**Carrier Rate and Production of Extended Spectrum β-lactamase by *Bacillus cereus* Isolated from Patients with Wounds Attending Government Hospitals in Ondo State.**

**Abstract**

The carrier rate and production of Extended Spectrumβ-lactamases by *Bacillus cereus* isolated from patients with wounds attending government hospital in Ondo State. The bacteria isolated from wound were *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Streptococcus pyogenes* and *Bacillus cereus*.Male wounds are the most carrier rate of *B. cereus* than female with percentage of 53.85 %. The age range of 45-54 years was the most carrier rate of *B. cereus* with percentage of 35.90 %. Farmer had the highest percentage carrier rate among the occupation observed. Skin infection was the common wound contaminated with *B. cereus* with percentage positivity of 30.77 %. Twenty five (64.01 %) out of the 39 isolate of *B. cereus* isolated from the respondent are Extended Spectrumβ-lactamases (ESBL) producer. *Bacillus cereus* is increasingly being acknowledged as a serious bacterial pathogen in immunosuppressed hosts. The resistance of *B. cereus* in the course of the study is ESBL mediated. The contamination of wound could be through contact with hospital environmental surfaces or the cross infection from health workers to patients. Therefore, the wound should be cover appropriately by trained personnel in order to avoid microorganism gaining entrance into the blood through wound especially *B. cereus* which could cause serious system diseases.

**Keywords:** Carrier, *Bacillus cereus*, β-lactamases, wounds, patients, hospital.

**Introduction**

*Bacillus cereus* is a spore forming and ubiquitous bacterium present in soil, foods, insect larvae, almost all surfaces and human skin (Auger *et al*., 2009; Tran *et al*., 2010). *B. cereus* can cause food poisoning and various opportunistic and nosocomial infections. It can cause two types of food poisoning, one resulting in vomiting through the action of the emetic toxin cereulide and the other resulting in diarrhea through the action of various enterotoxins (Kotiranta *et al.*,2000; Granum, 2001; Stenfors *et al*., 2008). The majority of *B. cereus*,cause diseases that affects the gastrointestinal tract resulting either in diarrhoea or emesis. The emetic syndrome is an intoxication with the emetic toxin cereulide, which is performed in food during vegetative *B. cereus* growth.

*B. cereus* causes non-gastrointestinal illness (Bottone, 2010). Non-gastrointestinal *B. cereus* outbreaks are less frequent, and most are identified as nosocomial in origin. The strains isolated from non-gastrointestinal infections have shown the ability to synthesize necrotizing exotoxin-like hemolysins and phospholipases. *B. cereus* is a common food poisoning organism, but also systemic and local infections have been reported, especially associated with immunologically compromised patients, neonates, drug addicts and patients with a history of traumatic or surgical wounds or catheters.

Besides food poisoning (Glasset *et al*., 2016), *B. cereus* induces local and systemic infections (Bottone, 2010; Veysseyre *et al*., 2015; Wright, 2016; Kato *et al*., 2017). The main described conditions are septicemia, endophthalmitis, pneumonia, endocarditis, meningititis and encephalitis, especially in immunosuppressed individuals such as neonates, resulting in the patient death in about 10% of cases (Ramarao *et al*., 2014). In addition, several cases of fulminant infections similar to anthrax, and affecting healthy persons, have also been reported (Hoffmaster *et al*., 2014; Marston *et al*., 2016). Predisposing factors include intravenous drug use, surgical or traumatic wounds, intravascular catheters and prematurity due to an immature immune response and to the presence of indwelling devices in the intensive care environment of neonates (Hilliard *et al*., 2003; Decousser *et al*., 2013; Ramarao *et al*., 2014; Benusic *et al*., 2015). Environmental reservoirs include air filtration/ventilation equipment, linen, medical devices and hands of the staff (Bottone , 2010; Sasahara *et al*., 2011).

Bottone (2010) also reported that *B. cereus* has also been recognised as the etiological agent for a variety of rare, but often fatal non-gastrointestinal local and systemic infections. Due to a premature or impaired epithelial barrier lining the gut, neonates, elderly and immunocompromised individuals are at special risk to suffer from *B. cereus* infections, which mainly result from postoperative and posttraumatic wound contaminations (Bottone, 2010). Cases of fulminant bacteraemia (Hilliard *et al.*,2003), meningitis (Lebessi *et al*.,2009), pneumonia (Avashia *et al*.,2007), severe ocular infections (keratitis, endolphthalmitis) (Callegan *et al.* 2002; Callegan *et al.,* 2007), endocarditis and cutaneous infections have been reported. Severe and lethal *B. cereus* infections are commonly connected to contaminated hospital linen, colonized indwelling catheters and nosocomial transmission (Hernaiz *et al*.,2003; Kuroki *et al*.,2009; Sasahara *et al*.,2011).

Local and systemic infections have been reported, mainly describing individual cases or single hospital setting (Glasset *et al*., 2018). The real incidence of such infection is unknown and information on genetic and phenotypic characteristics of the incriminated strains is generally scarce (Glasset *et al*., 2018).

Postoperative and posttraumatic wound infections and burns caused by *B. cereus* are associated with the production of a dermonecrotic vascular permeability factor, HBL. Many of the local infections are mild, but also severe deep infections with necrosis and purulence occur. Intravenous drug abuse, alcoholism and diseases lowering the immune responses are regarded as predisposing factors in osteomyelitis due to *B. cereus*, which has occasionally been described also in patients with a history of surgical or accidental trauma.

*B. cereus* is one of the most important microorganisms found in severe ocular infections: keratitis, endophthalmitis, and panophthalmitis. In 1993, Drobniewski listed 35 cases of significant ocular infections reported in this century. Endophthalmitis is a severe infection caused by the introduction of bacteria into the eye following trauma or surgery. Case reports of *B. cereus* endophthalmitis or panophthalmitis have been reported in the literature (Altiparmak *et al*.,2007; Martinez *et al*.,2007; Tobita and Hayano, 2007; Al-Jamali *et al*., 2008; Zheng *et al*.,2008). Among the organisms that infect the eye, *B. cereus* has one of the most rapidly evolving courses of infection and is one of the most destructive. Despite aggressive drug and/or surgical intervention, *B. cereus* endophthalmitis typically results in migration of bacteria throughout the eye and a remarkably rapid progression to a severe intraocular inflammatory response, resulting in loss of functional vision within 24 hour to 48 hour.

*B. cereus* can produce a variety of skin and soft tissue infections, including cellulitis (Dancer *et al*.,2002; Latsios *et al*.,2003) and necrotizing cellulitis (Sada *et al.* 2009; Hutchens *et al*., 2010). Wound infections, mostly in otherwise healthy persons, have been reported following trauma, surgery and burns (Shimoni *et al*.,2008; Ribeiro *et al*.,2010). Catheter use was often associated with *B. cereus* infection (Srivaths *et al*.,2004; Flavelle *et al*.,2007).

*B. cereus* endocarditis is a rare condition that is associated with intravenous devices or recreational drug injections (Abusin *et al*.,2008). Morbidity and mortality associated with *B. cereus* endocarditis are high among patients with valvular heart disease (Cone *et al*., 2005).

Some cases of *B. cereus* meningoencephalitis (Puvabanditsin *et al*.,2007; Manickam *et al.,* 2008) and bacteraemia(Hilliard *et al*.,2003) have been reported in neonates. Neonatal meningoencephalitis caused by *B. cereus* is rare, but it carries a high mortality. Cases of infection are often associated withmedical equipment or devices.

Some cases of *B. cereus* pneumonia have been reported. Pulmonary infections due to *B. cereus* are rare compared to those attributed to *B. anthracis,* but can be just as life threatening in immunocompromised persons. The majority of cases were in metalworkers and immunocompromised patients who have greater susceptibility to infection.

All these pathologies are characterized by massive tissue degradation/destruction as a result of unspecific cytolytic and tissue-reactive enzyme activity (Bottone, 2010). A role for the different *B. cereus* haemolysins, the collagenase, cereolysin O and the three phospholipases sphingomyelinase (SMase), phosphatidylcholinespecific phospholipase C (PC-Plc) and phosphatidylinositol-specific phospholipase C (PI-Plc) has been suggested (Beecher and Wong 2000). In many pathogenic bacteria phospholipase C is recognised as virulence factor contributing to tissue damage by degranulation of human neutrophils. In addition, recent studies focussed on haemolysin II (HlyII) and secreted proteases like the neutral protease and immune inhibitor metalloproteases (InhA1, InhA2 and InhA3) as novel contributors to *B. cereus* pathogenicity (Cadot *et al*.,2010). Results indicate that *B. cereus* likely uses several virulence factors concomitantly to enhance tissue degradation and circumvent host defence mechanisms (Cadot *et al*.,2010; Guillemet *et al*.,2010).

β-lactamases are hydrolytic enzymes which acts on the β-lactam ring of common antibiotics such as penicillins and cephalosporins and render them ineffective thus confer the bacterium *Bacillus cereus* resistance towards lactam antibiotics (Rishi *et al*., 2012). ß-lactamases are increasing in number and diversification of the group of enzymes is occurring that inactivatesß-lactam type of antibacterials (Gupta, 2007). Beta-lactamases are enzymes (EC number|3.5.2.6) produced by some bacteria and are responsible for their [resistance](https://en.academic.ru/dic.nsf/enwiki/833) to beta-lactam antibiotics like penicillins, cephalosporins, cephamycins, ertapenems and carbapenems. β-lactamases are hydrolytic enzymes which acts on the β-lactam ring of common antibiotics such as penicillins and cephalosporins and render them ineffective thus confer the bacterium *Bacillus cereus* resistance towards lactam antibiotics (Rishi *et al*., 2012). These antibiotics have a common element in their molecular structure: a four-atom ring known as a beta-lactam. The lactamase enzyme breaks that ring open, deactivating the molecule's antibacterial properties. Beta-lactam antibiotics are typically used to treat a broad spectrum of gram-positive and gram-negative bacteria. Beta-lactamases produced by gram-positive organisms are usually secreted especially when antibiotic are present in the environment. A common cause of antibiotic resistance in bacteria is an increased abundance of ß-lactamases (Avashia *et al*., 2007). This can be the result of genetic engineering, or it can be caused by the selection of resistant variants in the presence of antibiotics. The first ß-lactamase was identified in *Escherichia coli* prior to the release of penicillin for use in medical practice1. In Gram negative pathogens, ß-lactamase production remains the most important contributing factor to ß-lactam resistance (Medeiros, 1997). ß-lactamase can be classified based on two major approaches. One is based on the biochemical and functional characteristics of the enzymes and the second is based on the molecular structure of the enzyme (Gupta, 2007). Functional classification of the ß-lactamases is based on spectrum of antimicrobial substrate profile, enzyme inhibition profile, enzyme net charge, hydrolysis rate and other parameters. Bush *et al* 1995 presented the classification based on 4 major groups (Bush, 2001) and subgroups (a-f) (Bush *et al*., 1995). Water-soluble ß–lactamase type I has been reported to be expressed in high abundance in vegetative cells of this resistant strain and also to be secreted by the vegetative bacteria. According to this classification, most ESBLs belong to group 2 B e, which is ß-lactamases inhibited by clavulanic acid, which can hydrolyze penicillins, narrow and extended spectrum cephalosporins and monobactams (Gupta, 2007).

Extended-spectrum beta-lactamases (ESBLs) render beta-lactam antibiotics such as penicillins, cephalosporins, and monobactams ineffective (Ghazaei, 2019). A common cause of antibiotic resistance in bacteria is an increased abundance of ß -lactamases (Majiduddin *et al*., 2002). This can be the result of genetic engineering, or it can be caused by the selection of resistant variants in the presence of antibiotics. ß-Lactamase genes are found in the wild-type genomes of many bacteria, including *Bacillus* species. These chromosoma l ß-lactamases do not generally provide effective antibiotic resistance in wild-type bacilli, despite evidence that the genes are not completely silenced (Chen *et al*., 2003). Extended Spectrum ß**-**Lactamases (ESBLs) are mostly plasmid-mediated ß**-**lactamases that efficiently hydrolyze oxyimino-cephalosporins and monobactams, yet are inhibited by -lactamase inhibitors (Bush and Fisher, 2011) They were first detected in *Enterobacteriaceae*, and nowadays various groups of ESBLs are produced by these microorganisms, the most common being CTX-M and SHV enzyme types (Pitout and Laupland, 2008; Bush and Fisher, 2011). ESBLs are increasingly reported worldwide and have been linked to successful enterobacterial clones possessing great epidemic potential (Zahar *et al*., 2009; Bush and Fisher, 2011). Plasmids coding for ESBLs may also carry additional ß**-**lactamase genes as well as genes conferring resistance to other antimicrobial classes (Pitout and Laupland, 2008; Carattoli,2009). An alteration in the amino acid sequence around the active site of the enzyme due to mutation in the narrow-spectrum beta-lactamases (TEM-1, TEM-2, or SHV-1) gives rise to ESBLs (Rawat and Nair, 2010; Rishi *et al*., 2012).This can limit the chemotherapeutic options for ESBL-producing pathogens and facilitate the inter- and intraspecies dissemination of ESBLs (Zahar *et al*., 2009)

The resistance to ß-lactam antibiotics is an increasing problem worldwide and ß**-**lactamases production is the most common mechanism of drug resistance (Gupta, 2007). Both global and Indian figures showed a marked increase in the number of ß-lactamases producing organisms (Gupta, 2007). These enzymes extended spectrum ß-lactamases (ESBLs) are numerous and continuous mutation has led to the development of enzymes having expanded substrate profile (Gupta, 2007). The four major groups of ß**-**lactams penicillin, cephalosporins, monobactams and carbapenems have a ß-lactam ring which can be hydrolysed by ß**-**lactamases resulting in microbiologically ineffective compounds (Bush and Mobashery, 1999). The persistent exposure of bacterial strains to a multitude of ß**-**lactams has led to overproduction and mutation of ß-lactamases. These ß**-**lactamases are now capable of hydrolyzing penicillins, broad-spectrum cephalosporins and monobactams. Thus these are new ß-lactamases and are called as extended spectrum beta lactamases (ESBLs) (Bush, 2001). In Gram negative bacteria these enzymes remain in the periplasmic space, where they attack the antibiotic before it can reach its receptor site (Stratton, 2000). The first plasmid mediated ß-lactamase was described in early 1960 (Gupta, 2007). ESBLs have been isolated from a wide variety of *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Capnocytophaga ochracea* (Bradford, 2001). Classical ESBLs have evolved from the widespread plasmid-encoded enzymes families TEM, SHV, cefotaxime (CTX-M) and oxacillin (OXA) (Gupta, 2007).

The aim of this study was to evaluate the carrier rate and production of β-lactamase by *Bacillus cereus* isolated from patients with wounds attending government hospitals in Ondo State.

**MATERIALS AND METHODS**

**Collection of Ethical Clearance**

Ethical clearance for the collection of wound swab samples from patients attending University of Medical Sciences Teaching Hospital, Akure and Federal Medical Center, Owo was collected from Hospitals Management Board, Akure, Ondo State, Nigeria.

**Description of study location**

This research work was carried out from October 2018 to May 2019 in Ondo state, Nigeria. The study area for this research is Ondo, Nigeria. Ondo state covers an area of 15,195.2 sqaure kilometers and lies at latitude 7⁰10′ North and longitude 5⁰ 05′ east. Ondo State is a [state](https://en.wikipedia.org/wiki/States_of_Nigeria) in Nigeria created on 3 February 1976 from the former [Western State](https://en.wikipedia.org/wiki/Western_State_(Nigeria)). It originally included what is now [Ekiti State](https://en.wikipedia.org/wiki/Ekiti_State" \o "Ekiti State), which was split off in 1996. [Akure](https://en.wikipedia.org/wiki/Akure" \o "Akure) is the state capital. Each Nigerian state has several ministerial offices representing the federal government.Ondo state borders Ekiti state to the north, Kogi State to the northeast, Edo State to the east, Delta State to the southeast, Ogun State to the southwest, and Osun State to the northwest. The state contains eighteen local government areas, the major ones being [Akoko](https://en.wikipedia.org/wiki/Akoko" \o "Akoko), [Akure](https://en.wikipedia.org/wiki/Akure" \o "Akure), [Okitipupa](https://en.wikipedia.org/wiki/Okitipupa" \o "Okitipupa), [Ondo](https://en.wikipedia.org/wiki/Ondo_City" \o "Ondo City), and [Owo](https://en.wikipedia.org/wiki/Owo" \o "Owo). The ethnic composition of Ondo State is largely from the [Yoruba](https://en.wikipedia.org/wiki/Yoruba_people) sub groups of the [Akoko](https://en.wikipedia.org/wiki/Akoko" \o "Akoko), [Akure](https://en.wikipedia.org/wiki/Akure" \o "Akure), [Okitipupa](https://en.wikipedia.org/wiki/Okitipupa" \o "Okitipupa), [Ilaje](https://en.wikipedia.org/wiki/Ilaje" \o "Ilaje), Ondo and [Owo](https://en.wikipedia.org/wiki/Owo" \o "Owo) people. [Ijaw](https://en.wikipedia.org/wiki/Ijaw_people" \o "Ijaw people) such as Apoi and Arogbo populations inhabit the coastal areas, while a sizable number of the Ondo State people who speak a variant of the Yoruba language similar to [Ife](https://en.wikipedia.org/wiki/Ife) dialect reside in Oke-Igbo.

To supplement the efforts of the federal medical centre, Owo and University of Medical Sciences Teaching Hospital, Akure in this regard, there are other government health centres and private clinics. 'Abiye' health programme of Governor Mimiko administration was recognized by World Health Organization (WHO) as one of the best health programmes on maternal health programme with the establishment of Mother-Child hospital in Akure.

**Administration of questionnaire**

Questionnaires containing relevant information were administered to the Laboratory scientists to fill in the information about the patients. Swab Samples of woundfrom the patients attending University of Medical Sciences Teaching Hospital, Akure and Federal Medical Center, Owo, was collected between October 2018 and May 2019. Carrier rate and clinical data on B. cereus samples isolated from patients were retrospectively collected from University of Medical Sciences Teaching Hospital, Akure and Federal Medical Centre Owo, Ondo State, Nigeria.

**Data collection**

Clinical data on *B. cereus* samples isolated from patients were retrospectively collected from University of Medical Sciences Teaching Hospital, Akure and Federal Medical Center, Owo between October 2018 and May 2019. Each filled questionnaire and reported every cases of patient from which bacteria and *B. cereus* were isolated were used to generate epidemiological data.

**Collection of wounds pus samples**

Pus swabs samples from the wound of patient attending University of Medical Sciences Teaching Hospital, Akure and Federal Medical Centre Owo, Ondo State were collected according to the method described by (Ananth and Rajan, 2014). Informed consent was obtained from the suspected patients prior to specimen collection. Only one swab per patient was collected after carefully cleaning the wound with sterile water in order to prevent surface contamination. Four hundred (400) pus swabs from both inpatients and outpatients were obtained from wound sites before the wound was cleaned using 70% alcohol. The specimen was collected on sterile cotton swab without contaminating them with skin commensals. Different types of wound samples were collected namely accident wound, post-operation sepsis, skin infection, Abscesss and burn wound. All samples were collected from University of Medical Sciences Teaching Hospital, Akure and Federal Medical Centre Owo, Ondo State and properly labeled indicating the source and age of patients. The samples were transported to the laboratory after being obtained.

**Isolation of Bacteria from the wounds**

Isolation of bacteria from wounds was carried out according to the method describe by Ananth and Rajan (2014). Culture plates of Eosin methylene blue agar, MacConkey agar, Nutrient agar, Cetrimide agar and Mannitol salt agar were used. The swab sticks used for the collection of the samples were streaked directly on the labeled agar plates and incubated at 37 °C for 24 hours. After incubation, cultures were examined for significant growth. Subcultures were then made into plates of nutrient agar and incubated for another 24 hours.

**Isolation of *B. cereus***

Isolation of *B. cereus* from the patient attending University of Medical Sciences Teaching Hospital, Akure and Federal Medical Center, Owo, between October 2018 and May 2019 was carried out has described by Glasset (2018). B. cereus strains were locally identified by plating on specific agar media (Mossel Medium) and confirmed by using 16S cDNA sequencing.

**Detection of ESBL production**

The methods used in this study involved the testing of the isolates for ESBL production against oxyimino β-lactam antibiotics following the recommendations of Clinical and Laboratory Standards Institute (CLSI) formerly NCCLS ([CLSI, 2005](https://scialert.net/fulltextmobile/?doi=jm.2011.796.804#8801_an)).

**Double Disc Synergy Test (DDST)**

In the DDST, synergy was determined between a disc of amoxyclav (20 μg amoxycillin and 10 μg clavulanic acid) and a 30 μg disc of each 3rd generation cephalosporin (3GC) (ceftazidime 30 μg mL-1, cefotaxime 30 μg mL-1) placed at a distance of 30 mm apart on Mueller-Hinton agar swabbed with the resistant isolates and incubated at 37°C for 18 to 24 h. The organisms were considered to produce ESBL, if the zone size around the 3GC disc extended towards the amoxyclav disc.

**Cephalosporin clavulanate combination discs**

The phenotype confirmatory test for ESBL production was performed with the use of ceftazidime (30 μg), cefotaxime (30 μg) with and without clavulanic acid against the isolates. Only the isolates that showed synergy in the DDST procedure were included for the test. The discs were placed on pre inoculated Mueller-Hinton agar and incubated at 37°C for 18 to 24 h. A difference of ≥5 mm between the zone diameters of either of the cephalosporin disks and their respective cephalosporin/clavulanate disk is taken to be phenotypic confirmation of ESBL production.

**Results**

**Table 1:** **Rate of occurrence of different bacteria isolated from patients with wounds attending Government hospital**

|  |  |  |
| --- | --- | --- |
| **Bacteria** | **Number of Patient tested positive** | **% Positivity** |
| *Staphylococcus aureus* | 207 | 34.27 |
| *Klebsiella pneumonia* | 109 | 18.05 |
| *Pseudomonas aeruginosa* | 88 | 14.57 |
| *Escherichia coli* | 70 | 11.59 |
| *Proteus mirabilis* | 50 | 8.28 |
| *Streptococcus pyogene* | 41 | 6.79 |
| *Bacillus cereus* | 39 | 6.46 |
| Total | 604 | 100.01 |

Table 1 shows the rate of occurrence of different bacteria isolated from patients with wounds. The bacteria isolated were *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Streptococcus pyogenes* and *Bacillus cereus. Staphylococcus aureus* had the highest frequency distribution of 34.27 %

**Table 2: Rate of occurrence of different bacteria isolated from male and female patients with wounds attending Government hospital**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Bacteria | Number of Male Patient tested positive | % positivity of Male | Number of Female Patient tested positive | % Positivity of Female |
| *Staphylococcus aureus* | 108 | 34.18 | 99 | 34.38 |
| *Klebsiella pneumoniae* | 57 | 18.04 | 52 | 18.06 |
| *Pseudomonas aeruginosa* | 46 | 14.56 | 42 | 14.58 |
| *Escherichia coli* | 37 | 11.71 | 33 | 11.46 |
| *Proteus mirabilis* | 26 | 8.23 | 24 | 8.33 |
| *Streptococcus pyogene* | 21 | 6.65 | 20 | 6.94 |
| *Bacillus cereus* | 21 | 6.65 | 18 | 6.25 |
| Total | 316 | 100 | 288 | 100 |

Table 2 shows the rate of occurrence of different bacteria isolated from male and female patients with wounds. Male had the highest percentage positivity of *Escherichia coli* while female had the highest percentage positivity *Staphylococcus* *aureus,* *Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Streptococcus pyogenes* and *Bacillus cereus* compare to male*.*

**Table 3: Frequency distribution of bacteria according to wound types**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Bacteria | Type of Wound | | | | | |
| **Accident wound** | **Post-Operation sepsis** | **Skin infection** | **Abscess** | **Burn wound** | **Total** |
| *Staphylococcus aureus* | 59 | 44 | 38 | 42 | 24 | 207 |
| *Klebsiella pneumonia* | 28 | 20 | 17 | 26 | 18 | 109 |
| *Pseudomonas aeruginosa* | 22 | 16 | 19 | 20 | 11 | 88 |
| *Escherichia coli* | 10 | 17 | 19 | 17 | 7 | 70 |
| *Proteus mirabilis* | 12 | 7 | 15 | 9 | 7 | 50 |
| *Streptococcus pyogene* | 15 | 8 | 10 | 7 | 1 | 41 |
| *Bacillus cereus* | 9 | 10 | 12 | 7 | 1 | 39 |
| Total | 155 | 122 | 130 | 128 | 69 | 604 |

Frequency distribution of bacteria according to wound types is shown in Table 3. The bacteria isolated from accident wound, post-operation infection, Abscess and burn wound were recorded against each of the bacteria isolated

**Table 4: Carrier rate of *B. cereus* isolated from patients attending Government hospital based on gender**

|  |  |  |
| --- | --- | --- |
| Gender | *B. cereus* | % positivity |
| Male | 21 | 53.85 |
| Female | 18 | 46.15 |
| Total | 39 | 100 |

Carrier rate of *B. cereus* isolated from patients attending Government hospital based on gender is recorded on Table 4. It was observed that male are the highest carrier rate of *Bacillus cereus* isolated from patients with wounds with the percentage positivity of 55.85% while the female had 46.15 % compare to male

**Table 5: Carrier rate of *B. cereus* isolated from patients attending Government hospital based on age**

|  |  |  |
| --- | --- | --- |
| Age range | *B. cereus* | % positivity |
| 25-34 | 3 | 7.69 |
| 45-54 | 14 | 35.90 |
| 55-64 | 13 | 33.33 |
| 65-74 | 9 | 23.08 |
| Total | 39 | 100 |

Carrier rate of *B. cereus* isolated from patients attending Government hospital based on age range is presented in Table 5. Age range 45-54 had the highest percentage of 35.90 %, followed by 55-64, 65-74 and 25-34 with percentage positivity of 33.33 %, 23.08 % and 23.08 percent respectively. Other age range observed in the course of the research was 0 %.

**Table 6: Carrier rate of *B. cereus* isolated from patients attending Government hospital based on occupation**

|  |  |  |
| --- | --- | --- |
| Occupation | *B. cereus* | % Positivity |
| Farmer | 18 | 46.15 |
| Driver | 5 | 12.82 |
| Butcher | 6 | 15.38 |
| Business | 10 | 25.64 |
| Total | 39 | 100 |

Carrier rate of*B. cereus* isolated from patients with wounds attending Government hospital based on occupation is presented in Table 6. Farmer had the highest carrier rate percent positivity of *B. cereus* with percentage positivity of 46.15 % followed by those involved in Business, butcher and driver with percentage positivity of 25.64 %, 15.38 % and 12.82 %. *Bacillus cereus* was not isolated in other patient that engaged in other occupation observed in the course of the research.

**Table 7: Carrier rate of *B. cereus* isolated from patients attending Government hospital based on type of wound**

|  |  |  |
| --- | --- | --- |
| Type of wound | *B. cereus* | % Positivity |
| Accident | 9 | 23.08 |
| Post-Operation | 10 | 25.64 |
| Skin Infection | 12 | 30.77 |
| Abscess | 7 | 17.95 |
| Burn Wound | 1 | 2.56 |
| Total | 39 | 100 |

Carrier rate of *B. cereus* isolated from patients attending Government hospital based on type of wound is presented in Table 7. Skin infection wound had the highest carrier rate of *B. cereus* with percentage positivity of 30.77 % followed by Post-Operation wound, Accidental wound, Absecesse and Burn wound with carrier rate of 25.64 %, 23.08 %, 17.95 % and 2.56 % respectively.

**Table 8: Carrier rate of *B. cereus* isolated from patients attending Government hospital based on city of residence**

|  |  |  |
| --- | --- | --- |
| City of resident | *B. cereus* | % Positivity |
| Akure | 14 | 35.90 |
| Ilara | 3 | 7.69 |
| Owo | 6 | 15.38 |
| Akoko | 6 | 15.38 |
| Igbara Oke | 5 | 12.82 |
| Okitipupa | 3 | 7.69 |
| Bolunduro | 2 | 5.13 |
| Total | 39 | 100 |

Carrier rate of *B.cereus* isolated from patients attending Government hospital based on city of residence is presented in Table 8. Patients with wounds that reside in Akure had the highest carrier rate of 35.90 % of *B. cereus* percent while those that reside in Bolunduro had the lowest of 5.13 % in the course of the research.

**Table 9: β-lactamase production of *B. cereus* isolated from patients with wounds attending government hospitals**

|  |  |  |
| --- | --- | --- |
| *Bacillus cereus* Isolates | Double Disc Synergy Test (DDST) | Cephalosporin clavulanate combination discs |
| *B. cereus* 1AC | + | + |
| *B. cereus* 2AC | + | + |
| *B. cereus* 3AC | + | + |
| *B. cereus* 4AC | + | + |
| *B. cereus* 5AC | - | ND |
| *B. cereus* 6AC | + | + |
| *B. cereus* 7AC | - | ND |
| *B. cereus* 8AC | - | ND |
| *B. cereus* 9AC | - | ND |
| *B. cereus* 1 POS | + | + |
| *B. cereus* 2 POS | - | ND |
| *B. cereus* 3 POS | + | + |
| *B. cereus* 4 POS | + | + |
| *B. cereus* 5 POS | + | + |
| *B. cereus* 6 POS | + | + |
| *B. cereus* 7 POS | - | ND |
| *B. cereus* 8 POS | + | + |
| *B. cereus* 9 POS | - | ND |
| *B. cereus* 10 POS | + | + |
| *B. cereus* 1 SI | + | + |
| *B. cereus* 2 SI | + | + |
| *B. cereus* 3 SI | + | + |
| *B. cereus* 4 SI | + | + |
| *B. cereus* 5 SI | + | + |
| *B. cereus* 6 SI | - | ND |
| *B. cereus* 7 SI | - | ND |
| *B. cereus* 8 SI | + | + |
| *B. cereus* 9 SI | + | + |
| *B. cereus* 10 SI | + | + |
| *B. cereus* 11 SI | - | ND |
| *B. cereus* 12 SI | + | + |
| *B. cereus* 1 A | - | ND |
| *B. cereus* 2 A | - | ND |
| *B. cereus* 3 A | + | + |
| *B. cereus* 4 A | + | + |
| *B. cereus* 5 A | + | + |
| *B. cereus* 6 A | - | ND |
| *B. cereus* 7 A | + | + |
| *B. cereus* 1 BW | - | ND |

**LEGEND:** +**=** Positive**, -=**Negative,AW=Accident wound, POS**=** Post-Operation sepsis,SI= Skin Infection, A= Abscess,BW=burn wound, ND**=** Not determined

β-lactamase production of *B. cereus* isolated from patients with wound attending government hospitals is presented in Table 9. The β-lactamase production of *B. cereus* was observed. The positivity and negativity of the β-lactamase production was recorded against each isolates of *B. cereus.* Twenty-five (25) isolates of *B. cereus* were able to produce β-lactamase out of 39 isolates.

**Discussion**

The aim of the study was to present the carrier rate and extended spectrum ß-Lactamase production by *Bacillus cereus* isolated from patients attending government hospitals in Ondo State, Nigeria. *Bacillus cereus* can cause serious, life-threatening, systemic infections in immunocompromised patients (Aygun *et al*., 2016). In hospital, *B. cereus* is usually regarded by the physicians as an environmental contaminant (Glasset *et al*., 2018). However, it can cause fatal systemic infections among neonates, immunocompro-mised patients, and intravenous drug users (Bottone, 2010; Gurler *et al*., 2010). Other manifestations of severe disease are meningitis, endocarditis, osteomyelitis, and surgical and traumatic wound infections, but they are rare and mainly limited to case reports (Gurler, *et al*., 2010; Tatara *et al*., 2013). Wound provides a moist, warm, nutritive environment conducive to microbial colonization, proliferation, and infection (Shittu, 2002; Cooper, 2005; Fauci *et al*., 2008). Wound healing is a complex process that can be derailed by multiple factors including obesity, diabetes, smoking, vascular disease, infection, renal failure and malnutrition (Gould and Fulton, 2016).

Male wound are the most carrier rate of *B. cereus* than female with percentage of 53.85 %. The reason could be due to unhygienic rate of male than female. It could also due to fact that most of the male get involved in work related to soil (Farmer) where *B. cereus* inhabit. *Bacillus* spores are abundant in soil, fresh water, and hospital environment and even in normal gastrointestinal flora of prolonged hospitalized patients (Aygun *et al*., 2016). The age range of 45-54 years was the most carrier rate of *B. cereus* with percentage of 35.90 %. Farmer had the highest percentage carrier rate among the occupation observed, the reason could be due to the fact that most of wound sustained by farmer occur while on the farm, and the wound could be easily contaminated with soil which is natural reservoir of *B. cereus.* Skin infection was the common wound contaminated with *B. cereus* with percentage positivity of 30.77 %. Akure had the highest carrier rate of *Bacillus cereus* compare to other city with frequency distribution of 35.90 %.

Twenty five (64.01 %) out of the 39 isolate of *B. cereus* isolated from the respondent are ESBL producer. Aftab *et al*. (2014) reported that drug resistant bacteria are the most important therapeutic challenge in the field of infectious diseases. Many of them are multi drug resistant. Among them MRSA and ESBL producing gram negative bacteria are of major concern (Aftab *et al*., 2014). *Bacillus cereus* is increasingly being acknowledged as a serious bacterial pathogen in immunosuppressed hosts(Ginsburg *et al*., 2003). Patients with acute leukemia are particularly susceptible to bacteremia resulting from *B. cereus* (Akiyama *et al*., 1997). Over the past two decades there has been a clear shift from Gram-negative bacteria to Gram-positive and resistant bacteria being responsible for 60–70% of bacteremias identified in patients with neutropenia and cancer. There have been 16 reported cases of *B. cereus* septicemia in leukemic patients with only three recoveries (Akiyama *et al*., 1997). Five of the bacteraemias (Cases 4e8) occurred concomitantly and this outbreak appeared to be related to the use of non-sterile cotton wool (Ozkocaman *et al*., 2006). The authors were alerted by the microbiology laboratory about the unusual frequency of *Bacillus* spp. isolation in the haematology unit (Ozkocaman *et al*., 2006).

**Conclusion**

Twenty five (64.01 %) out of the 39 isolate of *B. cereus* isolated from the respondent are ESBL producer which could lead to resistance of *B. ce*i*reus*. Nongastrointestinal infections by *B. cereus* are relatively rare, but can occur particularly in patients whose defense is weakened. These include wound infections, ophtalmic infections, endocarditis, postoperative meningitis, urinary tract infection and liver infection. *B. cereus* isolated from patients, especially if immunosuppressed, should not be systematically disregarded as a contaminant, and its clinical significance should be raised. Inadequate attention could delay appropriate therapy and increase the risk of severe infections and poor outcome. Wound should be cover appropriately by trained personnel in order to avoid microorganism gaining entrance into the blood through wound.

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