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Antibiogram Pattern of *Bacillus cereus* isolated from Milk and Milk Products in Udaipur City of Rajasthan, India

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ABSTRACT: This study was conducted in Udaipur city of Rajasthan, and aimed to analyse the antibiogram of *Bacillus cereus* isolated from dairy products. A total of 160 samples, including raw pooled market milk (n=20), ice cream (n = 20), milk powder (n = 20), pasteurised milk (n = 20), paneer (n = 20), dahi (n = 20), khoa (n = 20), and butter (n = 20), were processed for the isolation, identification, and antibiotic susceptibility pattern of *Bacillus cereus*. All the isolates from milk and milk products were tested throughout the trial for susceptibility to a total of 12 different antibiotics. The antibiogram study showed that all the isolates had 100% sensitivity to amikacin and 100% resistance to ampicillin (100%), methicillin (100%) and penicillin-G (100%). The main challenge regarding in this study is that since milk is a perishable thing so we have to quickly analyse the samples and it is also used to make variety of products, so we need to collects large number of samples to know the antibiogram pattern.

Keywords: *Bacillus cereus*, milk, milk products, antibiogram, resistance, sensitive.

INTRODUCTION

The study on the antibiogram of *Bacillus cereus* isolated from dairy products was performed in Udaipur city of Rajasthan. Raw pooled market milk (n=20), ice cream (n=20), and other dairy products (n=80) were among the total of 160 samples. *Bacillus cereus* has been blamed for cases of food poisoning in a wide variety of countries. Pasteurisation only partially kills *Bacillus cereus* spores in milk (Aires *et al*., 2009; Sood *et al*., 2017), thus the bacterium is commonly found in milk. Out of the 80 samples of raw milk and locally dairy products studied, 19 (23.75) were contaminated with Bacillus cereus. Among the 19 positive samples, the bacterial percentage were 26.3%, 21%, 15.8%, 15.8%, 10.5%, 5.3%, 5.3% and 0% in raw milk, kashk, Skimmed cheese, yogurt, cream, UHT milk, butter, and ghee, respectively (Ali *et al*., 2016). In another study to characterize Bacillus cereus using culture-based and PCR techniques among 390 samples including milk, dairy products, water and environmental specimens. Antimicrobial activities of disinfectants and bioactive peptides were performed using European Suspension Test and liquid-broth method. Overall of 29.5 and 67.4% among dairy and environmental samples were positive for B. cereus. The prevalence of B. cereus in household milk, rice milk, water and street vendor milk was 37.5, 36.4, 33.3, and 30%, respectively (Ahmed *et al*., 2020).

According to Tallent *et al*. (2012); Abraha *et al.* (2017), *Bacillus cereus* is a Gram positive, facultative anaerobic, spore-forming, catalase positive, rod-shape, beta-hemolytic, and motile bacteria. According to Carlin *et al.* (2010) ; Ceuppens *et al.* (2013), *Bacillus cereus* has been isolated from dairy products, eggs, raw meat, processed meat, soil, and plant materials. At temperatures between 4 to 7 degrees Celsius, *Bacillus cereus* can grow (Grannum *et al.*, 2005). Emetic and diarrhoeal food poisoning syndromes are both brought on by *Bacillus cereus* (Oh *et al*., 2012). The diarrhoeal syndrome is brought on by enterotoxins like haemolysin BL (Beechar *et al.*, 1995), non-haemolytic enterotoxin (Lund and Granum 1997), and cytotoxin K (Ankolekar *et al.*, 2009), while the emetic syndrome results in vomiting (Waser *et al.*, 2007). Within 8 to 16 hours of ingesting hemolysin BL, abdominal pain and watery diarrhoea are symptoms of the diarrhoeal kind (Beecher *et al.*, 1995). There is currently a pressing need to identify the antibiogram of *Bacillus cereus*, which is commonly found in foods like milk and milk products. Amplification of β-lactamases, which makes bacteria resistant to penicillin, ampicillin, and cephalosporins, is the most frequent cause of antibiotic resistance in bacteria (Cormican *et al.*, 1998). Multiple drug-resistant *Bacillus cereus* are becoming more prevalent, which poses a serious risk to public health. The WHO now considers antibiotic resistance to be a global health emergency (Ghazaei, 2019). To prevent the spread of antibiotic-resistant bacteria from livestock to humans, it is important to think about how antibiotics are used in the first place (Faria-Reyes *et al.*, 2001). *Bacillus cereus* was isolated from store-bought milk and milk products in Udaipur, Rajasthan, and its antibiogram was evaluated for this study.

MATERIALS AND METHODS

Media used: Motility test medium, nitrate broth, brain heart infusion broth (BHIB), Muller-Hinton agar, sheep blood agar, polymyxin pyruvate egg yolk mannitol bromothymol blue agar (PEMBA),"and Simmons citrate agar were employed in the current investigation.

Sample collection: A total of 160 samples were gathered from various dairy establishments and sweet shops in Udaipur, Rajasthan, including 20 samples of raw pooled market milk, 20 samples of pasteurized milk, 20 samples of dahi, 20 samples of khoa, 20 samples of paneer, 20 samples of milk powder, 20 samples of ice cream, and 20 samples of butter.

Isolation: To isolate the bacteria, peacock blue-colored colonies were looked for on the plates of selective medium made of polymyxin pyruvate, egg yolk mannitol, and bromothymol blue agar (PEMBA). The isolates phenotypes were verified by biochemical analysis.

Antibiogram of *Bacillus cereus*: According to Bauer *et al.* (1966), an antibiotic susceptibility test was performed on every isolate of *Bacillus cereus*.

RESULTS AND DISCUSSION

Characteristic fimbriate peacock blue colonies (3-5 mm) surrounded by a blue zone of egg yolk were observed when *Bacillus cereus* was isolated on PEMBA media. All of the morphologically examined isolates seemed to be rod-shaped, Gram-positive bacilli that were either individually or in chains (Fig. 1 and 2).

Antibiotic susceptibility pattern of *Bacillus cereus.* To determine the antibiotic susceptibility pattern for all isolates of *Bacillus cereus* isolated from raw milk and milk product samples, the following 12 antibiotics (amikacin, ampicillin, cephalothin, ciprofloxacin, erythromycin, gentamicin, methicillin, kanamycin, nalidixic acid, penicillin G, streptomycin, polymyxin B) were used in the current study (Table 1 & Fig. 3).

Antibiotic susceptibility pattern of *Bacillus cereus* isolated from raw milk. The most effective antibiotics were amikacin (100%), gentamicin (100%) and streptomycin (100%), according to the analysis of antibiotic resistance on the six isolates of *Bacillus cereus* found in raw pooled market milk. The susceptibility pattern was moderate for erythromycin (100%), nalidixic acid (83.33%), polymyxin B (50%) ciprofloxacin (33.33%), and kanamycin (16.66%). The most resistance was seen against ampicillin, cephalothin, methicillin, and penicillin-G (100%).

Antibiotic susceptibility pattern of *Bacillus cereus* isolated from pasteurized milk. Antimicrobial resistance to 12 different antibiotics was evaluated in *Bacillus cereus* isolates (n=4) from pasteurised milk samples. The results showed that the isolates were highly susceptible to amikacin (100%), gentamicin (100%) and streptomycin (100%), followed by ciprofloxacin (75%), kanamycin (75%), polymyxin B (75%), and erythromycin (50%). Additionally, isolates were found to be 100% resistant to ampicillin, 100% resistant to methicillin, 100% resistant to penicillin G, and 75% and 50% resistant to cephalothin and nalidixic acid, respectively.

Antibiotic susceptibility pattern of *Bacillus cereus* isolated from dahi. From Dahi samples that were tested for antibiotic susceptibility, a *Bacillus cereus* isolate (n=1) was found. The isolate was 100% sensitive to the antibiotics amikacin, cephalothin, kanamycin, nalidixic acid, polymyxin B, streptomycin, gentamicin, and ciprofloxacin, while penicillin G, ampicillin, and methicillin showed the highest resistance (100%).

Antibiotic susceptibility pattern of *Bacillus cereus* isolated from paneer. The antibiotic susceptibility test was performed on the *Bacillus cereus* isolates (n=5) that were obtained from paneer samples. All of the isolates (100%) were sensitive to amikacin, ciprofloxacin, gentamicin, kanamycin, and streptomycin, which were the most effective antibiotics according to the antibiogram. These antibiotics were followed by erythromycin (80%), nalidixic acid (40%) and polymyxin B both (40%). Ampicillin, cephalothin, methicillin, and penicillin G were the antibiotics with the highest levels of resistance (100%).

Antibiotic susceptibility pattern of *Bacillus cereus* isolated from khoa. The antibiotic susceptibility profile of the *Bacillus cereus* isolates (n=9) isolated from khoa samples showed that all the isolates were sensitive to amikacin (88.88%), kanamycin (88.88%), ciprofloxacin (44.44%), and polymyxin B (22.22%). The isolates were also found to be 100% resistant to ampicillin, 100% resistant to cephalothin, 100% resistant to methicillin, and 100% resistant to penicillin G.

Antibiotic susceptibility pattern of *Bacillus cereus* isolated from milk powder. The isolates of *Bacillus cereus* (n=5) tested against 12 antibiotics showed 100% sensitivity to amikacin, 100% sensitivity to ciprofloxacin, 100% sensitivity to gentamicin, and 100% sensitivity to streptomycin. The reported isolates were found to be 100% resistant to ampicillin, 100% resistant to methicillin, and 100% resistant to penicillin G, 100% resistant to cephalothin.

Antibiotic susceptibility pattern of *Bacillus cereus* isolated from Ice-cream. Similar to this, the most effective antibiotics for 6 isolates of *Bacillus cereus* found in Ice-cream samples were amikacin (100%), gentamicin (100%), and streptomycin (100%), followed by ciprofloxacin and kanamycin (83.33%) each. However, the isolates were methicillin (100%), resistant to ampicillin (100%), penicillin-G (100%), and cephalothin (66.16%).

Antibiotic susceptibility pattern of *Bacillus cereus* isolated from butter. The antibiotics that *Bacillus cereus* isolates isolated from butter samples were found to be sensitive to include gentamicin (100%), streptomycin (100%), amikacin (87.50%), ciprofloxacin (87.50%), and kanamycin (87.50%). The most resistance was shown against ampicillin, methicillin, and penicillin G (100%).

The antibiogram of *Bacillus cereus* is examined using twelve antibiotics. The investigation revealed that amikacin (100%), gentamicin (100%), and streptomycin (100%) were the most effective antibiotics. The sensitivity of ciprofloxacin to isolates of milk and milk derivatives is 79.54%. In two isolates of raw milk, one isolate in each of pasteurised milk, butter, and ice cream, and four samples of khoa, the susceptibility to ciprofloxacin is intermediate. Milk and milk products are 86.36% sensitive to kanamycin. One isolate of butter exhibits resistance, while one isolate each of raw milk, pasteurised milk, khoa, milk powder, and ice cream exhibits intermediate susceptibility. A 52.27% isolate's nalidixic acid intermediate and a 45.45% isolate's polymyxin B intermediate. These results concur with those of Yusuf *et al*. (2018), who discovered 100% gentamicin, 96.6% ciprofloxacin, and 85.25 percent streptomycin sensitivity. According to Kumari and Sarkar (2014), 41% of nalidixic acid and 89% of streptomycin were determined to be intermediate. According to Tewari *et al.* (2012), 100% streptomycin, 63.7% gentamicin, and 90% kanamycin were found to be sensitive. Whong *et al.* (1988) discovered that 97% of gentamicin, 93% of kanamycin, and 89% of streptomycin were sensitive. According to Kwarteng *et al.* (2017), ciprofloxacin and gentamicin are both 100% sensitive.

All isolates exhibited the maximum (100%) resistance to ampicillin, methicillin, and penicillin-G. Cephalothin also exhibits strong resistance (90.90%), although butter, pasteurised milk, and two ice cream samples exhibit intermediate susceptibility. Erythromycin exhibits a middle level of susceptibility. In their 2014 study, Kumari and Sarkar discovered resistance to 69% kanamycin, 85% cephalothin, 98% penicillin G, and 99% ampicillin. Resistance was discovered by Tewari *et al.* in 2012. 81.8% ampicillin, 72.8% cephalothin, and 100% penicillin G. Owusu *et al.* (2017) discovered 98% ampicillin resistance. 100% ampicillin resistance was discovered by Abraha *et al.* (2014).

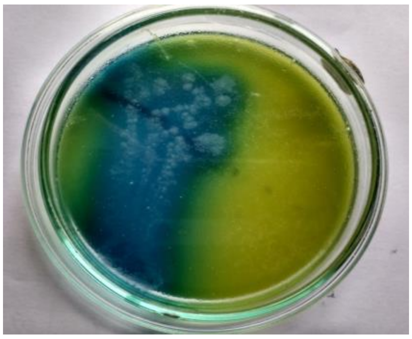

Fig. 1. Growth of the test culture on PEMBA plate, isolate showing peacock blue coloured colony.

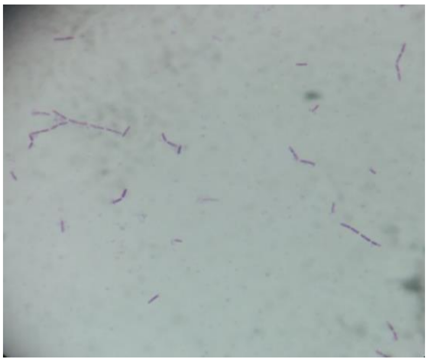

Fig. 2. Gram's staining of the isolate.

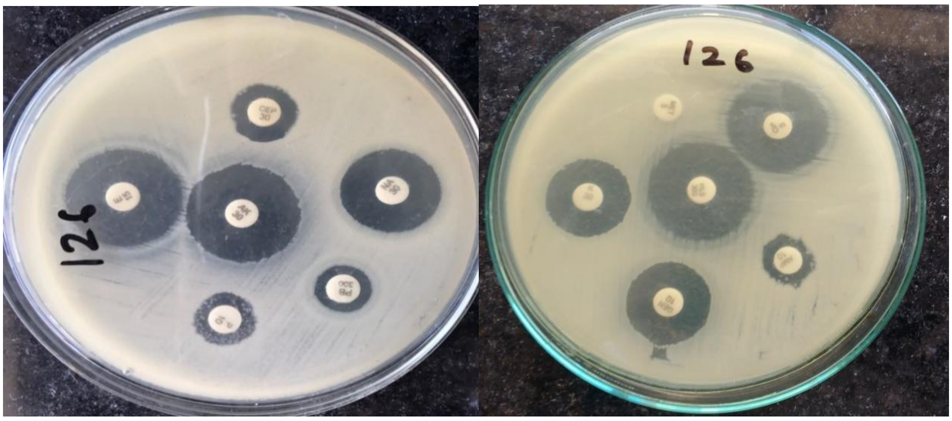

Fig. 3. Antibiogram of *Bacillus cereus* isolated from milk and milk products.

Table 1: Antibiogram of *Bacillus cereus* isolated from milk and milk products.

Antibiotics	Efficacy of antibiotics*	Source of *Bacillus cereus* isolates							
		"Raw milk (%)"	"Pasteurized milk (%)"	"Dahi (%)"	"Paneer (%)"	"Khoa (%)"	"Milk powder (%)"	"Ice cream (%)"	"Butter (%)""
Amikacin	R	0	0	0	0	0	0	0	0
	I	0	0	0	0	1(11.11)	0	0	1(12.50)
	S	6 (100)	4 (100)	1 (100)	5 (100)	8(88.88)	5(100)	6(100)	7(87.50)
Ampicillin	R	6 (100)	4 (100)	1 (100)	5 (100)	9 (100)	5 (100)	6(100)	8(100)
	I	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0
Cephalothin	R	6 (100)	3 (75)	0	5 (100)	9 (100)	5(100)	4(66.66)	7(87.50)
	I	0	1 (25)	0	0	0	0	2(33.33)	1(12.50)
	S	0	0	1 (100)	0	0	0	0	0
Ciprofloxacin	R	0	00	0	0	1(11.11)	0	0	0
	I	2 (33.33)	1 (25)	0	0	4(44.44)	0	1(16.66)	1(12.50)
	S	4 (66.66)	3 (75)	1 (100)	5 (100)	4(44.44)	5(100)	5(83.33)	7(87.50)
Erythromycin	R	0	0	0	0	1(11.11)	0	0	1(12.50)
	I	6 (100)	2 (50)	1 (100)	1(20)	8(88.88)	5 (100)	6(100)	5(62.50)
	S	0	2 (50)	0	4(80)	0	0	0	2(25.00)
Gentamicin	R	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0
	S	6 (100)	4 (100)	1 (100)	5 (100)	9 (100)	5 (100)	6(100)	8(100)
Kanamycin	R	0	0	0	0	0	0	0	1(12.50)
	I	1 (16.66)	1 (25)	0	0	1(11.11)	1(20)	1(16.66)	0
	S	5 (83.33)	3 (75)	1 (100)	5 (100)	8(88.88)	4(80)	5(83.33)	7(87.50)
Methicillin	R	6 (100)	4 (100)	1 (100)	5 (100)	9 (100)	5 (100)	6(100)	8(100)
	I	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0
Nalidixic acid	R	0	2(50)	0	1(20)	3(33.33)	1(20)	2(33.33)	3(37.50)
	I	5 (83.33)	2(50)	0	2(40)	6(66.66)	3(60)	2(33.33)	3(37.50)
	S	1 (16.66)	0	1 (100)	2(40)	0	1(20)	2(33.33)	2(25.00)
Penicillin G,	R	6 (100)	4 (100)	1 (100)	5 (100)	9 (100)	5 (100)	6(100)	8(100)
	I	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0
Polymyxin B	R	3 (50)	0	0	1(20)	3(33.33)	0	1(16.66)	1(12.50)
	I	3 (50)	1 (25)	0	2(40)	4(44.44)	4(80)	4(66.66)	2(25.00)
	S	0	3 (75)	1 (100)	2(40)	2(22.22)	1(20)	1(16.66)	5(62.50)
Streptomycin	R	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0
	S	6 (100)	4 (100)	1 (100)	5 (100)	9 (100)	5 (100)	6(100)	8(100)

*R- Resistant. I- Intermediate, S- Sensitive

CONCLUSIONS

It was determined that the high amount of *Bacillus cereus* contamination in milk and milk products poses a risk to human consumption. The widespread use of antibiotics as a growth stimulant in animals creates bacteria that pose a risk to the public's health. As a result of the findings, more has to be done to improve the hygienic conditions for milk production, processing, and packing.

FUTURE SCOPE

The findings are thought to have satisfied the study's goals and purview. Antibiogram analysis in milk has a bright future, as technological and scientific advances promise a deeper comprehension of microbial dynamics and antibiotic resistance in dairy environments. By combining AI, metagenomics, and quick testing techniques, the field will undergo a revolution that will improve food safety, dairy animal health, and ultimately protect the public from milkborne illnesses. To fully utilize antibiogram analysis in milk and adopt these breakthroughs for a healthier future, researchers, dairy companies, and public health authorities must work together.

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