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Pengembangan Sarana Deteksi Food Poisoning Melalui Biomarker Recombinant Staphylococcal Enterotoxin A
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PENGEMBANGAN SARANA DETEKSI *FOOD POISONING* MELALUI BIOMARKER *RECOMBINANT STAPHYLOCOCCAL ENTEROTOXIN* A

Hidayatun Nisa' Purwanasari

ABSTRAK

Foodborne disease atau penyakit yang ditularkan melalui makanan merupakan masalah kesehatan masyarakat yang serius, sering kali disebabkan oleh konsumsi makanan yang terkontaminasi mikroorganisme patogen seperti *Staphylococcus aureus* (*S. aureus*). Salah satu faktor virulensi utama dari *S. aureus* adalah kemampuannya memproduksi enterotoksin, terutama Staphylococcal Enterotoxin A (SEA), yang merupakan penyebab terbanyak kasus keracunan makanan secara global. Deteksi cepat dan akurat terhadap keberadaan SEA pada produk pangan, khususnya susu, menjadi penting untuk mencegah kejadian foodborne disease yang meluas. Penelitian ini bertujuan mengembangkan antibodi poliklonal terhadap protein rekombinan SEA (rSEA) sebagai bahan uji diagnostik berbasis biomolekuler untuk mendeteksi SEA secara spesifik dan aplikatif.

Penelitian dilakukan dalam tiga tahap utama. Tahap pertama mencakup karakterisasi genotipik 188 isolat lokal *S. aureus* yang berasal dari manusia (n=173) dan hewan (n=15). Identifikasi dilakukan melalui PCR terhadap gen 23S rRNA, nuc, dan coa. Selanjutnya, distribusi 12 gen enterotoksin (*sea, seb, sec, sed, see, seg, seh, sei, sej, sel, sem, sen*) dianalisis menggunakan multiplex-PCR. Hasil menunjukkan bahwa seluruh isolat terkonfirmasi sebagai *S. aureus* dengan gen enterotoksin paling dominan pada isolat manusia adalah *sec* (64,7%), *seh* (51,4%), dan *seg* (35,2%), sedangkan pada isolat hewan adalah *seh* (100%) dan *sec* (78%). Gen *sea* sebagai target imunisasi ditemukan pada 49% isolat manusia dan 20% isolat hewan berdasarkan PCR tunggal. Tahap kedua fokus pada pengembangan antibodi poliklonal mencit terhadap protein rekombinan SEA. Protein rSEA diperoleh dari hasil kloning dan ekspresi gen *sea* dalam sistem prokariotik di Sekolah Vokasi UGM. Mencit *Balb/c* diimunisasi *intraperitineal* menggunakan rSEA dan adjuvan Freund lengkap, dilanjutkan *booster*. Respon imun yang dihasilkan menunjukkan titer tinggi dengan nilai OD hingga 3.500. Analisis SDS-PAGE menunjukkan rSEA berukuran ±27 kDa, yang dikonfirmasi spesifik oleh antibodi melalui uji *Western blot*. Tahap ketiga bertujuan mengevaluasi validitas antibodi dalam mendeteksi SEA dari isolat bakteri skala laboratorium dan sampel lapangan (susu sapi perah). Sebanyak 50 isolat manusia diuji menggunakan ELISA *antigen capture* dan *dot blot*. Hasil ELISA menunjukkan sensitivitas 75%, spesifisitas 91,2%, akurasi 86%, dan nilai *Cohen's Kappa* 67% (kategori baik). Uji *dot blot* menunjukkan sensitivitas 75%, spesifisitas 88,2%, akurasi 84%, dan *Kappa index* 63% (kategori baik). Uji lapangan pada 30 sampel susu menghasilkan 24 isolat positif *S. aureus*. Dengan *cut-off* ELISA sebesar 0.810, antibodi mampu mendeteksi 5 isolat positif SEA dan 19 negatif, dengan sensitivitas 66,7%,

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spesifisitas 94,4%, akurasi 88%, dan *Kappa index* 61%. Uji *dot blot* mendeteksi 8 isolat positif dan 16 negatif, dengan sensitivitas, spesifisitas, dan akurasi masing-masing 83,3%, serta *Kappa index* 60%.

Dari hasil penelitian dapat disimpulkan bahwa antibodi poliklonal terhadap rSEA menunjukkan performa validasi yang baik dan dapat digunakan sebagai sarana diagnostik berbasis antibodi untuk deteksi SEA. Metode ELISA *antigen capture* lebih sesuai untuk uji skrining di laboratorium, sedangkan uji *dot blot* lebih aplikatif di lapangan.

Kata kunci: *Staphylococcus aureus*, *Staphylococcal Enterotoxin* A (SEA), antibodi poliklonal, ELISA *antigen capture*, *dot blot*, keracunan makanan

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DEVELOPMENT OF FOOD POISONING DETECTION THROUGH RECOMBINANT BIOMARKER *STAPHYLOCOCCAL ENTEROTOXIN* A

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ABSTRACT

Foodborne disease remains a major public health concern, often caused by the consumption of food contaminated with pathogenic microorganisms, such as *Staphylococcus aureus* (*S. aureus*). One of the key virulence factors of *S. aureus* is its ability to produce enterotoxins, particularly Staphylococcal Enterotoxin A (SEA), which is the most frequently implicated enterotoxin in food poisoning outbreaks globally. Rapid and accurate detection of SEA in food products, especially milk, is essential to prevent widespread foodborne illness. This study aimed to develop polyclonal antibodies against recombinant SEA (rSEA) protein as a biomolecular-based diagnostic tool for specific and practical SEA detection.

The study was conducted in three major stages. The first stage involved genotypic characterization of 188 local *S. aureus* isolates obtained from humans (n=173) and animals (n=15). Identification was confirmed using PCR targeting 23S rRNA, *nuc*, and *coa* genes. Further analysis of 12 enterotoxin genes (*sea, seb, sec, sed, see, seg, seh, sei, sej, sel, sem, sen*) was performed using multiplex PCR. All isolates were confirmed as *S. aureus*. The most prevalent enterotoxin genes among human isolates were *sec* (64.7%), *seh* (51.4%), and *seg* (35.2%), while in animal isolates, *seh* (100%) and *sec* (78%) dominated. The *sea* gene, selected as the target for immunization, was detected in 49% of human isolates and 20% of animal isolates via singleplex PCR. The second stage focused on the development of polyclonal antibodies against rSEA in mice. Recombinant SEA protein was produced through cloning and expression of the *sea* gene in a prokaryotic system at the Vocational School, Universitas Gadjah Mada. BALB/c mice were subcutaneously immunized with rSEA emulsified in complete Freund's adjuvant, followed by booster doses. The resulting immune response showed a high antibody titer, with OD values reaching up to 3.500. SDS-PAGE analysis revealed rSEA at ~27 kDa, which was confirmed as specific by Western blot using the produced antibody. The third stage evaluated the diagnostic performance of the antibody in detecting SEA from both laboratory-scale bacterial isolates and field milk samples. Fifty human *S. aureus* isolates were tested using antigen-capture ELISA and dot blot. ELISA antigen capture results showed 75% sensitivity, 91.2% specificity, 86% accuracy, and a Cohen's Kappa value of 63% (good agreement). Dot blot yielded 75% sensitivity, 88.2% specificity, 84% accuracy, and a Kappa value of 63% (good agreement). Field testing of 30 milk samples revealed 24 positive for *S. aureus*. Using an ELISA cut-off of 0.810, five isolates were detected as SEA-positive and 19 as negative, with 66.7% sensitivity, 94.4% specificity, 88% accuracy, and Kappa 61% (good agreement). Dot blot detected 8 positives and 16

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negatives, showing 83.3% sensitivity, specificity, and accuracy, with Kappa 60% (moderate agreement).

In conclusion, the polyclonal antibody against rSEA demonstrated good validation performance and can be utilized as a diagnostic reagent for SEA detection. ELISA is suitable for laboratory screening, while dot blot offers potential for field-level application.

Keywords: *Staphylococcus aureus*, Staphylococcal Enterotoxin A (SEA), polyclonal antibody, antigen-capture ELISA, dot blot, foodborne disease