PREVALENCE OF SALMONELLA, ESCHERICHIA COLI O157:H7, LISTERIA MONOCYTOGENES AND STAPHYLOCOCCUS AUREUS IN RAW BEEF IN KELANTAN

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Prevalence of *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus* in Raw Beef in Kelantan

by

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A thesis submitted in fulfillment of the requirements for the degree of Master of Science

Faculty of Veterinary Medicine
UNIVERSITI MALAYSIA KELANTAN

2014
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<tr>
<td>AMC</td>
<td>Amoxycillin-clavulanate</td>
</tr>
<tr>
<td>AMP</td>
<td>Ampicillin</td>
</tr>
<tr>
<td>(a_w)</td>
<td>Water activity</td>
</tr>
<tr>
<td>BAM</td>
<td>Bacteriological Analytical Manual</td>
</tr>
<tr>
<td>BCIG</td>
<td>5-bromo-4-chloro-3-indolyl- (b)-D-glucuronide</td>
</tr>
<tr>
<td>C</td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td>cfu</td>
<td>Colony forming unit</td>
</tr>
<tr>
<td>CIP</td>
<td>Ciprofloxacin</td>
</tr>
<tr>
<td>CLSI (formally NCCLS)</td>
<td>Clinical and Laboratory Standards Institute</td>
</tr>
<tr>
<td>CN</td>
<td>Gentamycin</td>
</tr>
<tr>
<td>CRO</td>
<td>Cefotaxime</td>
</tr>
<tr>
<td>CT-SMAC</td>
<td>Cefixime and tellurite sorbitol MacConkey agar</td>
</tr>
<tr>
<td>DA</td>
<td>Clindamycin</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>E</td>
<td>Erythromycin</td>
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<td>FDA</td>
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<tr>
<td>FOX</td>
<td>Cefoxitin</td>
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<tr>
<td>H(_2)O</td>
<td>Water</td>
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<tr>
<td>HUS</td>
<td>Hemolytic uremic syndrome</td>
</tr>
<tr>
<td>K</td>
<td>Kanamycin</td>
</tr>
<tr>
<td>MAR</td>
<td>Multiple Antibiotic Resistance</td>
</tr>
<tr>
<td>MDR</td>
<td>Multi Drug Resistance</td>
</tr>
<tr>
<td>mw</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>mpn</td>
<td>Most probable number</td>
</tr>
<tr>
<td>NA</td>
<td>Nalidixic acid</td>
</tr>
<tr>
<td>NCBI</td>
<td>National Centre for Biotechnology Information</td>
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<tr>
<td>NCCLS</td>
<td>National Committee for Clinical Laboratory Standards</td>
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OX  Oxacillin
P  Penicillin G
PCR  Polymerase chain reaction
PFGE  Pulsed field gel electrophoresis
pH  Measure of the acidity of a solution in terms of the activity of hydrogen (H+)
RD  Rifampin
RNA  Ribonucleic acid
S  Streptomycin
spp.  Species
STEC  Shiga toxin-producing *Escherichia coli*
S3  Sulphonamides
SXT  Trimethoprim-sulfamethoxazole
TE  Tetracycline
TPC  Total plate count
UK  United Kingdom
US  United States
vs  Versus
W5  Trimethoprim
XLT-4  Xylose Lactose Tergitol™ 4
LIST OF SYMBOLS

%  Percent
°C  Degree Celsius
°  Degree
β  Beta
®  Copyright
™  Trademark
g  Gram
h  Hour
L  Liter
ml  Milliliter
mg  Milligram
mm  Millimeter
min  Minute
n  Sample size
ng  Nanogram
r.p.m.  Revolutions per minute
sec  Second
U  Unit
V  Volt
Kelaziman Salmonella, Escherichia coli O157:H7, Listeria monocytogenes dan Staphylococcus aureus dalam Daging Lembu Mentah di Kelantan

ABSTRAK

Penyakit bawaan makanan disebabkan oleh Salmonella, Escherichia coli O157: H7, Listeria monocytogenes dan Staphylococcus aureus telah dikaitkan dengan pengambilan daging lembu kurang masak dalam makanan telah dilaporkan di seluruh dunia. Di Kelantan, penyembelihan lembu di rumah sembelih persendirian biasa diamalkan kerana rumah sembelih kerajaan berdaftar adalah terhad. Objektif kajian ini adalah untuk; i) Menentukan kualiti bakteriologi pada daging lembu mentah, ii) Menentukan kelaziman bakteria bawaan makanan (Salmonella, E. coli O157: H7, L. monocytogenes dan S. aureus) dalam daging lembu mentah dari kedai, rumah sembelih persendirian dan rumah sembelih kerajaan, iii) Mengenalpasti corak daya tahan bakteria terhadap antibiotik. Sampel daging lembu mentah dikumpulkan dari pelbagai premis berdasarkan kepada kiraan bakteria plat (TPC), kiraan koliform, analisis Salmonella, E. coli O157: H7, L. monocytogenes dan S. aureus. Pencilan bakteria seterusnya melalui ujian antibiotik dengan menggunakan kaedah Disc diffusion (Kirby-Bauer). Sebelas daripada 23 (47.8%) daging lembu mentah dari kedai tepi jalan mempunyai TPC melebihi had yang dibenarkan (<1.0 x 10^6 cfu/g). Manakala tiada daripada 19 (0%) daging lembu dari rumah sembelih kerajaan. Lapan belas daripada 23 (78.3%) sampel daging lembu dari kedai tepi jalan mempunyai kiraan koliform melebihi had yang dibenarkan (1000 mpn/g). Manakala satu daripada 19 (5.3%) daging lembu dari rumah sembelih kerajaan mempunyai kiraan koliform melebihi had yang dibenarkan. Salmonella spp. dikesan 44.0% (11/25), 16.7% (2/12), 5.3% (1/19) dalam daging lembu mentah dari kedai tepi jalan, rumah sembelih persendirian dan rumah sembelih kerajaan masing-masing. Daripada 14 Salmonella dipencil, yang paling tinggi ialah S. Mbandaka (5/14), S. Weltevreden (5/14) diikuti S. Albany (4/14).Tiada E. coli O157: H7 dan L. monocytogenes dikesan dalam 25 sampel daging lembu dari kedai tepi jalan, 12 rumah sembelih persendirian dan 19 rumah sembelih kerajaan. S. aureus dikesan 32% (8/25), 33.3% (4/12) dalam daging lembu dari kedai tepi jalan dan rumah sembelih persendirian masing-masing. Daripada 14 pencilan Salmonella, 21% (3/14) adalah sensitif kepada semua antibiotik yang diuji, 36% (5/14) tahan kepada satu kelas antibiotik, 14% (2/14) tahan kepada 2 kelas antibiotik dan 29% (4/14) adalah MDR. Analisis MAR indeks menunjukkan 2 pencilan S. Albany mempunyai nilai MAR indeks tertinggi (1.00) diikuti 2 pencilan S. Albany yang lain (0.69). Kesimpulannya, kajian ini menunjukkan bahawa pengguna di Kelantan mungkin terdedah kepada kualiti bakteriologi dan tahap keselamatan daging lembu mentah yang rendah dari kedai tepi jalan dan mengandungi Salmonella yang tahan rintangan antibiotik. Keputusan yang dibentangkan dalam kajian ini dapat membantu agensi yang berkaitan untuk dijadikan garis panduan bagi meningkatkan kualiti bakteriologi dan keselamatan daging lembu mentah rancit di Kelantan. Ia juga boleh dijadikan sebagai asas perbandingan untuk kajian pada masa hadapan.
Prevalence of *Salmonella, Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus* in Raw Beef in Kelantan

**ABSTRACT**

Foodborne illness caused by *Salmonella, Escherichia coli* O157:H7, *Listeria monocytogenes* and *Staphylococcus aureus* associated with the consumption of undercooked beef or foods containing beef have been reported worldwide. In Kelantan, slaughtering of cattle in backyard slaughterhouse is common practice because there are limited numbers of government abattoir. The objectives of this study were to; i) Determine the bacteriological quality of raw beef, ii) Determine the prevalence of foodborne bacteria (*Salmonella, E. coli* O157:H7, *L. monocytogenes* and *S. aureus*) in raw beef from retail stores, backyard slaughterhouses and government abattoirs, iii) Determine the antibiotic resistant pattern of the bacteria. Raw beef samples were collected from different types of premises and subjected to TPC, coliform count and analysis for *Salmonella, E. coli* O157:H7, *L. monocytogenes* and *S. aureus*. The bacteria were then subjected to antibiotic susceptibility test by using the standard disc diffusion method (Kirby-Bauer). Eleven of 23 (47.8%) raw beef from retail stores had TPC above the acceptable limit (<1.0 x 10^6 cfu/g). Whereas none of 19 (0%) raw beef from government abattoir had TPC above the acceptable limit. Eighteen of 23 (78.3%) beef samples from retail store had coliform count above the acceptable limit (1000 mpn/g). Whereas one of 19 (5.3%) raw beef from government abattoir had coliform count above the acceptable limit. *Salmonella* spp. was detected in 44.0% (11/25), 16.7% (2/12), 5.3% (1/19) of raw beef from retail stores, backyard slaughterhouses and government abattoir respectively. Of 14 *Salmonella* isolated, the most common were *S. Mbandaka* (5/14), *S. Weltevreden* (5/14) followed by *S. Albany* (4/14). No *E.coli* O157:H7 and *L. monocytogenes* were detected in raw beef from all different types of premises. *S. aureus* was detected in 32% (8/25), 33.3% (4/12) of raw beef from retail stores and backyard slaughterhouses respectively. Of 14 isolates of *Salmonella*, 21% (3/14) were susceptible to all antibiotics tested, 36% (5/14) presented a single type of resistance, 14% (2/14) were resistance to 2 classes of antibiotics and 29% (4/14) were MDR. The MAR index analysis indicated that 2 isolates of *S. Albany* had highest MAR index value (1.00) followed by another 2 isolates of *S. Albany* (0.69). In conclusion the results of this study indicate that consumers in Kelantan may access to raw beef from retail stores that are of low bacteriological quality and safety level and contain MDR *Salmonella*. The results presented in this study can help relevant agencies to establish guidelines to improve the bacteriological quality and safety of retail raw beef in Kelantan. It can also serve as a baseline information for future studies.