ORIGINAL RESEARCH

Susceptibility and Identification of *Bacillus cereus* on Chromogenic Selective Agar Isolated from Hospital Environments

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Abstract

Background: *Bacillus (B.) cereus* is widely distributed in different environments including hospitals and health centers that considered one of the highly resistant organisms to various antimicrobial agents. This investigation aimed to detect the incidence of *B. cereus* in hospital environments and their antibiotic susceptibility profile.

Materials and methods: A total of 114 swaps were collected from different places in the hospital and divided into two groups, floors 56 (49%) and equipment 58 (51%). Swabs cultured on blood agar, bacteria were identified according to the morphological characteristics and microscopic examination that confirmed by Bacillus ChromoSelect Agar. The antibiotics susceptibility test was carried out by following the Kirby Bauer method by using 7 antibiotics from various classes.

Results: From 114 specimens, 48 (42%) were positive for *B. cereus*. Antibiotic sensitivity test revealed highest resistance for ampicillin 33/48 (68.8%), cephalexin 31/48 (64.6%), and imipenem 25/48 (52.1%), and highest sensitive for ciprofloxacin 42/48 (87.5%) and gentamycin 40/48 (83.3%). The high positive correlation between cephalexin and ampicillin ($r = 0.78$, $p = 0.001$), tetracycline and ciprofloxacin ($r = 0.64$, $p = 0.001$), and no correlation between cephalexin and gentamycin ($r = -0.063$, $p = 0.67$).

Conclusion: This study revealed a high prevalence of *B. cereus* in hospital floors and equipment with high resistance to various antibiotics including β-lactams and tetracycline. The monitoring of hospital environments is an important tool in the prevention of hospital-associated infection by *B. cereus*.

Keywords: Antimicrobial agents, Antibiotics susceptibility, *Bacillus cereus,* Hospital environments.

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Introduction

In hospital settings, *Bacillus (B.) cereus* is known to produce severe extra-intestinal and nosocomial infections, which include bacteremia, endocarditis, meningoencephalitis, and pneumonia. These infections pose a substantial threat to public health. Severe infections, which are more common in immunocompromised patients, can occasionally lead to nosocomial *B. cereus* infections, which have been linked to the contamination of ventilator accessories, IV catheters, and bed linens.[1]

Gram-positive *B. cereus* is a foodborne pathogenic bacterium that forms endospores and is often found in natural habitats. It is notably common in dairy products and may even survive in host epithelial cells. There are two kinds of gastrointestinal disorders linked to food that are caused by these common soil bacteria. The emetic type, a food intoxication, presents as nausea and vomiting, whereas the diarrheal type is characterized by a preponderance of diarrhea. Diarrhea and stomach discomfort are caused by food infections involving enteropathogenic strains.[2]

Enterotoxins and emetic toxins are produced by the pathogenic bacteria *B. cereus*. It has demonstrated resistance to several antibiotics, including β-lactam drugs. Using bacteriophages is an alternate method of controlling *B. cereus* infection.[3]

From 2006 to 2018, *B. cereus* pollution was linked to a variety of illnesses in a number of nations, including the Netherlands, Norway, the United States, New Zealand, France, and Indonesia. It even resulted in a number of outbreak cases.[4] The establishment of resistance genes through horizontal gene transfer or long-term, careless antibiotic usage has both been linked to the discovery of antibiotic-resistant *B. cereus* strains.[5]

The aims of the current study are to detect the incidence of *B. cereus* in hospital environments especially on floors and medical equipment as well as to study some antibiotic susceptibility to *B. cereus* phenotypically.

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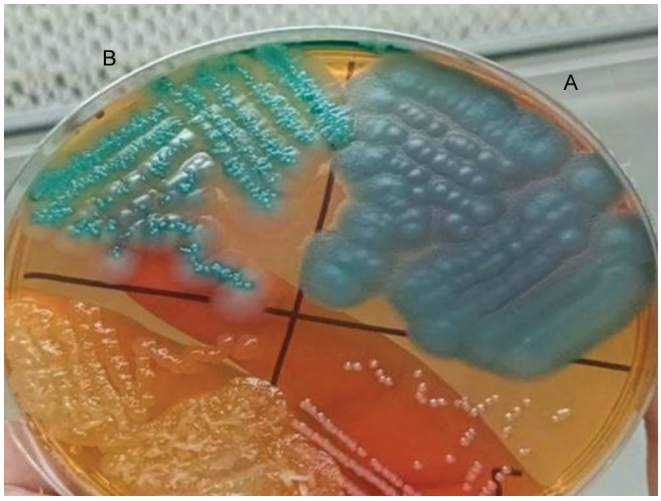

Fig. 1: *Bacillus cereus* on ChromoSelect Bacillus agar. (A) *Bacillus cereus*; (B) Other species of *Bacillus*

Materials and Methods

a total of 114 swaps were collected from different spaces and equipment of Al-Shomali Hospital in Babylon City, Iraq; during a period from September 2023 to January 2024. These swabs were cultured on blood agar (HIMEDIA, India). Species were identified according to the morphological features on culture media, and microscopic examination. A colony from each bacterial isolate was fixed on a slide and stained by gram stain to examine their reaction and shape according to.[6] Diagnosis of *B. ceruse* isolates is confirmed by Bacillus ChromoSelect Agar (Sigma-Aldrich, Germany).

Mueller Hinton agar (HIMEDIA, India) is a general media for the determination of the susceptibility of microorganisms to antimicrobial agents. The antibiotics susceptibility test was carried out by following the Kirby Bauer method described by CLSI, (2023); by using 7 antibiotics from various classes (Ciprofloxacin (5 µg), Tetracycline (30 µg), Amoxicillin/Clavulanic acid (20/10 µg), Ampicillin (10 µg), Gentamycin (10 µg), Imipenem (10 µg), Cephalexin (30 µg) (HIMEDIA, India).[7]

Data were analyzed by using SPSS 26. Correlation detected by Spearman's correlation test. The p-value < 0.05 is considered significant.

Results

In this study, from 114 specimens, 48 (42%) were positive for *B. cereus* isolated on selective chromogenic agar (Fig. 1). A total of 114 specimens were taken from different places and divided into two groups floor 56 (49%) and equipment 58 (51%) (Fig. 2 and Table 1).

Antibiogram profile for *B. cereus* bacteria exhibited significantly resistance to some of the tested antibiotics, highest resistance for ampicillin 33/48 (68.8%), cephalexin 31/48 (64.6%), and Imipenem 25/48 (52.1%), and highest sensitive for Ciprofloxacin 42/48 (87.5%), Gentamycin 40/48 (83.3%). Other antibiotics showed various sensitivity, tetracycline 30/48 (63%), Amoxicillin/Clavulanic acid 28/48 (58.3%) (Table 2 and Fig. 3).

The high positive correlation between cephalexin and ampicillin ($r = 0.78$, $p = 0.001$), tetracycline and ciprofloxacin ($r = 0.64$, $p = 0.001$), and no correlation between cephalexin and gentamycin ($r = -0.063$, $p = 0.67$) (Table 3 and Fig. 4).

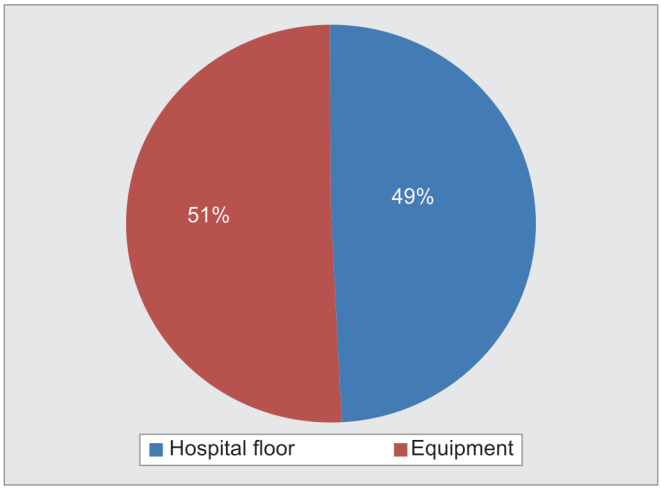

Fig. 2: Sources of collected specimens

Table 1: Number and percentage of positive and negative swaps for *B. cereus*

Place of specimens No. (%)	*Positive swab No. (%)*	*Negative swab No. (%)*
Hospital floor	18 (37.5)	38 (57.5)
Equipment	30 (62.5)	28 (42.5)
Total No. = 114	48 (100)	66 (100)

Table 2: Antibiotics susceptibility of *B. ceruse* isolates (No. = 48)

Antibiotics	*Abbreviation*	*Sensitive (%)*	*Resistance (%)*
Ciprofloxacin	CIP	42 (87.5)	6 (12.5)
Tetracycline	TE	30 (63)	18 (37)
Amoxicillin/Clavulanic acid	AUG	28 (58.3)	20 (41.7)
Ampicillin	AMP	15 (31.2)	33 (68.8)
Gentamycin	CN	40 (83.3)	8 (16.7)
Imipenem	IMP	23 (47.9)	25 (52.1)
Cephalexin	CEP	17 (47.9)	31 (64.6)

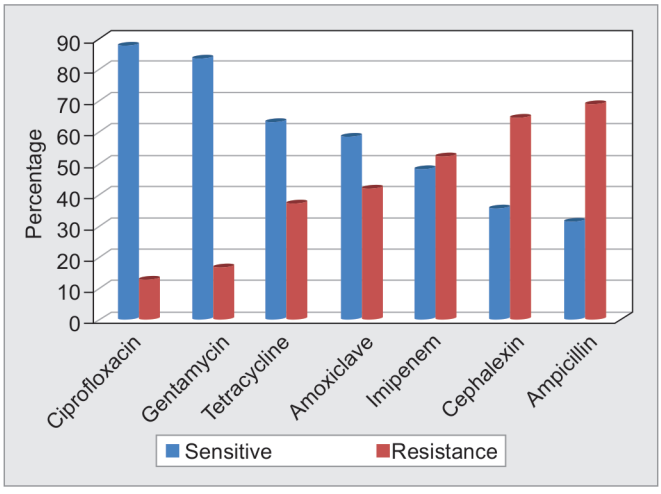

Fig. 3: Percentage of antibiotic resistance for *B. cereus*

Table 3: Correlation between different antibiotics

Antibiotics	*TE*	*AUG*	*AMP*	*CN*	*IMP*	*CEP*
Cip						
Correlation	0.648**	0.380**	0.057	0.700**	0.177	0.067
Sig	0.000	0.008	0.699	0.000	0.229	0.649
TE						
Correlation		0.716**	0.488**	0.791**	0.376**	0.341*
Sig		0.000	0.000	0.000	0.009	0.018
AUG						
Correlation			0.751**	0.616**	0.656**	0.474**
Sig			0.000	0.000	0.000	0.001
AMP						
Correlation				0.197	0.496**	0.782**
Sig				0.180	0.000	0.000
CN						
Correlation					0.353*	–0.063
Sig					0.014	0.673
IMP						
Correlation						0.459**
Sig						0.001

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed); AMP, ampicillin; AUG, amoxicillin/clavulanic acid, Cip, ciprofloxacin; CN, gentamycin; CEP, cephalexin; IMP, imipenem; TE, tetracycline

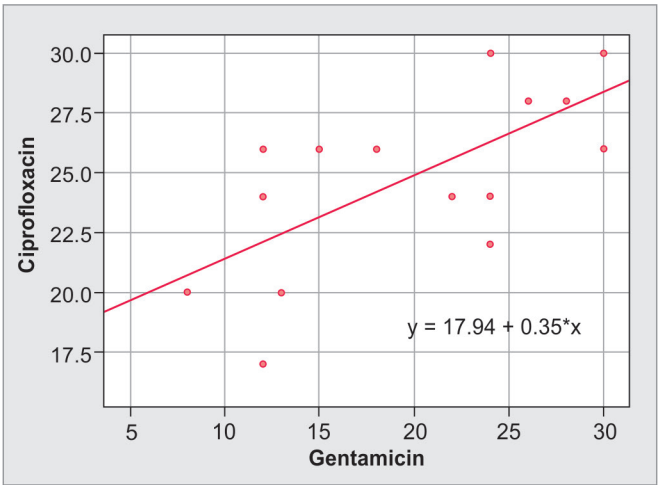

Fig. 4: Correlation between ciprofloxacin and gentamicin

Discussion

A complete picture of the transmission of bacteria and the danger of contracting diseases linked to health care might be obtained by comprehending the prevalence, antibiotic resistance, and relatedness of bacteria in hospital environments.[8,9]

One important factor in the spread of bacteria linked to nosocomial infections in healthcare settings is the environment. These bacteria can spread from person to person or through contact with inanimate objects, especially items that come into close proximity to patients.[10–12]

Food poisoning and diseases such as eye infections, sepsis, and deadly CNS infections are linked to Bacillus isolates, particularly *B. cereus*.[13,14] On the other hand, not much research has been done on the distribution of *B. cereus* isolates in Iraqi hospitals.

In the current study, from 114 specimens, 48 (42%) were positive for *B. cereus* isolated on selective chromogenic agar. Isolates are divided into two groups floor 56 (49%) and equipment 58 (51%). In equipment, B cereus was more common than on flooring. These results were in line with another study that found *B. cereus* to be the most common species (46.6%). The Internal Medicine Department accounted for the bulk of Bacillus isolates (25.6%), with the Emergency Department coming in second (18.8%).[15]

Another study revealed that *B. cereus* is considered a contaminant when isolated from clinical specimens. The hospital environment is widely and naturally contaminated with microorganisms of specifically human or environmental origin.[16]

In the current study, antibiogram Profile for *B. cereus* bacteria exhibited significant resistance to some of the tested antibiotics, highest resistance for ampicillin 33/48 (68.8%), cephalexin 31/48 (64.6%), and imipenem 25/48 (52.1%), and highest sensitive for ciprofloxacin 42/48 (87.5%), gentamycin 40/48 (83.3%); this results partially similar to Zhang et al.; results of antimicrobial susceptibility test, nearly all isolates showed resistance to β-lactams such as ampicillin (99.3%), penicillin (99.6%), and cephalothin (83.6%), whilst they gave less resistance to other β-lactams, such as cefotetan and imipenem (27.6 and 2.5%, respectively). Gentamicin and ciprofloxacin could effectively inhibit the growth of different strains. Regarding 133 antimicrobial resistance profiles, 58 isolates (21.1%) were resistant to ≥10 antibiotics.[17]

B. cereus isolates were reported in another investigation to be 100% resistant to amoxicillin, ampicillin, and colistin. On the other hand, 83.01% of the samples had resistance to ampicillin-sulbactam, 67.9% to streptomycin, 45.2% to spiramycin, 35.8% to lincomycin, 22.6% to tetracycline, and 5.6% to erythromycin.[18]

Conclusion

This investigation verified that hospitals with high rates of antibiotic resistance had a high percentage of *B. cereus*. In order to avoid hospital-acquired illnesses, monitoring the hospital environment is crucial.

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