each), ciprofloxacin (74.54 per cent) and tetracyciline (63.63 per cent).

Keywords: subclinical mastitis, isolated bacteria, antibiotic sensitivity test, Gir cows, Rajasthan

1. Introduction

Milk, is an essential nutritious diet to young mammals containing proteins, lipids, amino acids, vitamins, minerals and carbohydrates. Milk also serves as an optimum medium for the propagation of various pathogenic and spoilage microorganisms (Minst *et al*., 2012; Gatti *et al*., 2013) [11, 7]. These pathogens invade the mammary glands, develop and multiply, producing some toxic substances that results in inflammation, reduced milk production and altered milk quality, leading to a clinical condition known as mastitis (Oliver and Muranda, 2012; Rall *et al*., 2013) [12, 15]. The most common bacterial pathogens responsible for clinical or subclinical mastitis in animals can be divided into two broad categories: Namely the contagious pathogens (*Streptococcus* species, *Staphylococcus* species and *Mycoplasma* species) and environmental or coliforms pathogens which include *Escherichia coli and Klebsiella* species, usually found around the dairy farm (Hogan *et al*., 2011; Oliver and Muranda, 2012; Rall *et al*., 2013) [9, 12, 15]. Apart from these, several other pathogens such as *Lactobacillus Salmonella*, *Listeria*, *Pseudomonas*, *Corynebacterium*, *Campylobacter* and *Micrococcus* species have also been reported in raw milk worldwide (Waller *et al*., 2011; Hill *et al*., 2012; Santman-Berends *et al*., 2012; Bracke *et al*., 2013) [18, 8, 17, 4]. Subclinical mastitis is the most common form of mastitis among dairy cattle (Salvador *et al*., 2012) [16], with a prevalence of about 40-50 times more than the clinical mastitis (Roy *et al*., 2009; Mekibib *et al*., 2010) [14, 10]; which attracts for prompt attention in the dairy industry. However, the prevalence was reported to be influenced by factors such as breed, anatomical abnormality of the udder, stage of lactation, parity and management practice (Almaw *et al*., 2008) [1]. Antimicrobial resistance of mastitis pathogens to multiple drugs has been reported worldwide (Waller *et al*., 2011; Oliver and Muranda, 2012; Chaudhary and Payasi, 2013) [18, 12, 5]. This is because of indiscriminate use of the antibiotics by farmers, thereby rendering them ineffective and leading to permanent loss of the mammary tissues. The pathogens can transfer the resistance to a sensitive bacterium by conjugation known as R-plasmid mediated antibiotic resistance (Ahmad *et al*., 2001) [2]. The prevalence of antibiotic resistance usually varies between isolates from different samples and even between herds in the same farm (Chaudhary and Payasi, 2013; Rall *et al*., 2013) [5, 15]. Since antibiotics play animportant role in the control of mastitis, a sound surveillance system for antibiotic resistance that will ensure optimal result and minimize the risk of development and spread of resistance in dairy farms is very crucial. Thus, the aim of this study was to assess the determine the antimicrobial resistance from subclinical mastitis in Gir Cows milk samples.

2. Material and Methods
2.1 Sample Collection
A total of 188 quarter milk samples of milk from the 47 apparently healthy Gir cows were collected. The age of animals ranged between 5 to 12 years with lactation stage between first to sixth. Udder and teats of cows were cleaned with water and allowed to dry. The hands were also washed with soap and water and rinsed with spirit. The teat orifice was thoroughly swabbed with a cotton soaked in spirit. After discarding first few strips of milk approximately 30 ml of foremilk from each quarter was collected into sterilized test tubes. Precautions was taken to avoid contamination in milk samples. A total of 188 milk samples were collected. The milk samples were brought to the laboratory and analyzed immediately.

2.2 Antibiotic sensitivity test
The following antibiotic discs were used for the antiobiogram determination of the bacterial isolates

Amoxycillin-sulbactam	AMS	10/10mcg
Cefoperazone	CPZ	75mcg
Cefuroxime	CXM	30mcg
Ceftriaxone	CTZ	30mcg
Chloramphenical	C	30mcg
Ciprofloxacin	CIP	5mcg
Gentamicin	GEN	10mcg
Tetracycline	TE	30mcg

Antibiogram of the bacterial isolates was based on the Bauer *et al.* (1966) [3] disc method. Nutrient broth in the tubes was inoculated with the bacterial culture from slant. After 6 -7 hours, when the bacteria were in the exponential phase of growth, the broth culture was swabbed on the Mueller-Hinton agar plates by sterile cotton swabs When broth culture was dried, eight antibiotic discs were placed with the aid of an automatic disc dispenser in front of flame. The petriplates were incubated for 15 - 20 hours and observed for the zone of inhibition. The diameter of the zone of inhibition was measured with the help of a measuring scale and compared with standard scale of inhibition for each antibiotic disc as per the instructions provided by manufacturer Himedia

3. Results and Discussion
Antibiotic sensitivity pattern of bacterial isolates pave the way in suggesting the treatment and control of the mastitis. It also helps in preventing development of resistant strains against the drug. Which may be either intermediate or resistant to bacteria in antibiotic sensitivity test. It aids the physician to go for a direct approach of treatment with drug to which bacteria are highly susceptible. In vitro antibiotic sensitivity was carried out to determine the sensitivity of different isolates in the present study. Result of antibiotic sensitivity to different bacterial isolates from subclinical mastitis have been presented in Table 1. Amongest Staphylococci (27) tested, all the isolates (100 per cent) were found found sensitive to amoxycillin- sulbactam and cefoparazone followed by cefuroxime and chloramphenicol (92.60 per cent each), ceftriaxone (92.59 per cent), gentamicin (77.77 per cent), ciprofloxacin (74.07 per cent) and tetracycline (62.96 per cent).Thirteen isolates of streptococci were 100 per cent sensitive to amoxycillin-sulbactam and cefuroxime followed by chloramphenicol (92.30 per cent), cefoparazone and ceftriaxone (84.61 per cent each), gentamicin (76.92 per cent), ciprofloxacin and tetracycline (69.23 per cent each).Eight isolates of *E. coli* were 100 per cent sensitive to chloramphenicol, cefuroxime and cefoperazone, followed by amoxycillin-sulbactam and ceftriaxone (87.5 per cent each), gentamicin and ciprofloxacin (75 per cent each) and tetracyciline (62.5 per cent). Four isolates of Corynebacterium were 100 per cent sensitive to cepoparazone cefuroxime, ceftriaxone and amoxycillin- sulbactam followed by chloramphenicol and ciprofloxacin (75 per cent each); and gentamicin and tetracycline (50 per cent each).Three isolates of Bacilli were 100 per cent sensitive for amoxycillin-sulbactam, cefuroxime, cefoparazone, ciprofloxacin, ceftriaxone and (66.66 per cent) for gentamicin, chloramphenicol and tetracyciline. Overall sensitivity of different antibiotics to the isolated bacteria was highest for amoxycillin-sulbactam (98 per cent), followed by cefuroxime and cefoperazone (96.36 per cent each). Chloramphenicol and ceftriaxone (90.90 each), ciprofloxacin (74.54 per cent) and tetracyciline (63.63 per cent).

All antimicrobial use in the herd may affect the resistance of isolates by increasing the presence of these antimicrobial agents in the cow's environment. Indiscriminate use of antibiotic leads to resistance of mastitis causing bacteria. Hence, selection of antibiotic is necessary to advocate effective treatment. The incidence of resistant mastitis was higher which might be due to indiscriminate use of antibiotics and intramammary preparations containing combinations or alone broad-spectrum antibiotics (Pitkala *et al*., 2007) [13]. Edward *et al*., (2002) [6] suggesting a possible development of resistance from prolonged and indiscriminate usage of some antimicrobials. It is therefore, very important to implement a systemic application of an antibiotic susceptibility test prior to the use of antibiotics in both treatment and prevention of intra-mammary infections. Further, it highlights the need for preventing the indiscriminate use of antibiotics. This approach can lower irrational use of antimicrobial drugs and lower risk of antibiotic resistance for animal and human population.

Table 1: Overall sensitivity of different antibiotics in the isolated bacteria

S.No.	Antibiotic	Sencitivity (%)
1	Amoxycilline-sulbactam	98
2	Cefuroxime	96.36
3	Chloramphenicol	90.90
4	Cefoprazone	96.36
5	Ciprofloxacin	74.54
6	Gentamicin	74.54
7	Ceftriaxone	90.90
8	Tetracycline	63.63

Table 2: Antibiotic sensitivity of microorganism isolated from sub clinical mastitic samples

S. No.	Antibiotic	Response of antibiotic	Staphylococci (27)	Streptococci (13)	*E. coli* (8)	Corynebacterium (4)	Bacillus (3)
1.	Amoycillin-sulbactum	Sensitive	27(00.00)	13(100.00)	7(87.5)	4(100.00)	3(100.00)
		Intermediate	0(00.00)	0(00.00)	1(12.5)	0(00.00)	0(00.00)
		Resistant	0(00.00)	0(00.00)	0(00.00)	0(00.00)	0(00.00)
2.	Cefuroxime	Sensitive	25(92.60)	13(100.00)	8(100.00)	4(100.00)	3(100.00)

		Intermediate	2(7.40)	(00.00)	(00.00)	(00.00)	(00.00)
		Resistant	(00.00)	(00.00)	(00.00)	(00.00)	(00.00)
3.	Chloramphenicol	Sensitive	25(92.60)	12(92.30)	8(100.00)	3(75.00)	2(66.66)
		Intermediate	1(3.20)	1(7.70)	(00.00)	(00.00)	(00.00)
		Resistant	1(3.20)	(00.00)	(00.00)	1(25.00)	1(33.33)
4.	Cefoperazone	Sensitive	27(100.00)	11(84.61)	8(100.00)	4(100.00)	3(100.00)
		Intermediate	(00.00)	2(15.38)	(00.00)	(00.00)	(00.00)
		Resistant	(00.00)	(00.00)	(00.00)	(00.00)	(00.00)
5.	ciprofloxacin	Sensitive	20(74.07)	9(69.23)	6(75)	3(75)	3(100.00)
		Intermediate	(00.00)	1(7.69)	(00.00)	(00.00)	(00.00)
		Resistant	7(25.92)	3(23.07)	2(25)	1(25)	(00.00)
6.	Gentamicin	Sensitive	21(77.77)	10(76.92)	6(75.0)	2(50.00)	2(66.66)
		Intermediate	(00.00)	0(00.00)	(00.00)	1(25.00)	(00.00)
		Resistant	6(22.22)	3(23.07)	2(25.0)	1(25.00)	1(33.33)
7.	Ceftriaxone	Sensitive	25(92.59)	11(84.61)	7(87.5)	4(100)	3(100)
		Intermediate	(00.00)	2(15.40)	(00.00)	(00.00)	(00.00)
		Resistant	2(7.40)	(00.00)	1(12.5)	(00.00)	(00.00)
8.	Tetracycline	Sensitive	17(62.96)	9(69.23)	5(62.5)	2(50)	2(66.66)
		Intermediate	3(11.11)	1(7.69)	1(12.5)	(00.00)	(00.00)
		Resistant	7(25.92)	3(23.07)	2(25.0)	2(50.0)	1(33.33)

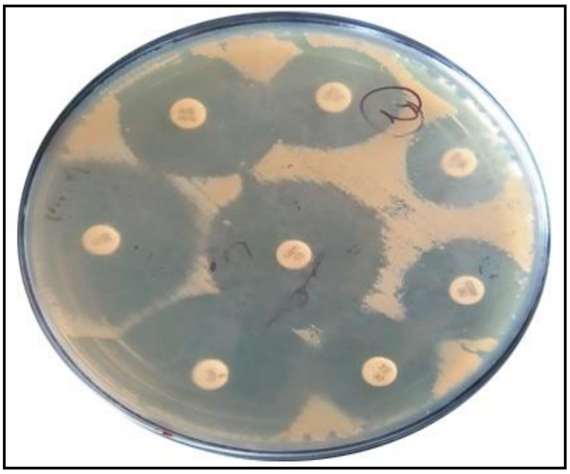

Plate 1: Antibiotic sensitivity pattern of bacteria isolated from subclinical mastitic milk sample

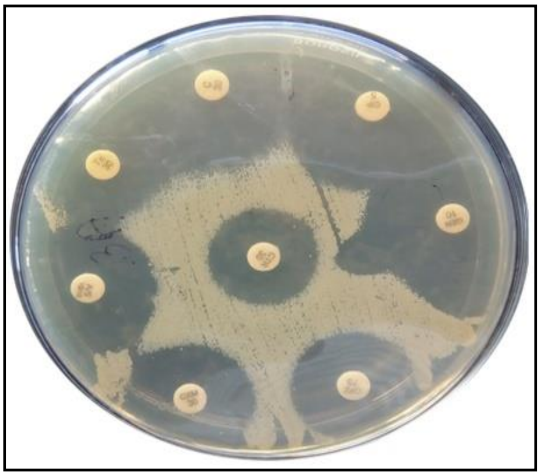

Plate 2: Antibiotic sensitivity pattern of bacteria isolated from subclinical mastitic milk sample

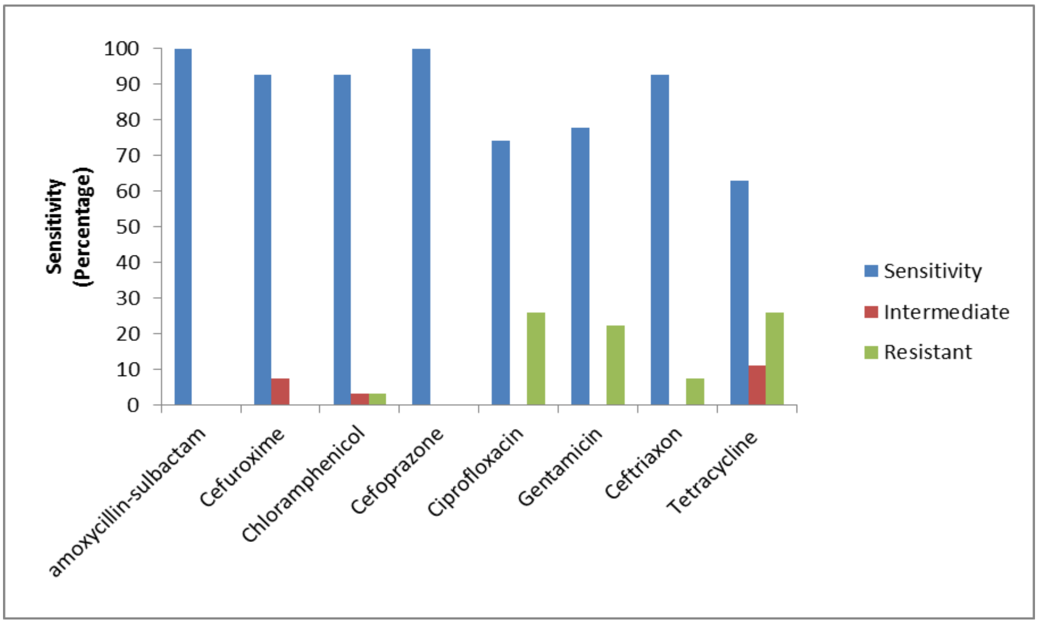

Fig 1: Antibiotic sensitivity of staphylococcus spp. isolated from subclinical mastitic sample

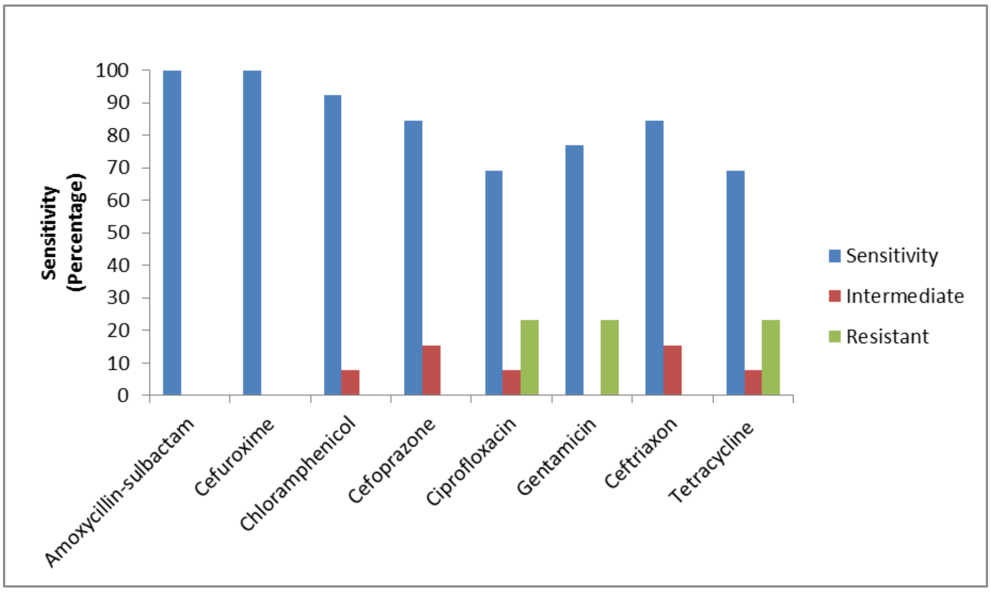

Fig 2: Antibiotic sensitivity of streptococcus spp. isolated from subclinical mastitic sample

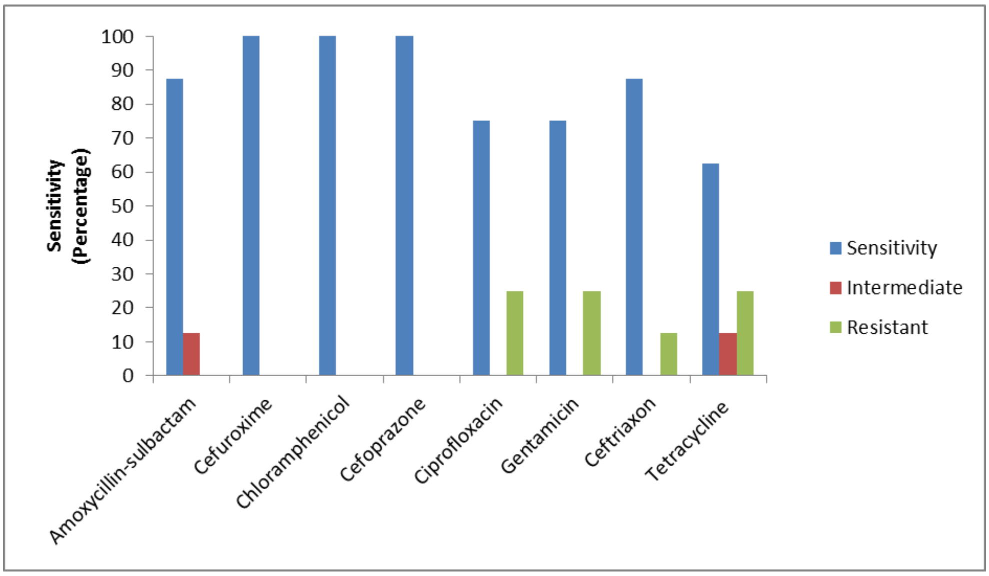

Fig 3: Antibiotic sensitivity of *E coli* spp. isolated from subclinical mastitic sample

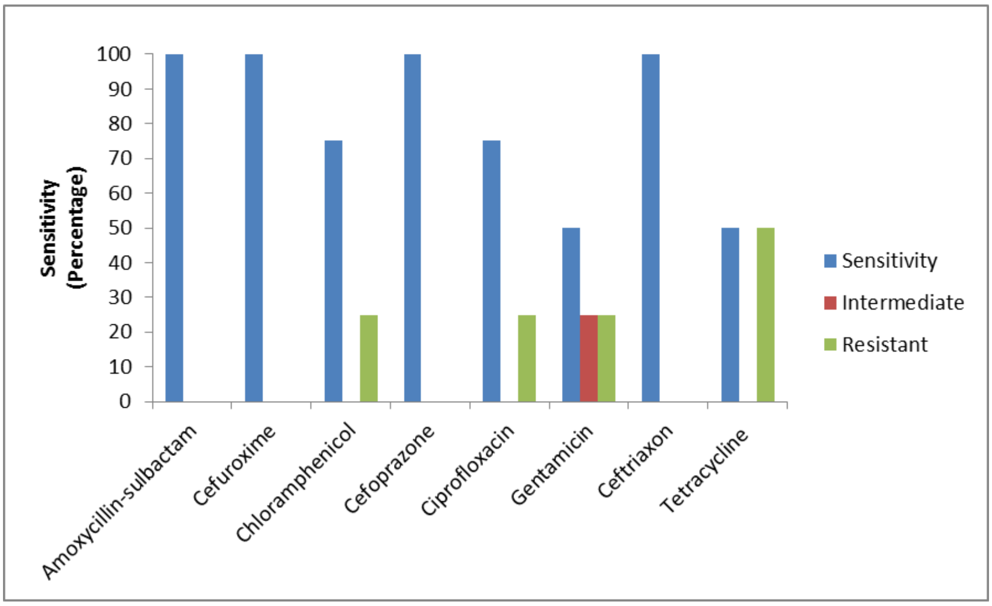

Fig 4: Antibiotic sensitivity of Corynebacterium spp. isolated from subclinical mastitic sample

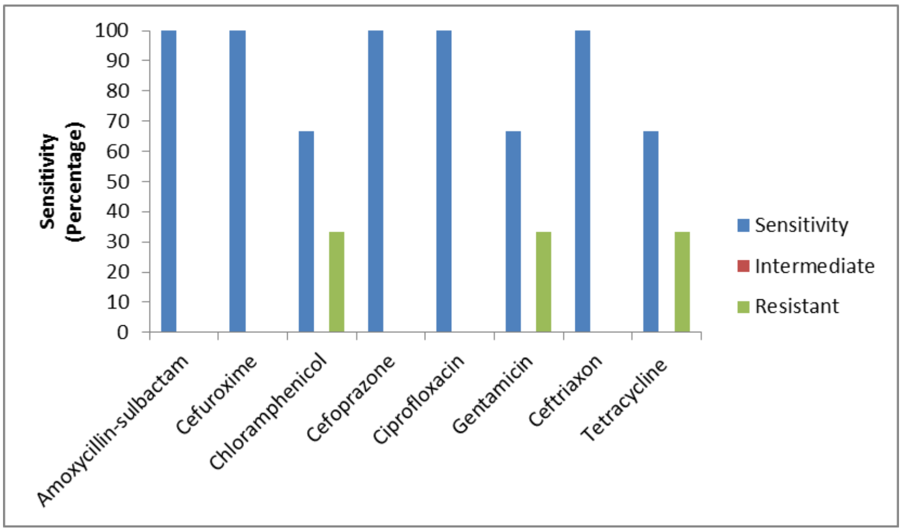

Fig 5: Antibiotic sensitivity of Bacillus spp. isolated from subclinical mastitic sample

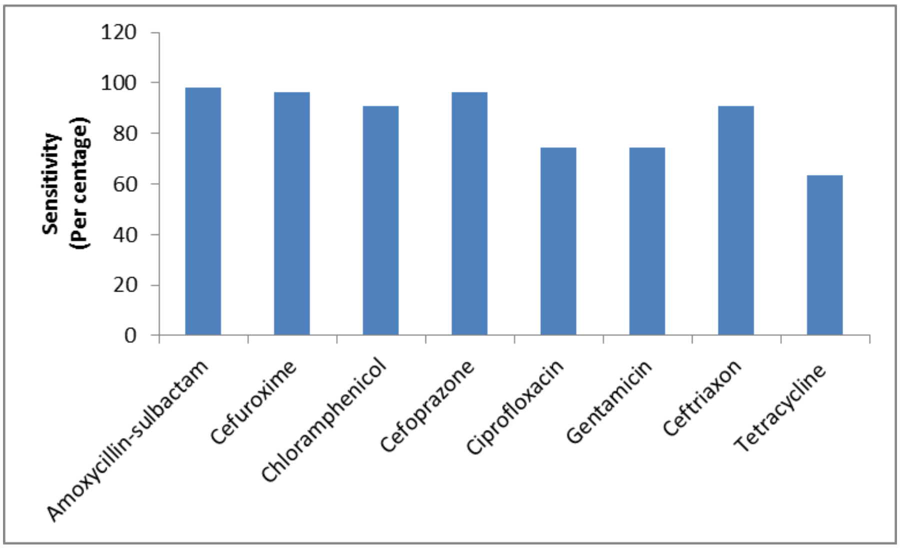

Fig 6: Overall sensitivity of different antibiotics in the isolated bacteria

4. Conclusion

In vitro antibiotic sensitivity revealed that the isolates of Staphylococci were highly sensitive to amoxycillin-sulbactam, cefuroxim and cefoperazone. Streptococci were highly sensitive to amoxycillin- sulbactam and cefuroxime, chloramphenicol, cefoparazone, ceftriaxone, gentamicin, ciprofloxacin and tetracyciline. *E. coli* were highly sensitive to cefoparazone, cefuroxime and chloramphenicol followed by ceftriaxone and amoxycillin-sulbactam. *Corynebacterium* were highly sensitive to cefoperazone, cefuroxime, ceftriaxonee and amoxycillin-sulbactam. *Bacillius* were highly sensitive to cefoperazone, cefuroxime, ceftriaxone and amoxycillin- sulbactam and ciprofloxacin. Overall sensitivity of different antibiotics to the isolated bacteria were amoxycillin-sulbactam (98 per cent) followed by cepoperazone, cefuroxime (96.36 per cent each), ceftriaxone and chloramphenicol (90.90 per cent each), gentamicin and ciprofloxacin (74.54 per cent each), and tetracyciline (63.63per cent).

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