### Tabel 1. Results of Biochemist Test

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indol</td>
<td>-</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
</tr>
<tr>
<td>Produksi urease</td>
<td>+</td>
</tr>
<tr>
<td>Produksi H₂S dari Triple Sugar Iron Agar (TSIA)</td>
<td>+</td>
</tr>
<tr>
<td>Citrat</td>
<td>+</td>
</tr>
<tr>
<td>Methyl Red</td>
<td>+</td>
</tr>
<tr>
<td>Voges-proskauer</td>
<td>-</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
</tr>
<tr>
<td>Oxydase</td>
<td>-</td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>-</td>
</tr>
<tr>
<td>Sucrose</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
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</table>

**Figure 2. Biochemist test (from the left) : TSIA, SIM, MR, VP, Citrat, Urea, Glucose, Lactose, Sucrose, and Mannitol**

H₂S On Triple Sugar Iron Agar (TSIA) medium, the sample was showing acid-acid, on urea, citrate positive, Voges Proskauer are negative, On Methyl Red positive, Glukose positive, catalase positive, oxidase negative and Lactose, Sucrose, manitol shows negative result. The positive culture was followed by PCR test to genetically identify the bacteria.

**Figure 3. a. Catalase test and b. Oxydase test**
Figure 4: Results of P. mirabilis with PCR

The PCR, 11 was assumed positive with PCR *P. mirabilis* (with positive control on *Proteus mirabilis* ATCC 43071)

From sensitivity test with antibiotics, those that are the most sensitive to *P. mirabilis* (in order with mm unit) are Imipenem 40 (S), Cefotaxime 34.4 (S), Choramphinicol 27.2 (S), and Ciprofloxacine 23.3 (S).

Figure 5. Inhibition Zone isolate *Proteus mirabilis*

1= Imipenem 10 μg, 2= Kloramfenikol 30 μg, 3= Cefotaxime 30 μg, 4= Ciprofloxacine 5 μg

*Proteus mirabilis* from the chicken meat in China were resistant to antibiotic Tetrasiklin (100%), sulfametaksalsol (80%), klorampenikol (66%), Nalidisic acid (66%), amphysilin (60%), streptomysin (56%), siprofloksasin (52%), kanamisin (46%), gentamicin (38%), sefriakson (36%), xefotaxime (34%), seftiofur
(22%), amoxyslin and In India antibiotic Clorampenikol not so sensitive to *Proteus mirabilis* from cattle meat (6)

Conclusion:

The presence of Pathogenic *P.mirabilis* bacteria in chichen meat is highly significant (22.9 %). The most sensitive antibiotics, Imipenem, mechanically prevent the cell membrane synthesis, while Cyprofoxacin and Cefotaxime works by blocking the acid-nucleic bacteria synthesis.

References

4. CLSI [Clinical Laboratory Standard Institute]. 2014. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. USA
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Dr Lucia Winata Muslimin

has attended the event and presented a poster at the

The 4th International One Health Congress and
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Abstract:
This study aims to accelerate zoonosis control system and secure food safety. Meat chicken breast samples, were acquired from traditional markets in Malaysia. The samples were inoculated to bacterial growth medium inside the ice-box and carried to the lab to be inoculated with Nutrient Agar, Trypsone Soy Broth (TSB), MacConkey Agar (MCA), Salmonella Shigella Agar (SSA), Muller Hinton Agar (MHA) test, Biochemical test and pathogenic test with blood Agar from the suspected Proteus mirabilis result, was followed by PCR ATCC 43071 test to genetically identify the bacteria. The result was then examined for sensitivity test with antibiotics: Ciprofloxacin, Chloramphenicol, Gentamicin, and imipenem. Among 48 samples, 13 was assumed positive with culture method and 11 was assumed positive with PCR Proteus mirabilis. The most sensitive antibiotics, Imipenem, Chloramphenicol, Cefotaxime and Ciprofloxacin.

Keywords: Antimicrobial Inhibition, Foodborne Disease, Proteus mirabilis

Introduction
Zoonosis is an infectious disease which can be transmitted from vertebrates to human. One of the important vectors of zoonotic and also pathogenic is Proteus mirabilis. Proteus mirabilis is a pathogen infection that is pathogen to human that cause the disease in human. In many developing countries, improper hygiene is not directly implemented when animals are slaughtered and meat products become contaminated. Contaminated meat may contain Proteus mirabilis which could cause diseases in humans if those food products are consumed uncooked. This research aims to accelerate zoonosis control system, secure food safety, and improve the nutritional quality.

Toxicity of Proteus mirabilis was resisted in 1946. In United States, 8 people was dead from 25 infected people and it is cause to be consumed with Proteus mirabilis. The news information in 2013 China Proteus mirabilis can be isolated from chicken meat and by products which make contamination. (14)

Materials and Methods
2.1. Sample Collection
Samples were collected aseptically in sterilized glass bottles and plastic bags with ice pack. Upon arrival in the laboratory, samples were analyzed immediately, from which were conducted the triplicate of assessment by the following methods: Polymyxin Agar Reaction (PCR) and Antibiotic Sensitivity Test.

2.2. Conventional Bacterial Culture
The samples were exposed to bacterial growth medium inside the ice-box and carried to the lab to be inoculated with (M) Nutrient Agar to measure the amount of Proteus mirabilis. Inoculate, we conduct IMVIC test, Motility, H2S and Motility test, TSI, citrate, urea, carbohydrate, and indole test. Fermentation test in glucose, Lactose, Sucrose, Mannitol and pathogenic test with blood Agar. Samples were incubated at 37°C for twenty-four hours.

The identification process was conducted in two ways: microscopically by observing the growing colony morphology, along with the hemolytic zones, and microscopically by observing the bacteria's shape using Gram staining to highlight the red color and were stained into the Blood agar medium twenty-four hours at 37°C, as well. The presence of Proteus was indicated by colonies were incubated at 37°C for 24 hours.

Polymyxin Chain Reaction (PCR)
Suspected Proteus mirabilis result was followed by PCR analysis to genetically identify the bacteria using methods protocol samples preparation PCR DNA according to previously procedure protocol PCR and with primers for Proteus mirabilis (ATCC 43071). The standardized method is forward: GAGCATCGCTGGTGCTCATTGTTGCT and reverse: CGTAATGTTAGCTTCACGTCGAGCATTG and with concentration of 0.8µl of each 5µl of DNA sample and 47µl of distilled water. (Wong, 2005) were added to the 10µl of DNA samples, 0.8µl of each primer and 2.0µl of PCR buffer containing AmpliTag Gold. Conditions thermostating were as follows: 95°C for 10 minutes; 40 cycles of 94°C for 30 seconds, 30 seconds of 50°C and 72°C for 10 minutes. Using 1.5% agarose gel containing ethidium bromide (Sigma, USA), 3µl of PCR product was analyzed by electrophoresis at 180V for 30 minutes. PCR Product length for M. genus 239 base pairs (bp) for Proteus mirabilis

Antibiotic Sensitivity Test
Antibiotic susceptibility tests were performed on all isolates to determine their antibiotic resistance profile (Vorly et al., 2006). Fresh overnight cultures were prepared and used for antibiotic sensitivity tests. An aliquot (100µl) of each overnight culture equivalent to 0.5% McFarland standard was spread plated on Mueller-Hinton agar (MHA). Sessile inoculation of the inoculum of several different antibiotic discs (13) were determined. Antibiotic discs were gently pressed onto the inoculated Mueller-Hinton agar plate to ensure contact with the culture. The plates were incubated aerobically at 37°C for 18-24 h (4). Inhibition zone diameters were measured and values calculated from the agar using the National Committee on (1979) were used to interpret the results. Bacteria isolates were then classified as resistant, intermediate resistant or susceptible to a particular antibiotic Imipenem, Chloramphenicol, and Ciprofloxacin.
Antibiotic Sensitivity pattern on zoonotic bacterial proteus mirabilis as a cause of food borne disease

Abstract:

The study aims to investigate human, animal, and food safety. Human fecal samples were acquired from five traditional markets inプレゼント州, New York. The samples were inoculated in growth medium inside the lab and further tested to identify the bacteria using standard methods. The data were statistically analyzed using a Fisher's exact test. The results showed a high level of antibiotic resistance in Proteus mirabilis, indicating a potential risk to human and animal health. Furthermore, the resistance pattern varied among different samples, highlighting the need for continued monitoring.

Introduction

Zoonotic diseases are infections that can be transferred from animals to humans. Proteus mirabilis, one of the most common gram-negative bacilli, is a zoonotic pathogen associated with foodborne diseases. The prevalence of antibiotic resistance in Proteus mirabilis poses a significant threat to public health. This study investigated the antibiotic sensitivity pattern of Proteus mirabilis isolated from human and food samples from New York.

Methods

Sample Collection

Sample collection was conducted at five traditional markets in New York City. Fecal samples were collected from individuals and food handlers, as well as from fresh produce, meat, and dairy products. The samples were stored at -20°C until further analysis.

Conventional Bacterial Culture

The samples were cultured on MacConkey agar plates and blood agar plates at 37°C. After 24 hours of incubation, colonies were observed, and the pathogenic bacteria were identified based on their morphological characteristics.

Polymerase Chain Reaction (PCR)

PCR was performed to confirm the presence of Proteus mirabilis in the samples. PCR amplification was carried out using specific primers for Proteus mirabilis. The amplified products were analyzed by agarose gel electrophoresis and confirmed by sequencing.

Results and Discussion

Our study revealed a high frequency of antibiotic resistance in Proteus mirabilis isolated from human and food samples. The resistance pattern varied among different samples, with some strains being resistant to multiple antibiotics, indicating a potential risk to public health. The findings highlight the need for continued monitoring and the implementation of effective control measures to prevent the spread of zoonotic diseases.
Antibiotic Sensitivity Pattern On Zoonotic Bacterial Proteus Mirabilis As A Cause Of Food Borne Disease

Abstract

submitted

Fellowship Application
Not Submitted

Requested Presentation Type
Oral Presentation

Paper ID 108

Paper Title
Antibiotic Sensitivity Pattern On Zoonotic Bacterial Proteus Mirabilis As A Cause Of Food Borne Disease

Theme
Food, land and water systems: Future challenges and pathways (Antibiotic Resistance: An Emerging Disease Problem?)

Keywords

Award Nomination


Biography

Authors

Author Type Submitting Author

Author Name Mrs Lucia Ratna Winata Muslimin

Presenting? Yes State N/A Country Indonesia

Affiliation/Organisation Name
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Antibiotic Sensitivity pattern on zoonotic bacterial *Proteus mirabilis* as a cause of food borne disease

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Abstract:

This study aims to accelerate zoonosis control system and secure food safety. Meat chicken breast samples were acquired from six traditional markets in Makassar, Indonesia. The samples were implanted to bacterial growth medium inside the ice-box and carried to the lab to be inoculated with Nutrient Agar, Tryptone Soya Broth (TSB), MacConkey Agar (MCA), Salmonella Shigella Agar (SSA), Muller Hinton Agar. IMVIC test, Biochemical Test and pathogenic test with blood Agar from the suspected *Proteus mirabilis* result, was followed by PCR test to genetically identify the bacteria. The result was then examined for sensitivity test with antibiotics: Ciprofloxacin, Chloramfenicol, Cefotaxime, and imipenem.

Among 48 samples. 13 was assumed positive with culture method and 11 was assumed positive with PCR *Proteus mirabilis*. The most sensitive antibiotics, Imipenem, chloramphphnicol, Cefotaxime and Ciprofloxacin

Keywords: Antimicrobial Inhibition, Food Borne Disease, *Proteus mirabilis*

1. Introduction

Zoonosis is an infectious disease which can be transmitted from vertebrae to human (11) One of the causal agent of zoonosis and also pathogenic is *Proteus mirabilis*. *Proteus mirabilis* is a pathogenic infection that is pathogenic to human that cause cystitis (9) also known as food borne diseases. In many developing countries, proper hygiene is not strictly implemented when animals are slaughtered and meat products become contaminated. Contaminated meat may contain *Proteus mirabilis* that could cause diseases in humans if these food products are consumed undercooked. This research aims to accelerate zoonosis control system, secure food safety and improve the environmental quality.

Toxicity of *Proteus mirabilis* was isolated in 1946 in United states, 9 people was dead from 19 infected people and it is cause by ham contaminated with *Proteus mirabilis* (3). The news information in 2013 in China *Proteus mirabilis* can be isolated from chichen meat and by product which make contamination. (14).

2. Materials and Methods

2.1. Samples Collection
Samples were collected aseptically in sterilised glass bottles and plastic bags with icepack. All samples were immediately transported to the Molecular Biology and Immunology Laboratory for Infectious Diseases, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia for analysis. Upon arrival in the Laboratory, samples were analysed immediately. Meat samples, from which we conducted three type of assessment by culture method Test, Polymerase Chain Reaction (PCR) and Antimicrobial Susceptibility Test. The samples from traditional markets and carried inside an ice box to the lab for future examination or testing.

2.2. Conventional Bacterial Culture

The samples were implanted to bacterial growth medium inside the ice-box and carried to the lab to be inoculated with (a) Nutrient Agar to measure the amount of bacteria. In addition, we conduct IMVIC Indole, Methyl Red, Voges Proskauer, TSIA, citrat, urea, catalase and oxidase test. Fermentation test in glucose, Lactose, Sucrose, Mannitol and pathogenic test with blood Agar samples were inoculated at 37°C for twenty four hours.

The identification process were conducted in two ways: macroscopically by observing the growing colony morphology along with the forming hemolysis zone, and microscopically by observing the bacteria's shape using Gram coloring to highlight the red coccoid, and colony were streaked into the Blood agar medium twenty-four hours at 37°C, as well. The presence P. mirabilis were indicated by colorless colony were incubated on the McConkey.

Polymerase Chain Reaction (PCR)

DNA EXTRACTION

Transfer cells (up to 1 x 10^7) to a 1.5 ml microcentrifuge tube then centrifuge for 5 minutes at 300 x g. Discard the supernatant then resuspend cells in 200 µl of PBS by pipette. Add 20 µl of Proteinase K. Add 200 µl of GSB Buffer then mix by shaking vigorously. For blood and cell samples, incubate at 60°C for 5 minutes, inverting the tube every 2 minutes. For amniotic fluid samples, incubate at 60°C for at least 20 minutes, inverting the tube every 5 minutes. During incubation, transfer required volume of Elution Buffer (200 µl/sample) to a 1.5 ml microcentrifuge tube and heat to 60°C then mix by pipetting. Incubate at 60°C for 5 minutes. Add 200 µl of absolute ethanol to the sample lysate and mix immediately by shaking vigorously for 10 seconds. If precipitate appears, break it up as much as possible with a pipette. Place a GS Column in a 2 ml Collection Tube. Transfer all of the mixture (including any insoluble precipitate) to the GS Column. Centrifuge at 14-16,000 x g for 1 minute. Following centrifugation, if the mixture did not flow through the GS Column membrane, increase the centrifuge time until it passes completely. Discard the 2 ml Collection Tube containing the flow-through then transfer the GS Column to a new 2 ml Collection Tube. NOTE: It is important that the lysate and ethanol are mixed thoroughly to yield a homogeneous solution. Add 400 µl of W1 Buffer to the GS Column. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the GS Column back in the 2 ml Collection Tube. Add 600 µl of Wash Buffer (make sure absolute ethanol was added) to the GS Column. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the GS Column back in the 2 ml Collection Tube. Centrifuge again for 3 minutes at 14-16,000 xg to dry the column matrix. Transfer the dried GS Column to a clean 1.5 ml microcentrifuge tube. Add 100 µl of preheated Elution Buffer, TE Buffer or water into the CENTER of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. Centrifuge at 14-15,000 x g for 30 seconds to elute purified DNA.

PCR PROCEDURE

Reaction was done in 50 µl total volume, containing 5 µl of 10X PCR buffer (Qiagen), 1 µl (5 mM) of dNTP Mix (Invitrogen, cat no.18427-088), 10 pmol of each UreC forward and UreC-reverse primer, 0.5 µl
(5U/μl) of Hotstart Taq DNA polymerase (Qiagen, Hilden, Germany, cat no. 203205), about 5 μL of DNA samples, and 2 μL of 25mM MgCl₂ (Qiagen). The PCRs were carried out using GeneAmp 9700 PCR system (Applied Biosystems, Singapore) starting with 15 min incubation at 95°C, followed by 40 cycles of 1 min denaturation at 94°C, 30 min annealing at 63°C, and 1 min extension at 72°C. The reactions were ended by incubation for 7 min at 72°C. PCR products can be used immediately or stored at -20°C for later use.

**AGAROSE GEL ELECTROPHORESIS**

Five μL of each PCR product plus 2 μL of 5X gel loading buffer (Qiagen) was loaded into the wells of 2% agarose gel (Bio-Rad), containing 0.2 μg/mL of ethidium bromide (Sigma-Aldrich) as a gel staining. As size markers, 2μg/μL of 100 bp DNA ladders (Invitrogen) was loaded in the first well of the gel. The DNA fragments were then visualized on GelDoc gel documentation system (Bio-Rad).

**DATA ANALYSIS**

PCR amplification results will be interpreted as positive or negative of *proteus mirabilis* based on banding pattern (bands) that appear after a 2% agarose gel electrophoresis.

**Antibiotic Susceptibility Test**

Antibiotic susceptibility tests were performed on all isolates to determine their antibiotic-resistance profiles (Kirby *et al.* 1966). Fresh overnight cultures were prepared and used for antibiotic sensitivity tests. An aliquot (100μL) of each isolate suspension equivalent to a 0.5% McFarland Standard was spread plated on Mueller Hinton agar (Oxoid, UK). Susceptibilities of the isolates to a panel of several different antibiotic discs (13) were determined. Antibiotic discs were gently pressed onto the inoculated Mueller Hinton agar to ensure intimate contact with the surface and the plates were incubated aerobically at 37°C for 18 h – 24 h (4). Inhibition zone diameters were measured and values obtained from the National Committee on (4) were used to interpret the results obtained. Bacteria isolates were then classified as resistant, intermediate resistant or susceptible to a particular antibiotic Imipenem, Chloramphenicol, and Ciprofloxacin. The sensitivity indication was specified by the forming translucent zone around the paper disc where its diameter was measured based on the disc product standard.

**Results and discussion**

Among 48 samples, 13 was assumed positive with culture method,

![Image of bacteria cultures](image.png)

**Figure 1. Culture of P.mirabilis in Mac Conkey**

As can be seen from figure 1, in McConkey Agar which resulted in colorless colony.