



# ANTIMICROBIAL RESISTANCE OF Bacillus cereus LOCAL ISOLATES BY PHENOTYPIC AND GENOTYPIC **SCREENING ANALYSIS**

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FOOD SCIENCE STUDY PROGRAM **GRADUATE SCHOOL IPB UNIVERSITY BOGOR** 2024





IPB University



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Bogor, October 2024

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### **RINGKASAN**

IZZADINI FARHANI. Antimicrobial Resistance of Bacillus cereus Local Isolates by Phenotypic and Genotypic Screening Analysis. Dibimbing oleh RATIH DEWANTI-HARIYADI dan HANIFAH NURYANI LIOE.

Kasus penyakit bawaan pangan, termasuk yang disebabkan oleh *Bacillus* cereus, semakin meningkat akibat globalisasi dan tingginya angka perdagangan pangan internasional. Bakteri pembentuk spora ini dapat bertahan dalam kondisi ekstrim sehingga mampu bertahan pada kondisi pengolahan yang umum. Disamping itu, *B. cereus* mampu memproduksi toksin sereulida penyebab emesis dalam pangan dan menghasilkan enterotoksin penyebab diare dalam tubuh. *B. cereus* dilaporkan menyebabkan penyakit bawaan pangan yang signifikan di seluruh dunia, termasuk di Indonesia.

Meningkatnya resistansi antibiotik pada bakteri patogen menimbulkan permasalahan kesehatan yang serius. Pada *B. cereus* angka resistansi antibiotik juga dilaporkan meningkat, terutama terhadap golongan β-laktam. Hal ini menjadi tantangan karena bakteri ini mampu mentransfer gen resistansi melalui rantai pangan. Penelitian ini bertujuan untuk mengevaluasi sifat resistansi antimikroba pada isolat lokal *B. cereus*, dengan fokus pada resistansi fenotipik dan keberadaan gen resistansi. Pengetahuan ini diharapkan dapat meningkatkan pemahaman tentang ancaman resistansi antibiotik dalam konteks kesehatan masyarakat serta keamanan pangan.

Penelitian ini dilakukan dalam tiga tahap, yaitu tahap persiapan kultur termasuk uji konfirmasi biokimia, pewarnaan Gram dan spora, dan uji katalase, tahap skrining resistansi antibiotik *B. cereus* menggunakan metode *Kirby-Bauer*, dan tahap deteksi gen penyandi resistansi antibiotik menggunakan PCR untuk semua isolat yang menunjukkan sifat resistansi. Dari dua puluh satu isolat lokal yang dianalisis, semua (100%; 21/21) rentan terhadap kloramfenikol dan siprofloksasin tetapi resistan terhadap ampisilin, sefoksitin, sefalotin, dan penisilin G. Resistansi intermediet terhadap eritromisin dimiliki oleh 95,2% dari isolat (20/21), sementara 28,6% (6/21) memiliki reistansi intermediet terhadap tetrasiklin. Deteksi gen penyandi resistansi antibiotik menunjukkan bahwa semua (21/21) isolat resistan terhadap antibiotik kelas β-laktam memiliki gen *bla*1. Gen *tet*B dan *tet*L tidak terdeteksi pada isolat yang resistan maupun sensitif terhadap tetrasiklin (0%; 0/21) yang mengindikasikan perlunya dilakukan pengujian terhadap gen *tet* lainnya.

Kata kunci: Analisis PCR, *Bacillus cereus*, gen resistansi, resistansi antibiotik, skrining

### **SUMMARY**

IZZADINI FARHANI. Antimicrobial Resistance of Bacillus cereus Local Isolates by Phenotypic and Genotypic Screening Analysis. Supervised by RATIH DEWANTI-HARIYADI and HANIFAH NURYANI LIOE.

Foodborne outbreaks, notably caused by Bacillus cereus, have become a growing concern due to globalization and increased international trade. The bacterium is capable of producing spores which are resilitent and can survive common food processing. Additionally, B. cereus has the ability to produce cereulide toxins in foods that can cause emetic illnesses as well as enterotoxins in human intestine leading to diarrhea. B. cereus has been implicated in significant foodborne illness cases worldwide, including in Indonesia.

The rise of antibiotic resistance in bacteria has raised concerns in public health. In B. cereus, resistance to  $\beta$ -lactam antibiotics posseses a serious challenge, as the bacterium can transfer resistance genes across the food chain. This study aimed to evaluate the antimicrobial resistance properties of several B. cereus isolates, focusing on their phenotypic resistance and the presence of resistance genes, to better understand and manage this threat in public health and food safety contexts.

This study was conducted in three stages, the first step was culture preparation which includes confirmation tests by Gram and spore staining, catalase test, the second step involved antibiotic resistance screening of B. cereus using Kirby-Bauer method, while the third step included detection of antibiotic resistance genes using PCR analysis for phenotypically resistant isolates. Of the twenty-one (21) local isolates analyzed, all (100%; 21/21) were susceptible to chloramphenicol and ciprofloxacin but resistant to ampicillin, cefoxitin, cephalothin, and penicillin G. Ninety five percent (20/21) isolates showed intermediate traits with regard to erythromycin resistance while 28.6% (6/21) had intermediate resistance tetracycline. The detection of resistance-encoding genes showed that 100% (21/21) of the isolates possessed the bla1 gene. None of the tetracyline resistance and sensitive isolates (0%; 0/21) own tetB and tetL genes suggesting that other tet genes may need to be tested.

Keywords: antibiotic resistance, Bacillus cereus, PCR analysis, resistance genes, screening.



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# ANTIMICROBIAL RESISTANCE OF Bacillus cereus LOCAL ISOLATES BY PHENOTYPIC AND GENOTYPIC SCREENING ANALYSIS

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### **FOREWORD**

The completion of this thesis would not be possible without the immense support author received from many marvelous parties. This thesis, titled "Antimicrobial Resistance of *Bacillus cereus* Local Isolates by Phenotypic and Genotypic Screening Analysis", was written as one of the requirements to obtain a Master's Degree in Food Science study program at IPB University. First and foremost praises and thanks to God, the Almighty, for He has given the author strength, knowledge, patience, and guidance throughout the thesis work thesis successfully and completely. Author would also like to convey her greatest gratitude and appreciation to:

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May this thesis will not only serve as a useful resource for other researchers and contribute to the scientific community but also inspire others to explore more regarding antibiotic resistance in local isolates.

Bogor, October 2024

Izzadini Farhani





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