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# Determination of prevalence and multidrug resistance phenotypes of *Bacillus cereus* in raw chicken meat and swabs of human subjects

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**Abstract**
**Background:** Food borne diseases are concomitant with high morbidity and mortality and pose a serious threat to public health world over. The increasing occurrence of multiple drug resistant bacterial species is also a matter of concern thereby hindering food safety.
**Aim:** This study was carried out to determine the prevalence and multidrug resistance phenotypes of *Bacillus cereus* in raw chicken meat and swabs of human subjects collected from urban and peri urban areas of Navsari city of South Gujarat.
**Methods:** A total of 280 samples, contained 175 raw chicken meat and 105 swabs comprising 35 each, from Handlers' hands, Butchers' knives and Chopping boards were collected and analyzed as per standard microbiological procedures. Recovered 42 isolates identified and confirmed as *B. cereus* were subjected to antibiogram assay using 16 selected antibiotics by agar disc diffusion method.
**Results:** Out of 280 samples examined, including 175 raw chicken meat and 105 swab samples total 42 samples (30/175 and 12/105) were positive for *B. cereus* with a prevalence of 17.14% and 11.42% *B. cereus* isolates respectively, and isolates also amplified group specific (*groEL*) gene and species specific (*gyrB*) gene by PCR. All the 42 isolates exhibited complete to moderate resistance phenotypic pattern to Penicillin G, Ampicillin, Trimethoprim, Cefotaxime and Ceftazidime followed by Clindamycin. The susceptibility of 42 *B. cereus* isolates showed significant difference in Pearson Chi-Square test ($p < 0.01$), which indicates the importance of different antimicrobial agents tested against the isolated bacterium. The susceptibility of isolates was significantly high towards Tetracycline, Chloramphenicol, Imipenem, Streptomycin and Amikacin followed by Gentamicin, Ciprofloxacin, Vancomycin, Erythromycin, Cefoperazone and Clindamycin.
**Conclusion:** This study indicates the prevalence and possible presence of multidrug resistant *B. cereus* in animal origin food and environment in high proportion is of public health significance.

**Keywords:** *B. cereus*, chicken meat, antibiotics, sensitivity, resistant, groEL, gyrB

## Introduction
Food borne infections, intoxications and toxi-infections are a serious public health hazard the world over. Among the various microbial species responsible for food borne diseases, *Bacillus cereus* has emerged as major food borne pathogen because of its ability to produce heat stable toxin and several other potential virulence factors. The organism is often present in starch rich foods such as rice as well as chicken, meat, milk and milk products (Jay, 2005) [18]. In India, occurrence of *B. cereus* has been reported from foods like milk (Garg *et al*., 1977; Chopra *et al*., 1980) [15,7] meat (Bacchil and Negi, 1984; Bacchil and Jaiswal, 1988) [3, 2] chicken meat (Tahmasebi *et al*., 2014; Aklilu *et al*., 2016) [30, 1] and various other foods (Kamat *et al*., 1989; Meena *et al*., 2000) [19, 21].
In the current scenario, organisms possess inherent potent toxigenic characteristics thereby developing resistance towards several antibacterial agents used in therapy worldwide. This is immensely challenging and necessitates the participation of complete medical network and public health agencies. *B. cereus* is capable of producing a broad spectrum *β*-lactamase and it is one of the most potent virulence elements that makes the strains resistant to Penicillin, Ampicillin and even to third generation Cephalosporins (Cormican *et al*., 1998) [10].
The occurrence of pathogen with multiple drug resistance with potent toxigenic *B. cereus* to Erythromycin and Tetracycline from the United States and Europe indicate the development of resistance, in addition pathogenic *B. cereus* resistance to Penicillin, Cephalosporin with other *β*-lactam antimicrobials (Myers *et al*., 1989) [23], exerts a selective pressure and acts as a riding force within the development of antibiotic resistant bacteria in food chain and its potential

transmission to humans (Faria-Reyes *et al.,* 2001) [11].

## Materials and Methods

### Samples

The samples were collected using random sampling method for a period of eleven months (from June-2017 to March-2018) from retail chicken outlets of urban and peri-urban area of Navsari city. A total of 280 samples comprising of 175 samples of raw chicken meat including different parts such as thigh, breast, wing, rib, heart, liver, neck and gizzard were collected. Also, 105 swab samples were collected 35 each, from butchers' hands, chopping board and knife. Types of samples, their numbers and sources are mentioned in Table 1.

**Table 1:** Details of the samples

| Sr. No. | Type of sample | | Number of samples | Total |
|---|---|---|---|---|
| 1 | Raw chicken meat | Thigh muscle | 39 | 175 |
| 2 | | Breast muscle | 41 | |
| 3 | | Wing muscle | 29 | |
| 4 | | Rib muscle | 18 | |
| 5 | | Heart portion | 9 | |
| 6 | | Liver portion | 11 | |
| 7 | | Neck muscle | 22 | |
| 8 | | Gizzard portion | 6 | |
| 1 | Swab samples | Handler's hand's | 35 | 105 |
| 2 | | Butchers knife | 35 | |
| 3 | | Chopping board | 35 | |
| Grand total | | | | 280 |

### Isolation and identification

The samples were processed to isolate the *B. cereus* as per the standard *Bacteriological Analytical Manual*, Food and Drug Administration [12] method (Rhodehamel and Harmon, 2001) [27] with some modifications.

Approximately 10 gm chicken meat sample was cut aseptically in small pieces, transferred into sterile mortar and triturated by using pestle after addition of 90 ml PBS Diluents (1:10) to make the homogeneous mixtures. This was followed by transferring 1 ml of the homogenate into tubes containing 9 ml PBS to carry out tenfold serial dilution of $10^{-2}$ to $10^{-6}$. From the tube containing $10^{-4}$ dilution 0.1 ml was spread over the entire surface of Mannitol Egg Yolk Polymyxin B (MYP) Agar plates. All plates were incubated in an upright position at 37 °C for 20 to 24 hours.

The swab samples collected from the hands of butchers, knives and chopping boards were collected in 5 ml nutrient broth and incubated aerobically at 37 °C for 24 h, and they were streaked on the MYP Agar plate and incubated at 37 °C for 24 h (Roy *et al*., 2013) [28]. Subsequently, the plates were examined for pink colonies surrounded by precipitate zone, which indicates the lecithinase production and were regarded as presumptive growth of *B. cereus*. These isolates were subjected to morphological, cultural and biochemical characterization using biochemical tests described in the *Burgey's Manual of Systemic Bacteriology* (2009) [8] for identrification of *B. cereus*.

### Determination of antimicrobial resistance

*In Vitro* antibiotic susceptibility test was performed using disc diffusion method described by CLSI (2017) [9] to find out the antibiotic resistance pattern of all *B. cereus* isolates against 16 different antibiotics *viz*., Cefoperazone, Penicillin G, Imipenem, Ampicillin, Cefotaxime, Ceftazidime, Clindamycin, Tetracycline, Erythromycin, Chloramphenicol, Gentamicin, Streptomycin, Amikacin. Ciprofloxacin, Trimethoprim and Vancomycins.

### Molecular confirmation

The *B. cereus* isolates and reference culture were grown individually in Luria Bertani broth for 24 hour at 37 °C. Then after total genomic DNA of individual isolate was extracted by using *mericon* DNA Bacteria plus Kit (Qiagen). The isolates were screened for the presence of *groEL* and *gyrB* gene by duplex PCR method (Park *et al*., (2007) [25].

### Statistical analysis

The data analysed by applying Chi-square test using IBM® SPSS® software (version 20.0) for antibiotic sensitivity pattern and distribution of toxin genes in *B. cereus* isolated from various sources and their significance of difference.

## Results

### Phenotypic characterization

The phenotypic colony characteristics in 42/280 (28.56%) *B. cereus* isolates on Mannitol egg Yolk Polymyxin B agar (MYP) as pink in colour surrounded by a precipitate zone were observed, which included 17.14% from raw chicken meat and 11.42% human swab samples. The isolates were subjected to various biochemical tests for confirmation. All 42 isolates were positive for Nitrate reduction test, Vogues Proskaur test, Tyrosine hydrolysis test and Lysozyme test and all were negative for Methyl Red test. The isolates were subjected for species differentiation and expressed Rhizoid growth, Positive Catalase test, motility test and Urease test and Negative Indole production test. Out of 42 isolates 22 (52.38%) were positive for haemolysis test in 5% sheep blood agar base. All 42 isolates expressed utilization of glucose anaerobically in phenol red glucose broth with production of yellow colour.

### Prevalence

In present study, total 42/280 (28.56%) *B. cereus* isolates, 17.14% and 11.42% were cultured from raw chicken meat and human swab samples, respectively. The detailed results mentioned in Table 2 and Table 3. The molecular confirmation of *B. cereus* isolates was carried out with species specific *gyrB* (475bp) gene and group specific *groEL* (400bp) gene by duplex PCR which were amplified by all 42 *B. cereus* isolates as shown in Fig.1. The sample wise area specific

results mentioned in Table 4 show that the highest prevalence was noted in samples collected from Location-6, followed by subsiding prevalence from Location-7, Location-3, Location-1, Location-5 near railway station, Location-2, Location-4 on Dandi road and Location-8. The prevalence of *B. cereus* contamination in samples collected from Location-9 was only 25% from were swab could not be collected.

**Determination of multidrug resistance phenotypes**

The multidrug resistance phenotypic pattern of all 42 *B. cereus* isolates is summarized in Table 5 and their significance of difference in Pearson Chi-Square is ($p < 0.01$), which indicates the significant difference of antimicrobial agent tested against *B. cereus* isolates that exhibited cent percent sensitivity towards Tetracycline, Chloramphenicol, Imipenem, Streptomycin and Amikacin, following descending patterns of sensitivity towards Gentamicin (97.61%), Ciprofloxacin (83.33%), Vancomycin (69.04%), Erythromycin (52.38%), Cefoperazone (30.95%) and Clindamycin (2.38%). An intermediate sensitivity pattern of *B. cereus* isolates was observed against Cefoperazone (69.04%), Erythromycin (47.61%), Clindamycin (45.23%), Vancomycin (30.95%), Ciprofloxacin (16.66%) and Gentamicin (2.38%). All *B. cereus* isolates were resistant towards Penicillin G, Ampicillin, Trimethoprim, Cefotaxime and Ceftazidime and 52.38% isolates were resistant towards clindamycin.

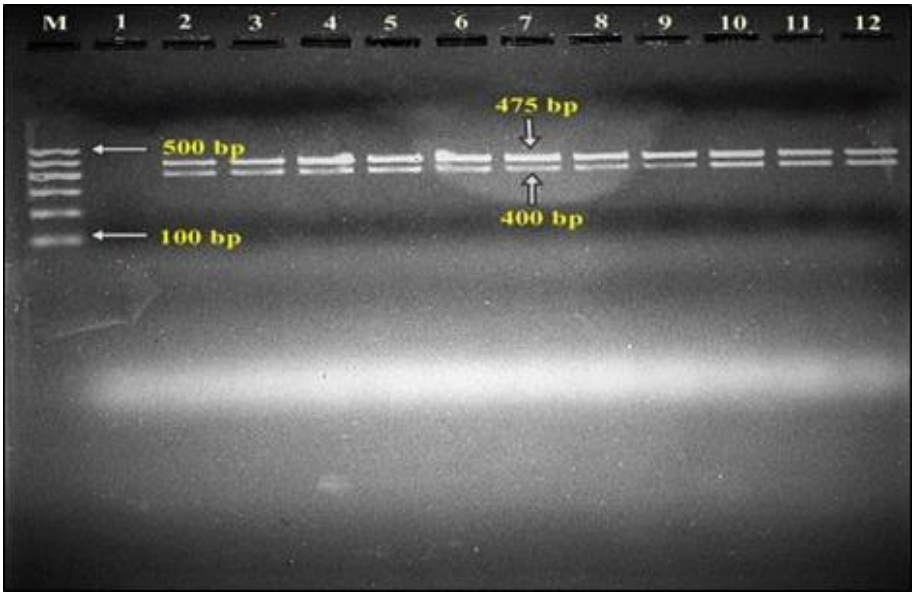


**Fig 1:** Agarose gel showing PCR amplified product of 400 bp for *groEL* gene in *B. cereus* group and 475 bp for *gyrB* gene in *B. cereus* isolates.

**Lane M:** 100 bp DNA ladder, Lane 1: Negative control (Reagent). Lane 2: Positive control (MTCC25061).

**Lane 3-12:** *B. cereus* isolates amplified species specific *groEL* (400 bp) and group specific *gyrB* (475 bp) gene

**Table 2:** Prevalence of *B. cereus in* raw chicken meat samples

| Sr. No. | Type of the sample | Organ wise collection | No. of samples examined | No. of samples positive | Percent value |
|---|---|---|---|---|---|
| 1 | Raw chicken meat | Thigh muscle | 39 | 8 | 20.51 |
| 2 | | Breast muscle | 41 | 4 | 9.75 |
| 3 | | Wing muscle | 29 | 4 | 13.79 |
| 4 | | Rib muscle | 18 | 2 | 11.11 |
| 5 | | Neck muscle | 22 | 4 | 18.18 |
| 6 | | Heart portion | 9 | 3 | 33.33 |
| 7 | | Liver portion | 11 | 4 | 36.36 |
| 8 | | Gizzard portion | 6 | 1 | 16.66 |
| Total | | | 175 | 30 | 17.14 |

**Table 3:** Prevalence of *Bacillus cereus in* swab samples

| Sr. No. | Type of swab samples | No. of samples examined | No. of samples Positive | Percent Value |
|---|---|---|---|---|
| 1. | Hand's swabs from chicken meat handlers | 35 | 5 | 14.28 |
| 2. | Butchers knife | 35 | 3 | 8.57 |
| 3. | Chopping board | 35 | 4 | 11.42 |
| Total | | 105 | 12 | 11.42 |

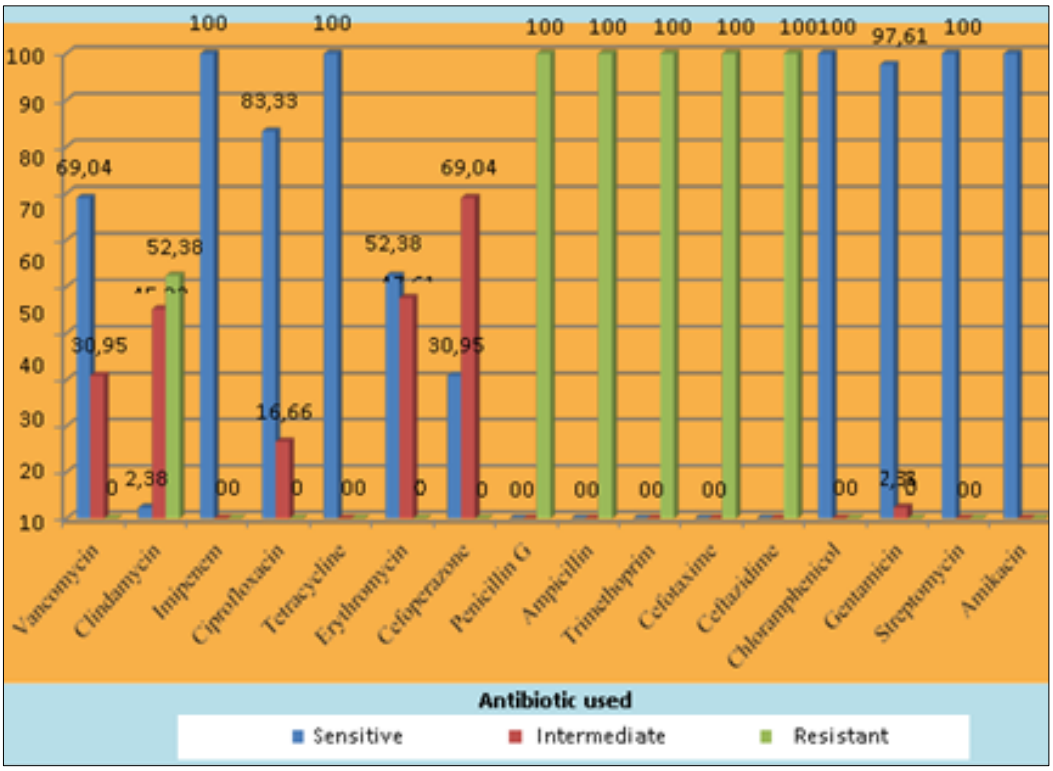


**Fig 2:** Antimicrobial susceptibility phenotypic pattern of *Bacillus cereus*

**Table 4:** Prevalence of *B. cereus* in raw chicken meat and the swab samples

| Location of collection of samples | Organ wise raw chicken meat samples | | | | | | | | Swab samples | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Thigh muscle n=8 | Breast muscle n=4 | Wing muscle n=4 | Rib muscle n=2 | Neck muscle n=4 | Heart portion n=3 | Liver portion n=4 | Gizzard portion n=1 | Chicken meat handlers n=5 | Butchers knife n=3 | Chopping board n=4 |
| Location -1 | 2(25) | 1(25) | - | - | - | - | - | - | 2(40) | - | 1(25) |
| Location -2 | 1(12.5) | 1(25) | - | - | - | - | - | - | - | 1(33.33) | - |
| Location -3 | - | 1(25) | 2(50) | - | 1(25) | - | 1(25) | - | 1(20) | 1(33.33) | - |
| Location -4 | 1(12.5) | - | - | - | - | - | - | - | 1(20) | - | 1(25) |
| Location -5 | - | - | - | 1(50) | 1(25) | - | - | - | - | - | 1(25) |
| Location -6 | - | 1(25) | 1(25) | 1(50) | - | - | - | 1(100) | - | 1(33.33) | 1(25) |
| Location -7 | 3(37.5) | - | 1(25) | - | - | 3(100) | 3(75) | - | 1(20) | - | - |
| Location -8 | 1(12.5) | - | - | - | 1(25) | - | - | - | - | - | - |
| Location -9 | - | - | - | - | 1(25) | - | - | - | * | * | * |

Note: ***** Not collected, Value in () indicate percentage, n= No. of Positive sample

**Table 5:** Antimicrobial resistance

| Sr. No. | Name of the antibiotic | Sensitive | Intermediate | Resistant |
|---|---|---|---|---|
| 1. | Vancomycin | 69.04* | 30.95* | 0 |
| 2. | Clindamycin | 2.38 | 45.23 | 52.38* |
| 3. | Imipenem | 100 | 0 | 0 |
| 4. | Ciprofloxacin | 83.33 | 16.66 | 0 |
| 5. | Tetracycline | 100 | 0 | 0 |
| 6. | Erythromycin | 52.38 | 47.61 | 0 |
| 7. | Cefoperazone | 30.95 | 69.04 | 0 |
| 8. | Penicillin G | 0 | 0 | 100 |
| 9. | Ampicillin | 0 | 0 | 100 |
| 10. | Trimethoprim | 0 | 0 | 100 |
| 11. | Cefotaxime | 0 | 0 | 100 |
| 12. | Ceftazidime | 0 | 0 | 100 |
| 13. | Chloramphenicol | 100 | 0 | 0 |
| 14. | Gentamicin | 97.61 | 2.38 | 0 |
| 15. | Streptomycin | 100 | 0 | 0 |
| 16. | Amikacin | 100 | 0 | 0 |
| $X^2$ | 2144.97** ($p$ = 0.00) | | | |

Note: * Figures in the table indicate% values, ** Highly Significant at $p$<0.01

## Discussion

Systematic bacteriological examination carried out on 175 raw chicken meat and organ samples resulted in the recovery of 30 (17.14%) *B. cereus* isolates. The findings of the present study were in coherence to the incidence of 18.4% and 18.3% recorded by Tahmasebi *et al*. (2014) [30], respectively. However, lower incidence of 16.67% was recorded by Aklilu *et al*. (2016) [1]. In contrast to the findings of present work, earlier studies on *Bacillus cereus* conducted by Bedi *et al.* (2004) [6]; Smith *et al.* (2004) [29]; Mira and Abuzied. (2006) [22]; Hafiz *et al*. (2012) [16]; Tewari *et al.* (2015) [32] and Bashir *et al*. (2017) [5] reported higher incidence of 56.3, 45, 100, 39.16, 30.9 and 24%, respectively. This could be due to variation in the sample size and different geographical environmental conditions.

In the present study, *Bacillus cereus* was found in 14.28% (5/35) samples of the hand swabs of chicken meat handlers. However, no similar incidence of these types of swabs samples has been reported, whereas Roy *et al*. (2013) [28] who isolated *B. cereus* from the swab samples of mobile phone of meat handlers and found incidence rate 84% which were higher than the present study.

The percentage occurrence of present *B. cereus* in swab samples collected from hands, knives and chopping boards of butchers was, 14.28, 8.57 and 11.42 per cent, respectively which was lower than that of Rosmawati *et al*. (2014) [34] who reported prevalence rates of 100%, 55.6% and 44.4%, respectively from the swab samples.

All 42 *B. cereus* isolates were sensitive to Tetracycline, Chloramphenicol, Streptomycin and Amikacin which was analogous to the findings of Banerjee *et al*. (2011) [4], Roy *et al.* (2013), Fossi *et al*. (2017), and Bashir *et al*.(2017) [14, 28, 5] who recorded 100% sensitivity of *B. cereus* to Tetracycline, Chloramphenicol, Streptomycin and Amikacin. All but one *B. cereus* isolates were sensitive towards Gentamicin in present study which is higher than the findings of Tewari *et al.* (2012) [33] who observed 58% sensitivity. However, Fossi *et al*. (2017) [14] reported cent percent sensitivity against Gentamicin. In coherence with the present study 100% resistance to Penicillin G, Ampicillin, Cefotaxime and Ceftazidime was documented by Aklilu *et al*. (2016) [1] and Fossi *et al*. (2017) [14]. In the present study 52.38% isolates exhibited sensitivity towards Erythromycin, which is lower than 100% sensitivity cited by Fossi *et al*. (2017) [14] and Kohneshahri *et al*. (2016) [20]. The difference in the result could be due to overuse of Erythromycin in the sampling areas of the present study. Organi *et al*. (2015) [24] recorded 100% sensitivity against *B. cereus* isolates to Clindamycin, whereas in the present study only 2.38% isolates were sensitive. Sensitivity of *B. cereus* to Vancomycin was 69.04% in this study which corroborated with the findings of Fossi *et al*. (2017) [14] stating 70% sensitivity of Vancomycin, in contrast 100% as reported by Kohneshahri *et al*., 2016 [20]. Cefoperazone showed susceptibility to 30.95% isolates in present study. Floristean. (2007) [13] reported 100% resistance of Cefoperazone in *B. cereus* isolates, which is in higher than present observations. In present study, 83.33% sensitivity was observed against Ciprofloxacin parallel to the reports of Tare A. (2010) [31] and Rather *et al*. (2011) [26] who found 64.28 and 98.97% sensitivity, respectively against Ciprofloxacin. However, Jawad *et al*. (2016) [17] reported 42% resistance to Ciprofloxacin, which is indicative of indiscriminate use of this antibiotics.

The determinant for the prevalence of *B. cereus* in the samples collected from urban and peri urban areas of Navsari is most probably the lack of hygienic practices of the meat outlets, poor personal hygiene of meat sellers, contaminated cutting equipments and chopping boards or surfaces as well as lack of awareness amongst the sellers about good management practices.

## Conclusion

This study aimed to provide information of the prevalence and antibiotic resistance pattern of *B. cereus* in raw chicken meat and human handlers' swab samples collected from urban and peri urban areas in Navsari city located in south Gujarat region. Out of 280 samples examined, including 175 raw chicken meat and 105 swab samples total 42 samples (30/175 and 12/105) were positive for *B. cereus* with a prevalence of 17.14% and 11.42% *B. cereus* isolates respectively. Antibiotics are commonly used as growth promoter in animal husbandry which has led to the development of antibiotic resistance amongst a large number of bacterial species. The emergence of multi drug resistant of *B. cereus* pose a serious threat to public health, therefore veterinarians working in the area should focus on judicious use of antibiotics. Bringing awareness among the public about the harmful effect of multi drug resistant micro flora will help to safeguard the human as well as animals from such type of threat.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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