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Antimicrobial Susceptibility of *Bacillus Cereus* Isolates from Different Infectious Sources

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ABSTRACT

This study has showed that there was a significant difference between the food and clinical Bacillus cereus isolates in relation to their antimicrobial susceptibility. A total of 120 samples (20 clinical and 100 food) were cultured and 45 B. cereus isolates have been confirmed by PEMBA selective agar as well as Vitek 2 system. Notably, food isolates were generally more susceptible to carbapenems (39.83% of total inhibitory activity) and fluoroquinolones (27.12%), whereas clinical isolates were generally less susceptible to these drugs (32.10% and 29.63%, respectively), in the CLSI M45 3rd Edition (2024) disk diffusion procedure. 100% of all isolates were resistant to trimethoprim. Empiric usage of carbapenems, in particular IMP-resistant B. cereus for B. cereus infections would be the most practical option to consider, as strains isolated from clinical sources were more resistant to IMP.

1- INTRODUCTION

Bacillus cereus is a spore-forming Gram-positive facultative anaerobe that has the remarkable ability to inhabit many environments from the soil and water to complex food production systems and hospitals and can cause many types of foodborne illnesses due to the presence of enterotoxins, emetic toxins (cereulide), or a combination of both enterotoxins and emetic toxins like virulence factors (Nhe, Hbl, and CytK)[1]. *B. cereus* is also known to cause serious and sometimes life-threatening infections outside of the gastrointestinal tract such as endophthalmitis, fulminant pneumonia and septicemia in neonatal and immune-compromised patients [2].

An inherent resistance challenge of *B. cereus* infections is the Beta-lactams resistance, conferred by high-grade chromosomal Beta-lactamase genes (bla1, bla2, and bla3), rendering almost any type of penicillins and cephalsorins ineffective [3,4]. At present, the treatments are the use of carbapenems, fluoroquinolones, and glycopeptides. In clinical setting, however, an emergence of reduced susceptibility strains to these antibiotics presents a great danger on the empirical treatment regimen [5].

Diagnosis is crucial to monitoring and treatment of disease. The use of PEMBA (Polymyxin Pyruvate Egg Yolk Mannitol Bromothymol Blue Agar) has a better diagnostic identification and specificity, due to the use of sodium pyruvate that will allow better recovery of sub-lethal stressed cells usually found in processed foods and the pH indicator's and lecithinase detection systems, differentiating *B. cereus* from closely related *Bacillus species* [6,7].

Several varieties of food have been reported to have become contaminated with *B. cereus*, including rice, milk and meat: when stored incorrectly, it allows the germination of spores and production of toxin in food. At the same time, it is recovered from clinical specimens such as blood, wounds, respiratory discharge and contaminated medical devices showing its environmental and clinical significance [8]. The information on antimicrobial susceptibility patterns of *B. cereus* from clinical and food sources is scare and there is no information on the antimicrobial susceptibility pattern of the *B.cereus* recovered from food samples of Iraq. The aim of the present study was to address this deficiency by (1) isolating and unambiguously identifying *B. cereus* from various sources in Iraq, and (2) detailed comparison of their antimicrobial susceptibility profiles. It represents the first comparative analysis of susceptibility of food isolates vs clinical isolates in this area, and added novel information to the understanding of the epidemiology and management of this pathogen.

2- MATERIALS AND METHODS

2.1 Sample Collection

Altogether 120 samples were taken (October to December 2025). Diarrhoea and vomiting patients in several hospitals of Baghdad and Najaf, Algeria namely Baghdad Educational Hospital, Specialized Burns Hospital, Ghazi Hariri Hospital, Al-Sadr Medical city were included and sent a total of 20 clinical samples (stoo samples were collected) from the patients aged from 10 to 40 years. The collected food samples were 100 samples from three types of food finger foods (30 samples), semi-pasteurized raw milk (30 samples), raw meat (30 samples) were collected from the local markets in the same governorates. Samples were collected and all samples were transported in a refrigerated container (4

°C) and processed within 2 hours of collection.

2.2 Isolation and Phenotypic Characterization.2.2 Isolation and Phenotypic Characterization.

Samples were first cultured on Nutrient Agar and 5% Sheep Blood Agar which was used to identify samples for beta-hemolytic activity. All samples were plated at the same time directly onto Polymyxin Pyruvate Egg Yolk Mannitol Bromothymol Blue Agar (PEMBA) (Oxoid, UK) for selective isolation and differentiation. Aerobic incubation of inoculated plates was done at 37 °C for 24–48 hours. Presumptive B. cereus colonies were surrounded by a zone of opacity/clearing and were characterized by their distinctive color that was peacock-blue as a result of the absence of mannitol fermentation. Subsequently, isolates were sub-cultured to get pure cultures for further analysis. First, the phenotype was identified by Gram stain to confirm that it was gram-positive and spore-forming rod morphology.

2.3 Definitive Identification by VITEK® 2 System

All the isolates were successfully identified as the final step by the VITEK® 2 automated microbial identification system (bioMérieux, Marcy-l'Étoile, France). BCL (*Bacillus*) card applied as per SOPs of the manufacturer. A standardized inoculum (also in 0.45% sterile saline) of about 1.8 × 10^8 CFU/mL was prepared from a fresh, pure overnight culture by using the VITEK® 2 DensiCHEK™ turbidimeter. The system is capable of kinetic fluorogenic and colorimetric measurements during incubation at 36 °C to allow high confidence species level identification by comparing biochemical profiles.

2.4. Antimicrobial Susceptibility Testing (AST) 2.4

Susceptibility testing was performed by the Kirby-Bauer disk diffusion assay, which was carried out in accordance with the procedures detailed in CLSI document M45—3rd Edition (2024) for infrequently isolated or fastidious bacteria [9]. A bacterial suspension was made in sterile 0.85% saline, and diluted to a turbidity standard of 0.5 McFarland using a turbidimeter calibrated for 0.5 McFarland. Final concentration: approximately 1.5 × 10^8CFU/mL. The surface of 150 mm diameter Mueller-Hinton Agar (Oxoid, UK) was evenly swabbed with the inoculum. The choice of antibiotics did not include the β-lactam class (particularly penicillins and cephalosporins), because of the known intrinsic resistance of *B. cereus* [9–11]. After 24 hours of incubation at 37 °C, the diameters of the zones of complete inhibition were measured in millimeters with the calibrated digital caliper. The results are means of three experiments.

2.5 Statistical Analysis

The results are reported as the mean inhibition zone diameter (mm) ± SD of results obtained from three or more independent determinations. Comparative susceptibility profiles were prepared graphically and also in tabular form to give a pictorial presentation of the susceptibility.

3-Results

3.1 Isolation and Identification of *Bacillus cereus*

Forty-five (45) (37.5%) *B. cereus* isolates were confirmed from 120 samples. S. aureus was the only microorganism to grow on the PEMBA selective agar, giving 100% specificity. In all 45 isolates the colony pattern had a characteristic peacock blue colour, and a clear zone around the colony

showed lathosterol, which is called a zone of lathosterc activity (Fig. 1). Strong beta-hemolytic activity on Blood Agar had been demonstrated, indicating their pathogenic potential. Table I shows the number of positive isolates distributed by wards. The result with highest isolation rate was from rice samples (46.7%), followed by milk (40.0%), clinical stool sample (36.7) and meat (26.7). The results of the distribution of positive growth between clinical and food sample are summarized in table 1.

Table I: Distribution of Positive Growth Among Clinical and Food Samples

Type of isolate	No. of Samples	positive Growth	Negative growth
Clinical	30	11	19
Food (rice)	30	14	16
Food (meat)	30	8	22
Food (milk)	30	12	18

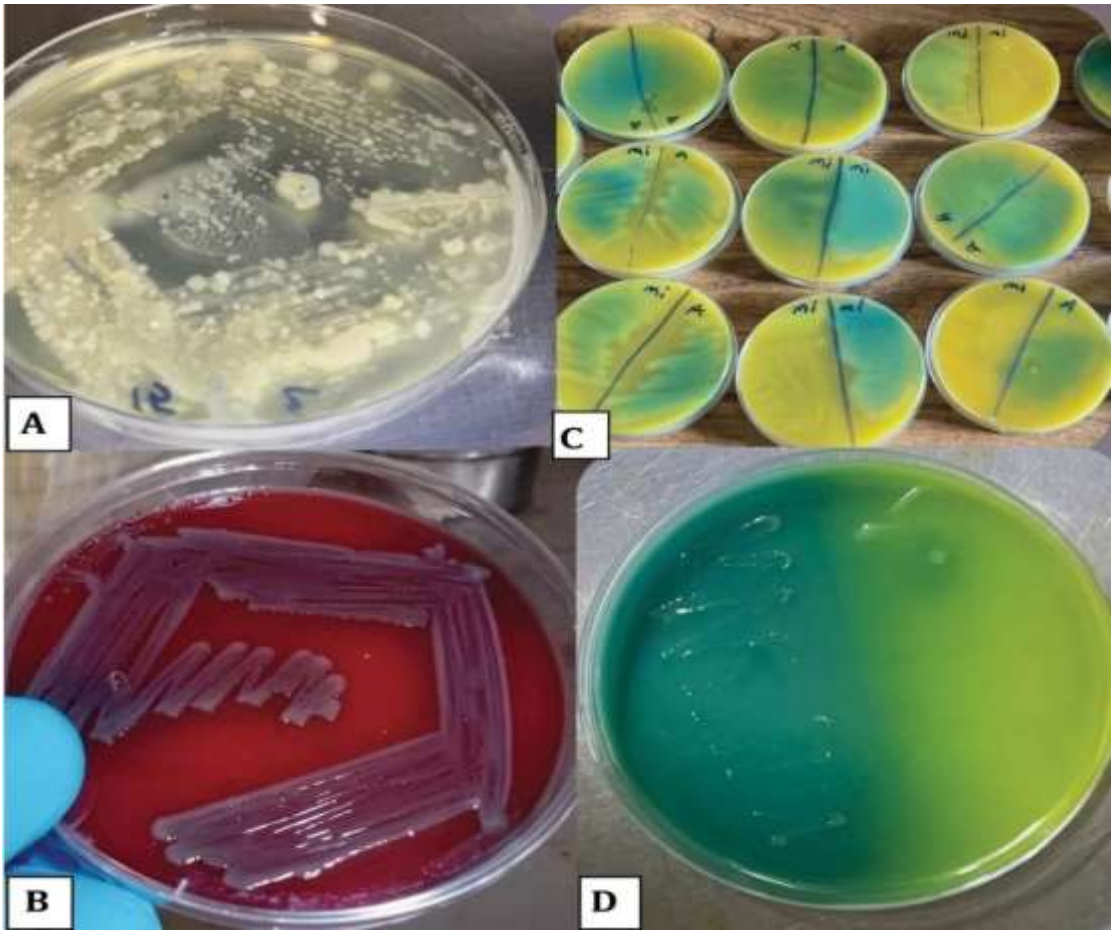
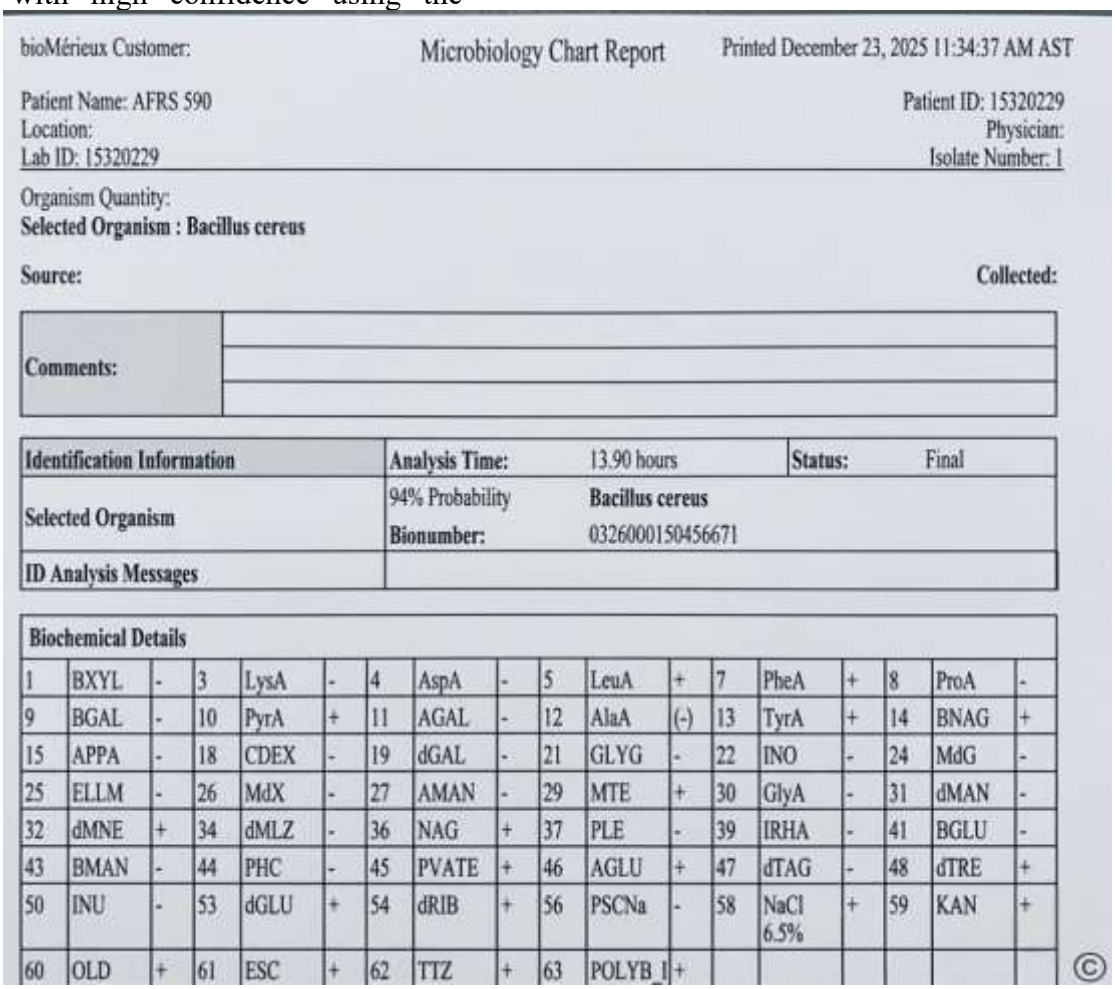


Fig 1: Colony Morphology and Cultural Characteristics of *Bacillus cereus* Isolates. (A) Creamy, flat colonies with irregular margins on Nutrient Agar. (B) Wide zone of beta-hemolysis on Blood Agar. (C, D) Peacock-blue colonies surrounded by a halo of precipitation on PEMBA Agar, indicative of mannitol non-fermentation and lecithinase activity.

Identification of Bacillus cereus by Vitek-2 System

the isolates were identified as Bacillus cereus with high confidence using the VITEK® 2 automated identification system (Fig 2).



bioMérieux Customer: Microbiology Chart Report Printed December 23, 2025 11:34:37 AM AST

Patient Name: AFRS 590 Patient ID: 15320229
Location: Physician:
Lab ID: 15320229 Isolate Number: 1

Organism Quantity:
Selected Organism : Bacillus cereus

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	13.90 hours	**Status:**	Final
Selected Organism	94% Probability	**Bacillus cereus**		
	Bionumber:	0326000150456671		
ID Analysis Messages				

Biochemical Details

1	BXYL	-	3	LysA	-	4	AspA	-	5	LeuA	+	7	PheA	+	8	ProA	-
9	BGAL	-	10	PyrA	+	11	AGAL	-	12	AlaA	(-)	13	TyrA	+	14	BNAG	+
15	APPA	-	18	CDEX	-	19	dGAL	-	21	GLYG	-	22	INO	-	24	MdG	-
25	ELLM	-	26	MdX	-	27	AMAN	-	29	MTE	+	30	GlyA	-	31	dMAN	-
32	dMNE	+	34	dMLZ	-	36	NAG	+	37	PLE	-	39	IRHA	-	41	BGLU	-
43	BMAN	-	44	PHC	-	45	PVATE	+	46	AGLU	+	47	dTAG	-	48	dTRE	+
50	INU	-	53	dGLU	+	54	dRIB	+	56	PSCNa	-	58	NaCl 6.5%	+	59	KAN	+
60	OLD	+	61	ESC	+	62	TTZ	+	63	POLYB_I	+						

Fig 2: The result of *B. cereus* identification by VITEK 2 system.

3.2 Identification by VITEK® 2 System

The results of the presumptive identification consistently reached 100% confidence with excellent probability (>95%) when using Vitek® 2 system, and confirmed the phenotypic identification made on selective media. Behaviors of Antimicrobial Susceptibility between Clinical and Food-derived Strains.

3.3 Antimicrobial Susceptibility of Clinical and Food-Derived Strains.

A comparative analysis of IZD (Table II, Fig. 3) showed that there were distinct susceptibility profile differences between food derived and clinical isolates. Collectively, the food isolates were more susceptible than clinical isolates for all the antibiotics tested, except trimethoprim. When used to treat the two groups of isolates, the most effective agent against both was imipenem, which had a mean IZD of 52 ± 2.0 mm against milk isolates, in comparison with a mean IZD of 30 ± 1.8 mm with clinical isolates. Trimethoprim showed absolute intrinsic resistance with the IZD of 0.0 mm in 100% of the tested strains.

Table 2:Antibiotic susceptibility patterns on clinical and food Bacillus cereus isolates on Mueller-Hinton agar according Clsi standars

Antibiotics	Inhibition zone diameter of clinical isolates	Inhibition zone diameter of food isolates(milk)	Inhibition zone diameter of food isolates(rice)	Inhibition zone diameter of food isolates(meat)
Vancomycin (30µg)	20mm (S)	27mm (S)	21mm	18mm
Imipenem (10 µg)	30mm (S)	52mm (S)	33mm	28mm
Meropenem (10 µg)	22mm (S)	42mm (S)	31mm	30mm
Amikacin (30 µg)	21mm (S)	26mm (S)	25mm	21mm
Ciprofloxacin (5 µg)	26mm (S)	33mm (S)	29mm	21mm
Levofloxacin (5 µg)	22mm (S)	31mm (S)	30mm	18mm
Tetracycline (30 µg)	21mm (S)	25mm (S)	24mm	20mm
Trimethoprim (5 µg)	Resistance	Resistance	Resistance	Resistance

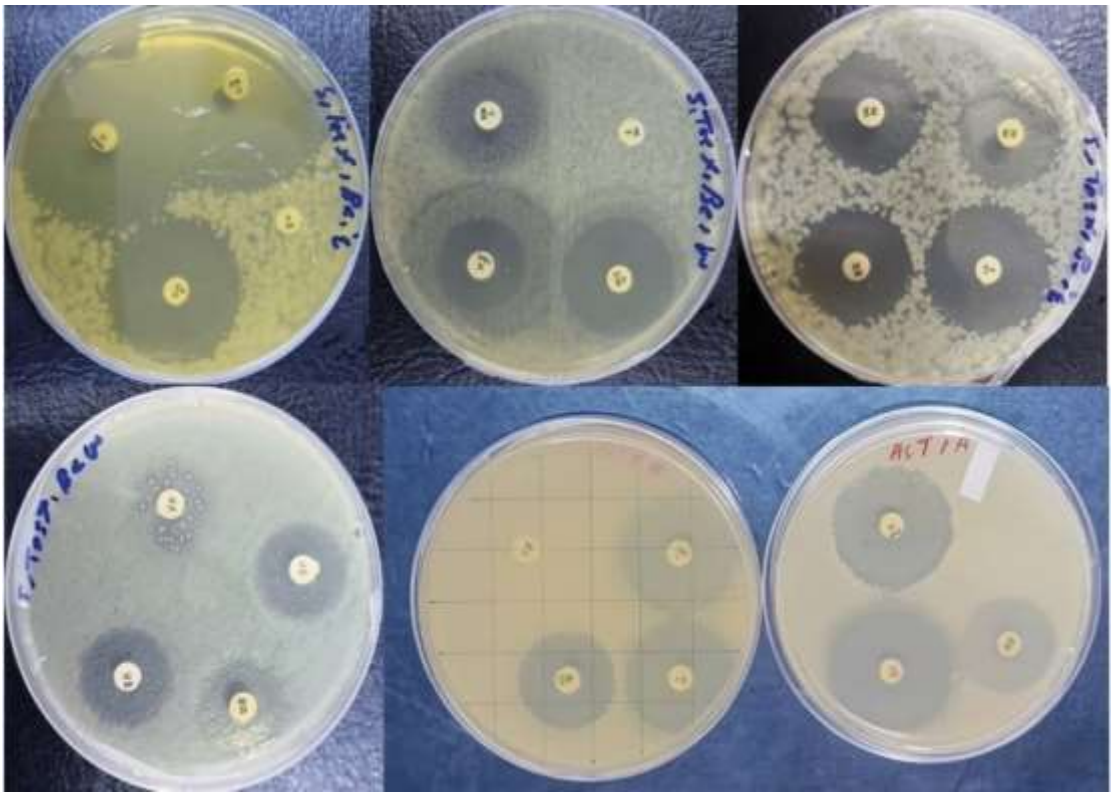


Fig 3: Disk Diffusion Assay on Mueller-Hinton Agar Showing Representative Inhibition Zones for a Food-Derived (A) and a Clinical (B) *B. cereus* Isolate. Note the visibly larger zones around carbapenems and fluoroquinolones in the food isolate compared to the clinical isolate.

3.4 Comparative Analysis of Antimicrobial Activity

The overall contribution of each antibiotic class was quantile by determining the

proportional inhibitory activity (Fig. 4). Carbapenems (IPM + MEM) seemed to be the most active drugs against food isolates, accounting for 39.83% of total measured inhibition, whereas the fluoroquinolones (CIP + LEV) had 27.12%. Clinical isolates on the contrary exhibited a comparatively lower contribution for carbapenems (32.10%) and the fluoroquinolones (29.63%), while a comparatively higher contribution for other drugs, which indicated a change in the resistance pattern could be attributed to selection pressure in clinical settings.

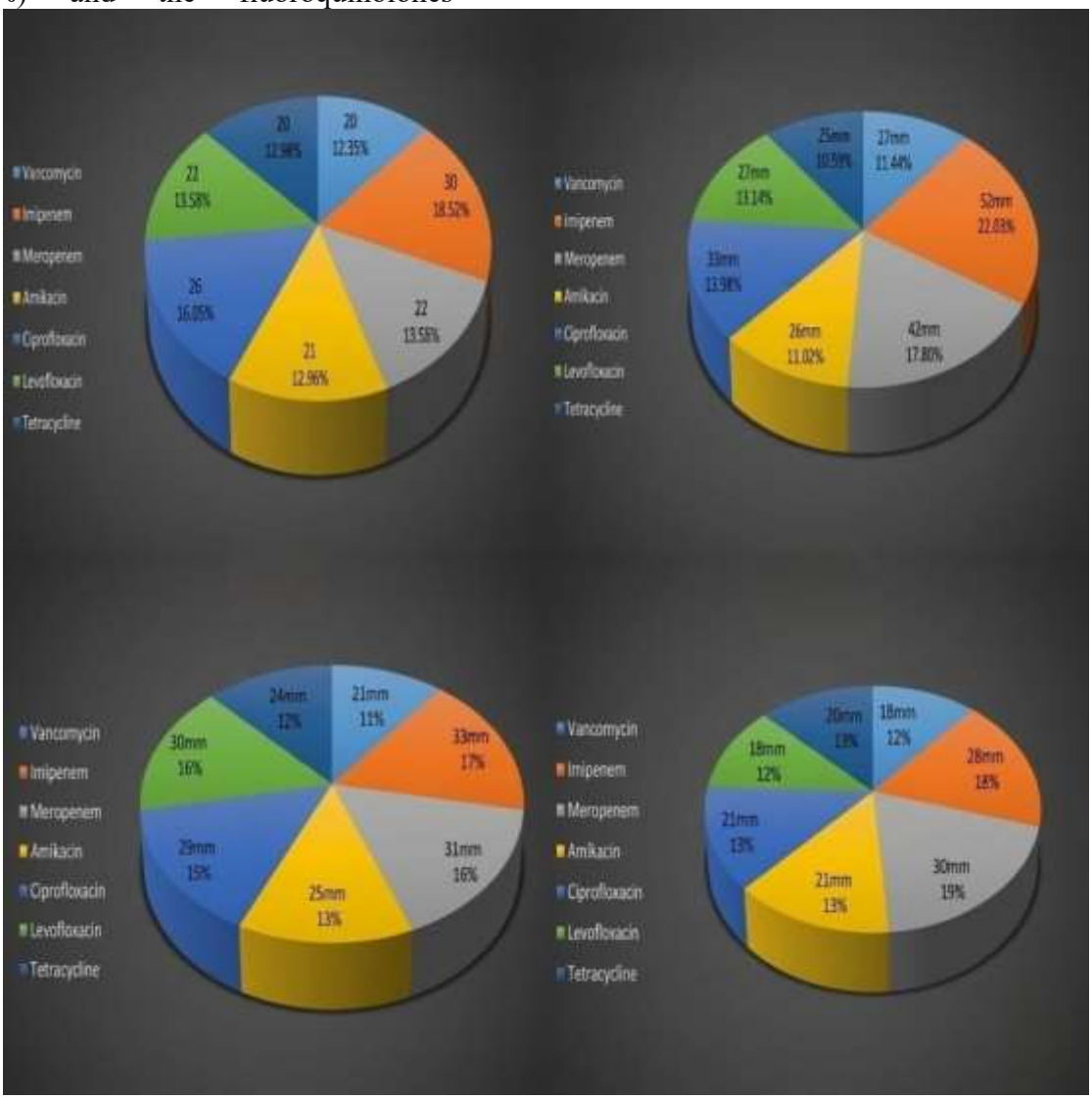


Fig4: Proportional Contribution of Tested Antibiotics to Overall Inhibitory Activity Against Clinical and Food-Derived *Bacillus cereus*

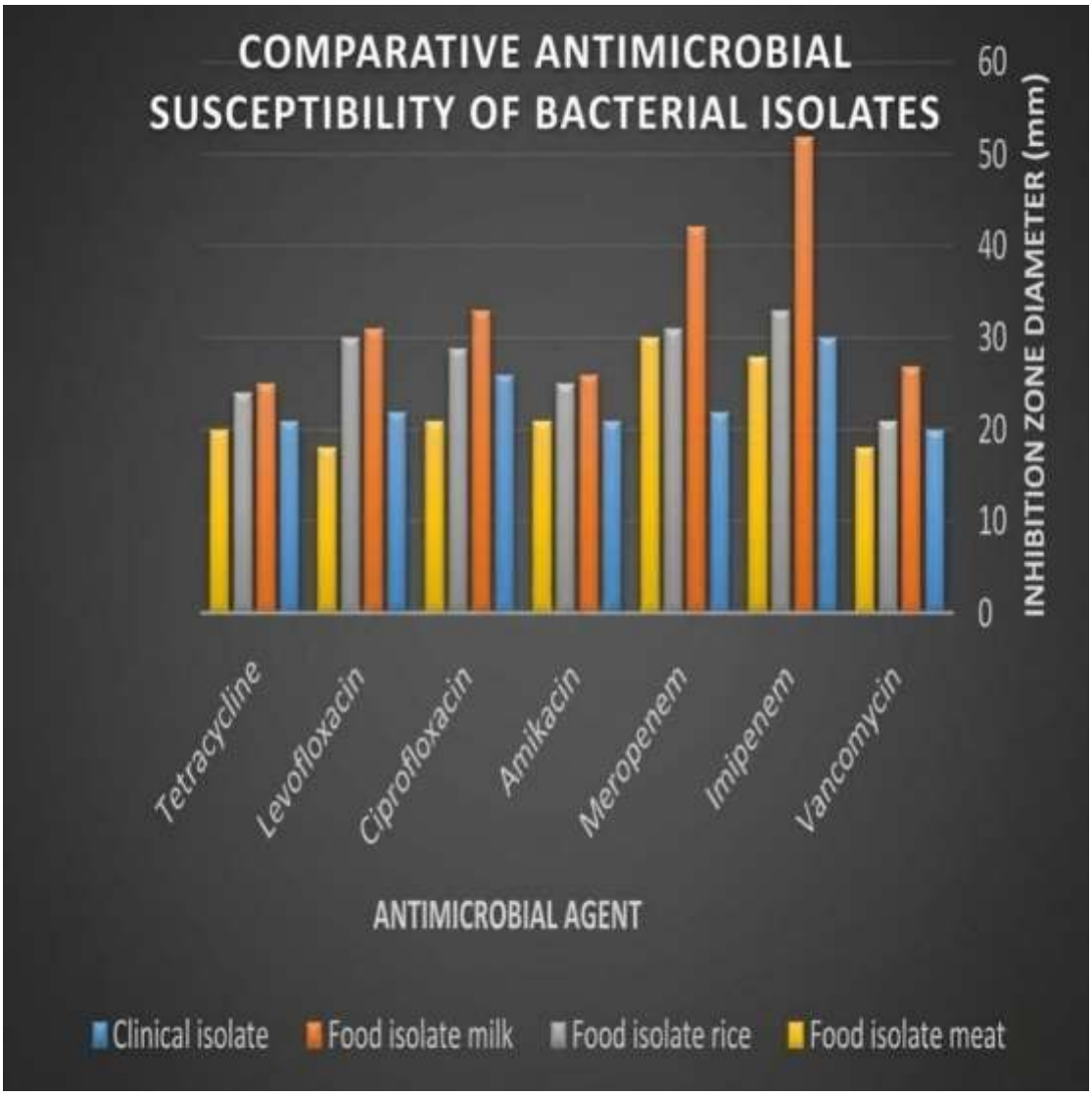


Fig5: Comparative Analysis of Antibiotic Susceptibility in Food and Clinical Bacillus Cereus Isolates (Measured by Inhibition Zone Diameter)

4-Discussion

This study has shed light on comparative data for the prevalence of *B. cereus* in food and clinical samples and its resistance to antimicrobial agents in Iraq for the first time. This overall isolation rate of 37,5% highlights the ubiquity of this pathogen in food products and clinical samples in the region. The validity of the process used for the diagnostic specificity was tested by using the PEMBA selective agar, which has been found to be very helpful to identify specific *B. cereus* colonies due to the peacock-blue colony morphology and precipitation of lecithinase [13]. The detection of lecithinase (Phospholipase C) was specifically tested in PEMBA as it is a virulence factor and a diagnostic marker which establishes the capacity to hydrolyse phospholipids from the host cell membrane contributing to necrosis of infected tissues and spread in the blood stream [6,7]. This enzyme seems to be very highly expressed and both food and clinical isolates are also beta-hemolytic, indicating that it has a very strong pathogenic potential.

The Vitek 2 system offered complete species-level identification and high confidence to identify all isolates as B. cereus-group species. This approach is free from any subjective interpretation or long turn-around time that are associated with the traditional biochemical techniques and provides a good discriminatory capability between *B. cereus* and related species by employing multi substrate kinetic analysis [15]. This step contributed to the high degree of replicability and validity of the findings of the study.

A finding with very great significance to this work is the significant difference in the susceptibility of clinical isolates versus food isolates. This was mechanistically evident for imipenem, as the mean IZD for clinical isolates (30 mm) was significantly lower than for the most susceptible food isolates (52 mm, for milk isolates). This difference is due to two competing effects. The well described chromosomal genes (bla1, bla2 and bla3) coding beta-lactamases that are intrinsic to the organisms confer resistance to most beta-lactams, but not to carbapenems such as imipenem or meropenem, which are usually not subject to hydrolysis of the beta-lactam ring [3,4]. This is borne out by the continued susceptibility of food isolates. These decreased clinical susceptibility is therefore suggestive of acquired or adaptive resistance mechanisms, presumably due to the extended selective pressure in hospital settings, hence this suggests to us that they are resistant to the drug used for treatment. It also involves the possibility of increased expression of multidrug efflux pumps that actively pump the drug out of the cell, and decreased permeability of the outer membrane, which has been documented in nosocomial Gram-positive bacteria [16]. The resistance of the total population (0% susceptibility) is used as an internal control to ensure the consistency of a stable resistance mechanism present intrinsically in all tested isolates, from whatever they have been isolated from.

Also, there is an apparent lack of correlation between in vitro efficacy and in vivo therapeutic efficacy. High resistance of B. cereus endospore formation to high concentrations of antimicrobial agent makes it a challenging organism to treat. This kind of spores enable the organism to remain in patients and on medical equipment, and can result in relapse following treatment termination [17,18]. The smaller inhibition zone seen with clinical isolates is a frightening indicator, then, of increased adaptability and possible treatment failure.

Both carbapenem resistance and beta-lactam resistance patterns have been described in other countries and are reflecting similar PMNs as observed in our study [5,10]. This study is the first to be quantitative and prove the susceptibility

shift to be significant between food and clinical biovars in Iraq. Overall, the data support the main hypothesis that the food environment versus the hospital environment renders a bacterium's resistance phenotype in a profoundly differential way, via selective pressure.

5- CONCLUSION

On the present study, it was found that the main differences between *B. cereus* clinical and food isolates are associated with less antimicrobial sensitivity among clinical isolates in Iraq. Imipenem and meropenem appear to be the most active carbapenems in vitro, and should be used as first line treatment in empirical therapy of serious *B. cereus* infections. The fact that susceptibility was reduced in clinical strains further highlights the need for regular antimicrobial resistance monitoring and New Zealand's Department of Health to ensure appropriate antibiotic use and prevent the increasing resistance to this opportunistic pathogen.

Ethical Approval

This study did not involve human participants or animals. All experimental procedures were conducted in accordance with institutional and laboratory biosafety guidelines.

Conflict of Interest

The authors declare that they have no conflict of interest.

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This research received no external funding.

Data Availability

All data supporting the findings of this study are available within the manuscript.

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