# Antimicrobial resistance and toxigenic profiles of bacteria isolated from tropical shrimps (*Farfantepenaeus notialis* and *Penaeus monodon*) in Cameroun

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**Research note**

**Keywords:** Spoilage, shrimps, Bacillus, resistance genes, enterotoxins

**Posted Date:** April 23rd, 2020

**DOI:** https://doi.org/10.21203/rs.3.rs-22912/v1

**Version of Record:** A version of this preprint was published at BMC Research Notes on July 29th, 2020. See the published version at https://doi.org/10.1186/s13104-020-05184-1.

## Abstract

# Objective

Post-harvest shrimp losses are a big problem due to the proliferation of spoilage bacteria. Presence and multiplication of these bacteria promotes the emergence of food-borne illnesses. This study was carried out to characterize specifics spoilage bacteria from tropical brackish water shrimp and black tiger shrimp shrimps stored in ambient temperature (25 °C).

# Result

*22 Bacillus spp*; 09 Coagulase negative staphylococci (SCN) and 04 enterobacteria such as 01 *Pantoea genus*; 01 *Serratia plymutica* and 02 *Serratia rubidaea* have been identified. Resistance and virulence genes were searched. All isolates expressed resistance to at least three of antibiotics tested. 03 strains of enterobacteria were succeptible to cetfazidim and Amoxycyclin-Clavulanic Acid. Bacillus showed complete succeptibility to Cefixim, ertapenem and cetfazidim. Staphylococcocci were susceptible to clyndamycine. *Pantoea spp* was resistant to all antibiotics but exhibited intermediate succeptibility to Amoxycyclin-Clavulanic Acid. 04 strains of staphycolocci were positives to mecA resistances genes. None enterobacteria were positives to tetracycline resistance genes. All the strains of Bacillus contained enterotoxin genes, high prevalence of 21 strain to hemolytic enterotoxins and 17 strains to cell-lyse factor production like sphingomyelinase activities. The majority of Bacillus strains present in the study poses a potential risk of food poisoning due to the prevalence of toxin genes found.


## Introduction

Shrimps are great component of global seafood production. In general, they contain appreciable quantities of digestible proteins, essential amino acids, bioactive peptides, long-chain polyunsaturated fatty acids (Venugopal, 2009). In Cameroun, tropical brackish water shrimp (*Farfantepenaeus notialis*) and black tiger shrimp (*Penaeus monodon*) are species widely consumed (MINEPIA, 2014). Shrimps are generally safe for consumption but their exposure to diverse habitats and handling practices may occasionally entail health risks (Pang 2002; Yang et al., 2017, Don et al., 2018). Many retailers inappropriately stored shrimps in addition to poorly handling practices causing postharvest degradation (Nga Ombede et al., 2018). Specific spoilage organisms after catch will change characteristics of food as consequence of contamination (Leisner, 2000). Many studies showed emergence of foodborne diseases due to spoilage bacteria (Chaves et al., 2011). Gram-positive bacteria are slightly dominant of tropical warm water (Al Bulushi et al., 2010). This suggests their great implications in spoilage, especially in ambient temperature (Dabadé et al., 2015). Foodborne illness could result from the participation of Gram-positive bacteria for the majority of species with enterotoxins and resistance genes (Chaves et al., 2011). The aim of this study is then to explore antibiotics resistance and virulence factor of spoilage bacteria isolated from shrimps stored in ambient temperature (25 °C).

# Material And Methods

Collection and storage of samples

Experiments were carried out with tropical brackish water shrimp (*Farfantepenaeus notialis*) and black tiger shrimp (*Penaeus monodon*) during November and December 2018. Freshly harvested shrimp purchased from Youpwe fisheries market in Douala-Cameroon city were transported to laboratory in iced box. They were packed into four groups of 250 g portions per species in sterile plastic bags and kept at -20 °C further Microbiological test. Every two days, one plastic bag from each species was randomly taken for enumeration and isolation of total spoilage bacteria. Shrimps were stored at 25 °C for 24 h until spoilage odor off production.

Identification of spoilage bacteria

Enumeration was done after storing at room temperature. 25 g of shrimp per specie were aseptically added to 225 mL of peptone water and homogenized (ISO, 2013). Seven fold dilutions of each homogenate were prepared and 0.1 mL of $10^{-7}$ dilution was used for enumeration and isolation on nutrient agar. Some plates were aerobically or anaerobically incubated at 37 °C. Thereafter counting, three characteristic strains per plate were purified by streaks in Muller Hinton plate. Pure strains were stored at -80 °C in 20% glycerol. Identification of bacterial isolates was carried out bacteriologically and biochemically. API 20E gallery was used for Gram- negative identification.

Detection of resistance profile

An overnight bacterial pre-culture was diluted to obtain a turbidity of 0.5 McFarland. Kirby Bauer techniques were used to perform susceptibility testing (CA-SFM, 2012). Broth cultures were aseptically swabbed on Mueller Hinton agar. Antibiotic disks were placed on the agar plates and incubated 24 hours at 37 °C. Inhibition zone diameters were measured after 24hours. Susceptibility or resistance was determined according to CA-SFM (2012). Antibiotic used were amoxicillin (30 µg), amoxycyllin-clavulanique acid (30 µg), cefixim (5 µg), ceftazidim (30 µg), ceftriazone (30 µg), ciprofoxacin (5 µg), clyndamycine (10 µg) colistin (25 µg), ertapenem (10 µg), erythromycin (15 µg) fosfomycine (50 µg), impenem (10 µg), nalidixic acid (30 µg), oxacillin (1 µg), penicillin (10ui), pristinamycin (15 µg) and vancomycine (30 µg).

Molecular identification of antibiotic resistance genes

Isolates DNA was extracted using Qiagen blue extraction kit. PCR mixture of 23 µl contained 03 µl of genomic DNA, 0,5 µl of each primer, 0.5 µl dNTP, 2.5 µl Buffer with MgCl2, 0,125 µl Taq DNA Polymerase, and 16 µl distilled water. For enterobacteria and Staphylococci strains, PCR detection was performed for different resistance genes(Macrolide-resistance genes (ermA, ermB, ermC, mphC), vancomycin resistance genes vanA and vanB and Methicillin resistance genes for staphylococcus and tetracycline resistance (TetB, TetC, TetD, TetE, tetG and TetM/O/S) gene for enterobacteria was screened. PCR Detection of ermA-B-C, mphC, vanA-B and TetB-C-D-E-G-M genes. PCR conditions included initial denaturation for 5 minutes at 94 °C, followed by 40 cycles of denaturation for 45 s at 94 °C, annealing for 55 s at 45 °C, extension for 90 s at 72 °C and final extension for 10 min at 72 °C. PCR conditions for mecA were in accordance with Igbinosa and Beshiru. (2019). For Bacillus, virulence and enterotoxins genes were investigated. Genes encoding hemolysin (hbl-D/A), non-hemolytic enterotoxin (nheB), *B. cereus* enterotoxin T (bceT) and

enterotoxin FM (entFM) were screened. Table S1 show the primers used in the study. Virulence factors targeting of genes coding for two phospholipases associated with cell lysis, sphingomyelinase (sph) and phosphatidylinositol-specific phospholipase C (piplc), were also investigated in accordance with Mohammadou et al. (2014) and Matarante et al. (2004). PCR products were analyzed on 1.5% (w/v) agarose gel stained (120 volts at 35 min) with ethidium bromide and visualized by UV. Table S2 shows the primers of virulence factor genes detection.

# Results

### Identification of spoilage bacteria

Shrimps contained more than $10^7$germs/g after 24 hours in ambient storage. Figure S1 shows the microbial population present in tropical shrimps. Results showed main presence of Gram-positive bacteria (*Bacillus spp* and Negative Coagulase staphylococci (SCN), especially high frequency of Bacillus species (80%). Isolated Enterobacteria were Serratia and Plantoea strains.

# Resistance profile

All the 09 SCN isolates were susceptible to clyndamycine. They show complete resistance to oxacillin and penicillin. The strains were resistant to ciprofloxacin, colistin and vancomycin at 55,56%, to nalidixic acid at 88,89%. 15 isolates showed resistance to ceftazidim, ceftriaxon, cefixim and amoxicillin. Table 1 shows the antimicrobial susceptibility profile of staphylococci species.

Table 1
Antimicrobial susceptibility of staphylococci species

| **Antibiotics** | **S≥** | **R<** | **S %** | **I %** | **R %** |
|---|---|---|---|---|---|
| Ciprofloxacin | 25 | 22 | **3** (33,33%) | **1** (11,11%) | **5** 5,56%) |
| Pristinamycin | 22 | 19 | **5** (55,56%) | **1** (11,11%) | **3** (33,33%) |
| Penicillin | 29 | 18 | **0** | **0** | **9** (100%) |
| Fosfomycine | 14 | 14 | **6** (66,67%) | **0** | **3** (33,33%) |
| Erythromycin | 22 | 17 | **5** (55,56%) | **1** (11,11%) | **3** (33,33%) |
| Clyndamycin | 15 | 15 | **9** (100%) | **0** | **0** |
| Nalidixic Acid | 20 | 15 | **1** (11,11%) | **0** | **8** (88,89%) |
| Vancomycin | 17 | - | **4** (44,44%) | **0** | **5** (55,56%) |
| Oxacillin | 21 | 21 | **0** | **0** | **9** (100%) |
| Colistin | 15 | 15 | **4** (44,44%) | **0** | **5** (55,56%) |
| S = sensible; R = resistant; I = intermediate | | | | | |

Enterobacteria showed complete resistance to ceftriazon, cefixim, amoxicillin and ertapenem. Only strains of *Planteoa spp* showed intermediate resistance to amoxicillin - clavulanic acid and complete resistance to all other antibiotics tested. *Bacillus* strains were susceptible to impenem. 27,27% of isolates and 59,09% of Bacillus strains exhibited intermediate resistance to ciprofloxacin and amoxicillin-clavulanic acid. Table 2 shows antimicrobial susceptibility profile of Enterobacteria and Bacillus species.

Table 2
Antimicrobial susceptibility of Enterobacteria and Bacillus species

| **Antibiotics** | | | **Enterobacteria** | | | ***Bacillus spp*** | | |
|---|---|---|---|---|---|---|---|---|
| | **S≥** | **R<** | **S** | **I** | **R** | **S** **%** | **I** **%** | **R** **%** |
| Amoxicillin - clavulanic Acid | 23 | 16 | 3 | 1 | - | **8** (36,36%) | **13** (59,09%) | **1** (4,55%) |
| Vancomycin | 17 | - | 2 | - | 2 | **7** (31,82%) | **-** | **15** (68,18%) |
| Ceftazidim | 21 | 19 | - | 1 | 3 | **-** | **-** | **22** (100%) |
| Impenem | 21 | 18 | 3 | - | 1 | **22** (100%) | **-** | **-** |
| Ciprofloxacin | 25 | 22 | 1 | 2 | 1 | **6** (27,27%) | **6** (27,27%) | **10** (45,45%) |
| Ceftriazone | 26 | 23 | | | 4 | **0** | **1** (4,55%) | **21** (95,45%) |
| Cefixim | 25 | 22 | | | 4 | **0** | **0** | **22** (100%) |
| Amoxicillin | 23 | 16 | | | 4 | **0** | **1** (4,55%) | **21** (95,45%) |
| Ertapenem | 28 | 26 | | | 4 | **0** | **0** | **22** (100%) |

S = sensible; R = resistant; I = intermediate

# Resistance and enterotoxins-virulence genes

Bacillus strains isolated were positive to at least two enterotoxins. Only one Bacillus strain was positive to hemolysin gene. 03 strains were negative to sph and piplo virulence gene. All Bacillus strains (08) own *Bacillus cereus* enterotoxin T and also the sph gene. 04 isolates of staphylococci own MecA resistant gene. All enterobacteria strains were tested negative to tetracycline resistance gene. Figures 1 shows the results of PCR gene migration.

# Discussion

## Identification of spoilage bacteria

This study was carried out to explore resistance and virulence genes of spoilage bacteria isolated from shrimps in Cameroun. Results of this study are similar to those of Dabadé et al. (2015) who mainly found Gram positives, especially lactic bacteria and Enterobacteria in black tropical brackish water shrimp. Authors revealed that, these microorganisms are H2S-producing dominant group of bacteria potentially spoilage bacteria of tropical shrimps in Benin. Finding in this study suggests that Bacillus and staphylococci species are probably spoilage bacteria to tropical shrimp. Srinivasan and Saranraj. (2017) also found *Vibrio cholerae, Pseudomonas fluorescens, Salmonella* Typhi, *Staphylococcus aureus* and *Escherichia coli* but these microorganisms are common in polluted water. Staphylococci species in shrimp were explained by Al Bulushi et al. (2010) who related that the frequencies of Staphylococci species in marine environments depend on geographic location. Bacillus species in coastal fisheries is common, despite the relative abundance of *Staphylococcus spp*. (Al bulushi et al., 2010). Abundance of Bacillus in coastal waters and marine fisheries depends on temperature; depth and decline distance from the coast and, suggests that Bacillus species in coastal water originate from runoff (Rüger et al., 2000). Mohammadou et al. (2014) reported Bacillus species such as *Bacillus cereus, Bacillus thuringiensis* and *Bacillus anthracis* well-known as food poisoning in spoilage food. Gram-positive bacteria was more abundant than Gram-negative in tropical water and their influence in spoilage remain little probed (Al bulushi et al., 2010). Findings of this study also showed low frequency of Enterobacteria such as Serratia and Plantoea strains who grow in a broad range of temperatures and substrates (Garrity et al., 2005). Many species related with food spoilage, have been described as opportunistic human pathogens (Mardanehand and Mohammad, 2013; Mahlen, 2011; Déletoile et al., 2009).

## Antimicrobial susceptibility profile

Plantoea and Serratia genus presence in food is often neglected, when compared to classic multidrug-resistant Gram-negative pathogens (Melo et al., 2018, Halpern and Lev-Yadun, 2013, Dennis et al., 2019) likewise in this study. They are opportunistic pathogens among the most common causes of nosocomial diseases and transmissible by food ingestion. Resistance diversity of Bacillus strains in the study is not similar to the works of Mohammadou et al. (2014) who showed relative susceptibility of Bacillus strains to antibiotics unlike our results. Chaves et al. (2011) also found susceptibilities of Bacillus isolated from Brazilian food to gentamicin and tetracycline. Drug resistance of strains in this study may be attributed to bacterial spores of Bacillus species. Many studies also carried out the protective potential of bacillus strains in safe fermented food. Mohammadou et al. (2014) found that *B. subtilus* was able to produce bacteriocins such as subtilin in Mbuja.

## Resistance and enterotoxins-virulence genes

Tetracycline resistance gene can be explored to current and emerging multidrug-resistant pathogens including carbapenem-resistant Enterobacteriaceae. None enterobacteria presented tetracycline

resistance genes, although they were all resistant to Ertapenem. Many researches has been directed towards MecA genes as presented in this study on the genus of staphylococci and results are similar to those of Igbinosa and Beshiru. (2019) and Ali. (2014). MecA gene is present in all *S. aureus* strains and is known to encode penicillin binding protein 2a (PBP2a). Beta-lactam resistance is mostly attributed to mutations in mecA gene, but other genetic elements may also be considered for the explanation of the mechanism of resistance. Half of Bacillu*s* strains were positive to HBL genes. These results are similar to those of Chaves et al. (2011). The same authors demonstrated prevalence of hemolytic toxins in Bacillus strains isolated from fermented foods. According to Mohammadou et al. (2010), none *Bacillus* strains showed enterotoxins in Mbuja foods in Cameroon. In this study, only Bacillus strains do not exhibit enterotoxin NHE genes. Many authors correlate prevalence of enterotoxins HBL-NHE (Boonchai et al., 2008). According to Trans et al. (2010), wall peptidase (sph genes and PIPLC genes) are involved in adhesion, biofilm formation and virulence. Prevalence of entFM genes in more than half of Bacillus strains is located on the chromosome and appears to be common to *Bacillus thuringiensis* and *B. cereus*. Detection of EntFM is similar to the results of Arima et al. (2011). Presence of Bcet gene is evidence of presence of *B. cereus* among isolates. Agata et al. (1995) also demonstrated that prevalence of this gene carried out other strains than *B. cereus*. The majority of the strains isolated pose a potential risk of food poisoning due to their resistance and the prevalence of toxin genes found in Bacillus strains.

### Limitations

Not applicable

## Abbreviations

SCN: Coagulase Negative Staphilococci

## Declarations

**Ethics approval and consent to participate:** Not Applicable.

**Consent for publication:** All the coauthors consent to publish this manuscript.

**Availability of data and materials:** All data generated or analysed during this study is included in this published article and supplementary information files.

**Competing interests:** Authors declare no competing interest.

**Funding:** This study has been self-funded by the authors.

**Authors' contributions:** SNNO, FT and VD drafted the protocol. HK, ED, RPJMN, JPG, CT, AY performed the analysis. HK, ED and VD carried out the statistical analysis. SNNO, VD, ED wrote the draft of publication. AY and JD supervised the work and reviewed the manuscript. All the authors read the final version of the manuscript.

**Acknowledgements:** The authors are grateful to Prof Pascal HOUNGNANDAN who allowed this collaboration.

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# References

1. Abriouel H, Ben Omar N, Lopez RL, Canamero MM, Ortega E, Galvez A. Differentiation and characterization by molecular techniques of Bacillus cereus group isolates from poto poto and degue, two traditional cereal-based fermented foods of Burkina Faso and Republic of Congo. J Food Prot. 2007;70(5):1165–73.
2. Agata N, Ohta M, Arakawa Y, Mori M. The bceT gene of Bacillus cereus encodes an enterotoxic protein. Microbiology. 1995;141(4):983–8.
3. Al Bulushi IM, Poole SE, Barlow R, Deeth HC, Dykes GA. Speciation of Gram-positive bacteria in fresh and ambient-stored sub-tropical marine fish. Int J Food Microbiol. 2010;138(1–2):32–8.
4. Alfaro B, Hernandez I. Evolution of the indigenous microbiota in modified atmosphere packaged Atlantic horse mackerel (Trachurus trachurus) identified by conventional and molecular methods. Int J Food Microbiol. 2013;167(2):117–23.
5. Ali HH. Isolation and identification of Staphylococcus bacteria from fish of fresh water and its antibiotics sensitivity in mosul city. Basrah Journal of Veterinary Research. 2014;13(1):33–42.
6. Boonchai N, Asano SI, Bando H, Wiwat C. Study on cytotoxicity and nucleotide sequences of enterotoxin FM of Bacillus cereus isolated from various food sources. Journal of the Medical Association of Thailand = Chotmaihet thangphaet. 2008;91(9):1425–32.
7. Chaves JQ, Pires ES, Vivoni AM. Genetic diversity, antimicrobial resistance and toxigenic profiles of Bacillus cereus isolated from food in Brazil over three decades. Int J Food Microbiol. 2011;147(1):12–6.
8. Cunningham DJ, Leber A. 2018. Enterobacter, Cronobacter, and Pantoea species. In *Principles and Practice of Pediatric Infectious Diseases* (pp. 824–827). Elsevier.
9. Dabadé DS, den Besten HM, Azokpota P, Nout MR, Hounhouigan DJ, Zwietering MH. 2015. Spoilage evaluation, shelf-life prediction, and potential spoilage organisms of tropical brackish water shrimp (Penaeus notialis) at different storage temperatures. *Food microbiology*, *48*, pp. 8–16.
10. Delétoile A, Decré D, Courant S, Passet V, Audo J, Grimont P, Arlet G, Brisse S. 2009. Phylogeny and identification of Pantoea species and typing of Pantoea agglomerans strains by multilocus gene

sequencing. *Journal of clinical microbiology*, *47*(2), pp. 300–310.Grossman, T.H., 2016. Tetracycline antibiotics and resistance. *Cold Spring Harbor perspectives in medicine*, *6*(4), p.a025387.

11. Don S, Xavier KM, Devi ST, Nayak BB, Kannuchamy N. Identification of potential spoilage bacteria in farmed shrimp (Litopenaeus vannamei): Application of Relative Rate of Spoilage models in shelf life-prediction. LWT. 2018;97:295–301.
12. Hsieh YM, Sheu SJ, Chen YL, Tsen HY. Enterotoxigenic profiles and polymerase chain reaction detection of Bacillus cereus group cells and B. cereus strains from foods and food-borne outbreaks. J Appl Microbiol. 1999;87(4):481–90.
13. Igbinosa EO, Beshiru A. Characterization of antibiotic resistance and species diversity of staphylococci isolated from apparently healthy farm animals. African Journal of Clinical Experimental Microbiology. 2019;20(4):289–98.
14. Leisner JJ, Gram L. 2000. Spoilage of fish. In *Encyclopedia of food microbiology* (pp. 813–820). Academic Press.
15. Mahlen SD. Serratia infections: from military experiments to current practice. Clin Microbiol Rev. 2011;24(4):755–91.
16. Mardaneh J, Dallal MMS. Isolation, identification and antimicrobial susceptibility of Pantoea (Enterobacter) agglomerans isolated from consumed powdered infant formula milk (PIF) in NICU ward: First report from Iran. Iranian journal of microbiology. 2013;5(3):263.
17. Melo DH, Souza BV, Nascimento JS, Amorim AMB, Medeiros LM, Mattoso JMV. 2018. A reddish problem: Antibiotic-resistant Serratia marcescens in dairy food commercialized in Rio de Janeiro. *International Food Research Journal*, *25*(2).
18. Mohammadou BA, Le Blay G, Mbofung CM, Barbier G. 2014. Antimicrobial activities, toxinogenic potential and sensitivity to antibiotics of Bacillus strains isolated from Mbuja, an Hibiscus sabdariffa fermented seeds from Cameroon. *African Journal of Biotechnology*, *13*(35).
19. Nga Ombede SN, Kaktcham PM, Seydi M, Zambou Ngoufack F. 2019. Changes in sensory, physicochemical, and microbiological properties of fresh captured tropical pink shrimps (Penaeus duorarum notialis) inoculated with Lactobacillus plantarum Lp6SH, Lactobacillus rhamnosus Yoba, and their cell-free culture supernatants during storage at 4° C. *Journal of food safety*, *39*(1), p.e12579.
20. Saranraj P, Stella D, Reetha D. Microbial spoilage of vegetables and its control measures: A Review. International Journal of Natural Product Science. 2012;2(2):1–12.
21. Srinivasan T, Saranraj P. Isolation and identification of spoilage causing microorganisms in an Indian mackerel fish (Rastrelliger kanagurta). Int J Adv Res Biol Sci. 2017;4(7):1–7.
22. Tauxe RV. Emerging foodborne pathogens. Int J Food Microbiol. 2002;78(1–2):31–41.
23. Tran SL, Guillemet E, Gohar M, Lereclus D, Ramarao N. CwpFM (EntFM) is a Bacillus cereus potential cell wall peptidase implicated in adhesion, biofilm formation, and virulence. J Bacteriol. 2010;192(10):2638–42.

24. Rüger HJ, Fritze D, Spröer C. New psychrophilic and psychrotolerant Bacillus marinus strains from tropical and polar deep-sea sediments and emended description of the species. Int J Syst Evol MicroBiol. 2000;50(3):1305–13.
25. White DG, Zhao S, Simjee S, Wagner DD, McDermott PF. Antimicrobial resistance of foodborne pathogens. Microbes infection. 2002;4(4):405–12.
26. Yang SP, Xie J, Qian YF. Determination of spoilage microbiota of Pacific white shrimp during ambient and cold storage using next-generation sequencing and culture-dependent method. Journal of food science. 2017;82(5):1178–83.
27. Yang SP, Xie J, Qian YF. Determination of spoilage microbiota of Pacific white shrimp during ambient and cold storage using next-generation sequencing and culture-dependent method. Journal of food science. 2017;82(5):1178–83.

# Figures

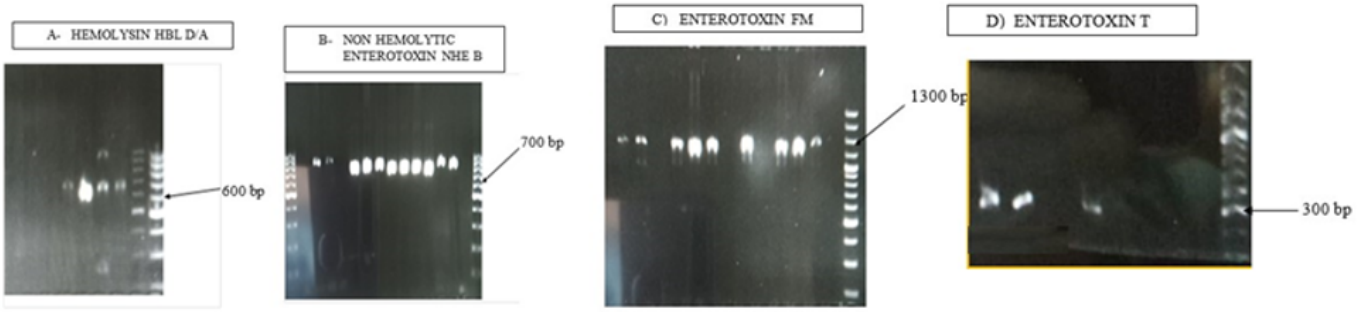


Fig 2a: hbl D/A(623 pb) and nheB gene (769pb)

Fig2b: entFM (1269 pb) and enterotoxin T B. cereus (428bp) genes

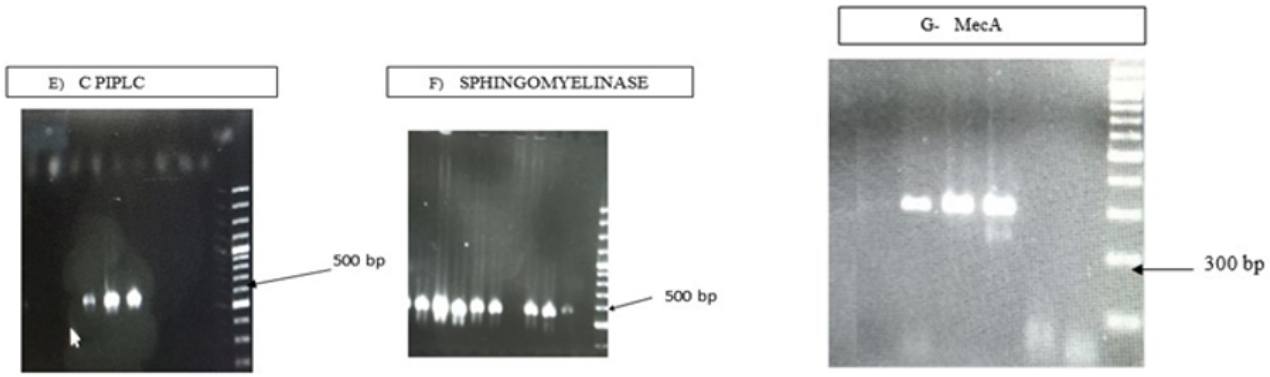


Fig 2c: piplc gene (569 bp) and sph (558 bp)

Fig2d: MecA (310 bp) gene

**Figure 1**

PCR gene migration

# Supplementary Files

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