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Antibiogram of Escherichia coli isolated from semi-closed system farmed Asian clam (*Corbicula fluminea*)

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Abstract

In the present study, antibiogram of *Escherichia coli* isolated from farmed Asian clam, *Corbicula fluminea* was characterised. Asian clam or locally known as 'etak' is processed to became smoked clam and consumed as snack by Kelantanese. However, there are many diarrhoea cases after consuming smoked clam. Furthermore, there are also insufficient information about the *E.coli* level in Asian clam farm and effective antibiotic in controlling the bacteria in the litereature. Hence, this study was carried out to provide information of antibiogram of *E.coli* to be reference in the future. Eosin Methylene Blue agar (EMB) was used to isolate *E. coli*. A total of 100 isolated bacteria were subjected to antibiotic sensitivity test using disk diffusion method. A total of 18 types of antibiotics namely novobiocin (30µg/disk), fosfomycin (50 µg/disk), tetracycline (30µg/disk), lincomycin (15µg/disk), flumequine (30µg/disk), sulphamethoxazole (25 µg/disk), amoxycillin (25 µg/disk), chloramphenicol (30 µg/disk), oleandomycin (15 µg/disk), spiramycin (100 µg/disk), ampicillin (10 µg/disk), oxytetracycline (30 µg/disk), doxycycline (30 µg/disk), nalidixic acid (30 µg/disk), florfenicol (30 µg/disk), erythromycin (15 µg/disk), kanamycin (30 µg/disk) and oxolinic acid (2 µg/disk). The findings of the present study showed total plate count of *E. coli*was 6.45 x 10^3 colony forming unit (CFU/100g) of sampled Asian clam. Hence, the clam is needed to be under cleansing treatment before can consider safe for human consumption. Antibiotic results showed 51 % was recorded as antibiotic resistance case, 44 % antibiotic sensitive case and 5 % as antibiotic intermediary sensitive case. None of the tested antibiotics was successfully inhibited the growth of the present bacterial isolates indicating more antibiotics are needed to be screen in the future study to find out the most effective antibiotic in controlling isolated *E. coli*.

1. INTRODUCTION

Asian clam (*Corbicula fluminea*) is a small size clam with round to triangle shape that inhabit in freshwater aquatic ecosystem. It is one of the native clam species from *Corbiculidae* family originating from Southern and Eastern Asia. In 1987, the first record of *C. fluminea* to be consumed as food was reported after it is being introduced by Chinese immigrant in Japan (Magara *et* al., 2001) and starting from that, the clam has become one of the food source for Asian people (Aweng et al., 2017). In Malaysia especially Kelantanese processed Asian clam to become smoked Asian clam and consumed as snack during past time. However, there are official and non-official reports claimed that many people suffered diarrhoea after consumed contaminated smoked Asian clam. One of the bacteria that responsible to the disease is *E. coli* where the bacterial is normally found in contaminated environment or food in humans has contributed to 47.3 food-borne disease incidence rate per 100,000 population in Malaysia (Department of Statistical Malaysia, 2016). Entero toxigenic *E. coli*, entero pathogenic *E. coli*, enter invasive *E. coli*, ente rhemorrhagic *E. coli,* shiga toxin-producing *E. coli*, entero aggregative *E. coli*. and diffusely adherent *E. coli*. are included among the six categories of latest discovered of diarrheagenic *E. coli.* (Costa, 2013). Commonly antibiotics that used for *E. coli* infection treatment are nitrofurantoin, ciprofloxacin and ampicillin (Alanazi et al., 2018). Till present, there is limited information on food poisoning after the consumption of Asian clam due to *E. coli* infection and effective antibiotic to control it. Therefore, this study was conducted to monitor the safety level of Asian clam consumption and identify effective antibiotic in controlling *E. coli* infection.

2. MATERIALS AND METHODS

2.1 Sampling

A total of 80 Asian clam *Corbicula fluminea* (size ranged from 12.0-17.5mm) were randomly collected from Asian clam farm located in Universiti Malaysia Kelantan Jeli campus. The water parameter i.e. temperature, dissolve oxygen, pH and salinity were recorded by using multiparameter (YSI, USA) and was recorded as 27ºC,

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7.06 mg/L, 7.88 and 0.03, respectively. 10 g of flesh of Asian clams were collected in sterile zipper plastic bag and homogenized manually with hand. Then, 1 mL of the sample was serially diluted for ten times in tubes filled with 9 ml of 0.85% physiological saline.

2.2 Bacteria isolation and identification

100µl from each dilution of the sample was spread on Eosin Methylene Blue (EMB) agar (Himedia, India). The plates were incubated for 24h to 48 h at 27°C in incubator (Jeiotech, Korea). Total plate count was carried out for only to the plate has bacterial colony ranging 30 to 200.The bacteria were further used for identification by using commercial identification kits (BBL, USA).

2.3 Antibiotic sensitivity test

Isolated bacteria from samples were inoculated in Trytone Soy Broth (TSB) (Himedia, India) and incubated at 27°C for 24 to 48h in incubator (Jeiotech, Korea). Next, soaked sterile cotton bud in each bijou bottle and swabbed on Tryptone Soy Agar (TSA) (Himedia, India). Selected antibiotics for this test were novobiocin (30µg) NV30, penicillin (1.5 µg) P15, tetracycline (30µg) TE30, lincomycin (15µg) MY15, flumequine (30µg) UB30, sulphamethoxazole (25 µg) RL25, amoxycillin (25 µg) AML25, chloramphenicol (30 µg) C30, oleandomycin (15 µg) OL15, spiramycin (100 µg) SP100, ampicillin (10 µg) AMP10, oxytetracycline (30 µg) OT30, doxycycline (30 µg) DO30, nalidixic acid (30 µg) NA30, florfenicol (30 µg) FFC30, erythromycin (15 µg) E15, kanamycin (30 µg) K30, oxolinic acid (2 µg) OA2. The plates were incubated at 27°C for 24 to 48h in incubator (Jeiotech, Korea). The result was categorized into sensitive (S), intermediate sensitive (I) and resistance (R) based on the zone of inhibition (mm) according to Clinical Laboratory and Standards Institute (CLSI, 2017).

2.4 Multiple antibiotic resistant (MAR) index

MAR index more than 0.2 showed that Asian clam is highly exposed to antibiotic whereas MAR index less than 0.2 was less exposed to antibiotic. MAR index was calculated by using the following formula (Lee and Wendy, 2012).

$$MAR\ index = \frac{Total\ antibiotic\ resistance\ cases}{(Total\ number\ of\ antibiotics\ use\ x\ Total\ bacteria\ isolates)}$$

3. RESULTS AND DISCUSSION

The result of present study showed total plate count of *E. coli* was 6.45 x 10^3 colony forming unit (CFU/100g) of sampled Asian clam. The bacteria were identified as *E. coli*. The isolated bacteria was appeared green metallic sheen on EMB medium. Antibiotic test results of the present study showed that total antibiotic resistance case was 51%. This was followed by total antibiotic sensitive case (44%) and total antibiotic intermediary sensitive case (5%). None of the tested antibiotics were successfully inhibited all of the bacterial isolates. Furthermore, all bacterial isolates were resistant to lincomycin and sulphamethoxazole. Almost half or more than half of the present bacterial isolates were resistant to oleandomycin, spiramycin, ampicillin, erythromycin, kanamycin and fosfomycin. Flumequine performed the highest antibiotic case (88%) against bacterial isolates of the present study. This was followed by doxycycline (80%). Oxytetracycline, chloramphenicol and florofenicol shared similar antibiotic sensitive case result (70%). Almost half or more than half of the present bacterial isolates were sensitive to nalidixic acid, oxolinic acid, novobiocin, tetracycline and amoxicillin. Based on the antibiotic test result, MAR index of the present study was calculated as 0.48.

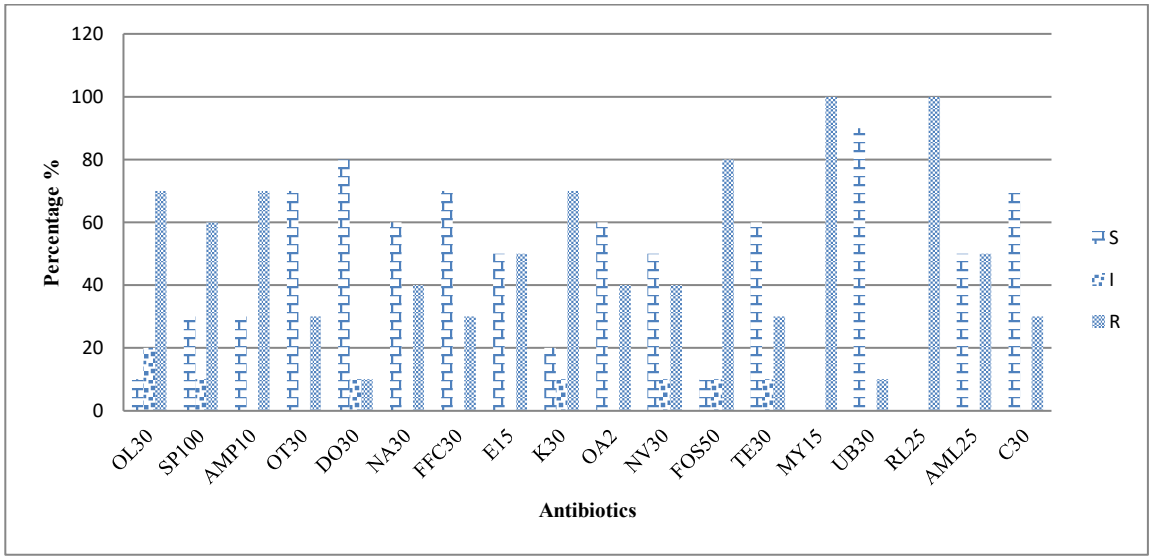

Figure 1: Antibiotic sensitivity test of *Escherichia coli* isolated from Asian clam
S – sensitive, I – intermediate sensitive, R - resistance

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This study was carried out to monitor the safety level of farmed Asian clam for human consumption. In the present study, bacteriology analysis showed that the present farmed Asian clam was fall in to Class C ground based on the European Union (EU) classification of bivalve/mollusk harvesting areas. This meaning that sampled Asian clam need to be purified any fecal bacterial content in cleansing tank with minimum 2 months' time before the stock can be sold (Laing, 2009).Generally, all bivalves are pedal and filter feeder where accumulate small particle of organic matters including bacteria (Kramer et al., 2016). Hence the likelihood to consume contaminated bivalve is high. The findings of the present study suggest that in order to minimize risk food poisoning case due to the clam consumption. Furthermore there is must to make sure the clam is well cook before use.

Antibiotic resistance case showed the highest percentage in the present study. None of the tested antibiotics was able to inhibit all the present bacterial isolates whereas high value of MAR index indicating the sampled Asian clams were highly exposed to the tested antibiotics. Flumequine and doxycycline are among the best antibiotics where can inhibit the growth of more than 80% of the present bacterial isolates. These antibiotics were reported widely used in poultry industry in *E. coli* treatment (Akbar et al., 2009). The incidence of antibiotic resistance case among bacteria withdraws a setback in bacterial diseases treatment. Hence scientist has to continue to find new generation of antibiotic to overcome the problem. Antibiotic resistance gene developed in the bacteria is recognized as the culprit of the incidence (Svara and Rankin, 2011). This gene is carried in the plasmid of the bacteria and can transfer the gene information to other bacteria. New antibiotic resistance gene will be continued developed if the bacteria exposed to the antibiotic in which may be due to misuse or overuse the antibiotic during treatment. In spite of the facts, there is a suggestion to use antibiotic alternately to avoid continuous exposure of certain antibiotic to bacteria community.

Instead of depend on antibiotics in the treatment of bacterial infections, plant extracts were found can be alternative antibacterial agent to the commercial antibiotics. Many studies have been done to evaluate the potential of plant extracts inhibiting the growth of bacteria. For instance, *Allium sativum* was found can inhibit the growth of *Edwardsiella tarda* (Lee and Najiah, 2008), *Michelia champaca* seed and flower extracts possess antimicrobial activity against various bacteria species (Lee et al., 2011) and Lee and Wendy (2011) revealed that *Andrographis paniculata* leaf can be used as antimicrobial agent for many species of bacteria. Other studies like Lee et al. (2011) and Lee and Wendy (2013) claimed that *Ficus deltoidea* leaf and *Cymbopogon nardus* essential oil, respectively, can inhibit the growth of various bacteria. Other than plant extracts, graphene oxide was found can be used as antimicrobial agent (Lee et al., 2014). However, all the mentioned studies are preliminary findings where need to further explore before they may come to commercial sense.

4. CONCLUSION

Based on the present study findings, we may conclude that the farmed Asian clams need to be fully cook before use in order to avoid food poisoning to happen. Furthermore, the Asian clam should undergo cleansing process to get rid or minimize bacteria coliform that colonized in the clam.

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