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ASSESSMENT OF ANTIMICROBIAL RESISTANCE OF *Salmonella* spp. ISOLATED FROM FARMED ASIAN CLAM *Corbicula fluminea*

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ABSTRACT

In the present study, *Salmonella* spp. was successfully isolated from Asian clam *Corbicula fluminea* by using Xylose Lysine Deoxycholate (XLD) selective agar. A total of 200 bacterial colonies from live Asian clams were isolated and subjected to antimicrobial sensitivity test by using disc diffusion method. A total of 18 antibiotics was applied in the present study, namely oxolinic acid, nalidixic acid, erythromycin, tetracycline, doxycycline, oleandomycin, oxytetracycline, spiramycin, ampicillin, kanamycin, fosfomycin, florfenicol, lincomycin, novobiocin, chloramphenicol, amoxycillin, flumequine and sulphamethoxazole. The findings of the present study showed that total antibiotic sensitive case for *Salmonella* spp. isolated from *C. fluminea* sample was 50% or 1800 cases. This was followed by antibiotic resistance case 45% or 1620 cases and intermediary antibiotic sensitive case (5% or 180 cases). Based on the results of the present study, tetracycline, doxycycline, oxytetracycline and flumequine showed the highest inhibition of isolated *Salmonella* spp. The multiple antibiotic resistance (MAR) index was 0.36, indicating the sampled clams were highly exposed to the tested antibiotics.

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INTRODUCTION

Asian clam *Corbicula fluminea* is a freshwater bivalve mollusc species from the family Corbiculidae, native to Southeast Asia, Africa and the Mediterranean. *C. fluminea* can widely be found in Asia and it is consumed as food and human health supplement due to its nutrition profile. This species is a filter feeder and accordingly could accumulate toxic substances or different microorganism in high concentration in case they are present in aquatic environment (Menninger, 2013). In spite of the fact, *Salmonella* was found responsible for food-associated illnesses around the world with hundreds of outbreaks occurring in developing countries every year (Martinez-Urtaza et al., 2003; Martinez-Urtaza et al., 2004; Zhao et al., 2006). Incidences of *Salmonella* spp. in seafood reported worldwide were associated with outbreaks of fever, nausea, vomiting and diarrhoea. These contaminations of seafood caused by *Salmonella* spp. is of great concern to public health in the investigated area, as there was a total of 604,000 deaths reported from diarrheal disease in South East Asia in 2002 (Hamdan et al., 2008). Currently, information on *Salmonella* spp. accumulation in *C. fluminea* is still scarce and this study was conducted to obtain information on the *Salmonella* spp. abundance in Asian clam with emphasis on its antimicrobial resistance in the investigated geographical area. These findings will allow better understanding of antimicrobial substances application in the treatment of *Salmonella* infection diseases in humans, as well as they will provide the information on the quality of bivalve farming/harvesting areas in this region.

MATERIALS AND METHODS

A total of 80 Asian clams *Corbicula fluminea* (weight: 15.29±0.31 g; length: 15.46±0.11 mm) were randomly sampled from the farm located at University Malaysia Kelantan Jeli Campus. Water from the farm was sampled and water parameters, i.e. temperature, dissolved oxygen, pH and salinity, were measured by using multiparameter (YSI, USA) and recorded as 27.0°C, 7.06 mg/L,7.88 and 0.03ppt, respectively. The flesh of *C. fluminea* was aseptically removed and homogenised manually (Asai et al., 2008). A ten-fold serial dilution is prepared by using 1 ml of homogenised flesh with addition of 9 ml 0.85% physiological saline (Lee et al., 2010). The analyses were performed in triplicate.

For bacteria isolation and identification, 100 µl of ten-fold serial diluted samples were spread onto Xylose Lysine Deoxycholate (XLD) plates (Oxoid, England). All the inoculated plates were incubated at 27°C for 24 h in an incubator (Jeiotech, Korea) (Lee et al., 2010). Bacteria colonies were counted and expressed as colony forming units (CFU/ml) for both samples. The formula is as followes (Chouhan, 2015):

$$\textit{Number of CFU/ml} = \frac{\textit{Number of colonies counted} \times \textit{Dilution factor}}{\textit{Volume of sample taken}}$$

The bacteria colonies which grew on the XLD plates were further selected for identification by using commercial identification kit (BBL, USA).

Antibiotic sensitivity test. The identified bacterial isolates were cultured in Tryptone Soy Broth (TSB) (Himedia, India) and incubated for 24 h at 27°C in an incubator (Jeiotech, Korea). The bacteria solutions were then swabbed on Tryptone Soy Agar (TSA) (Himedia, India) using disc diffusion method. Tested antimicrobial substances were: doxycycline (30 µg) DO 30, lincomycin (15 µg) MY 15, oxolinic acid (2 µg) OA 2, nalidixic acid (30 µg) NA 30, flumequine (30 µg) UB 30, florfenicol (30 µg) FFC 30, oxytetracycline (30 µg) OT 30, sulphamethoxazole (25 µg) RL 25, ampicillin (10 µg) AMP 10, oleandomycin (15 µg) OL 15, chloramphenicol (30 µg) C 30, erythromycin (15 µg) E 15, spiramycin (100 µg) SP 100, fosfomycin (50µg) FOS 50, tetracycline (30 µg) TE 30, amoxycillin (25 µg) AML 25, kanamycin (30 µg) K 30 and novobiocin (30 µg) NV 30 (Oxoid, England). Interpretations of the results were done in accordance with standard measurement of inhibitory zones in millimetres (mm) as sensitive (S), intermediary sensitive (I) and resistant (R).

Multiple antibiotic resistance index. Multiple antibiotic resistance (MAR) index of the bacteria colonies were calculated based on the formula (Lee et al., 2010):

$$\text{MAR index} = X / (Y \times Z)$$

X = total of antibiotic resistance cases
Y = total of antibiotics used in this study
Z = total bacteria isolates.

MAR index value that equals to or is less than 0.2 means that tested antibiotics were seldom or never used for the treatment of the samples. Meanwhile, MAR index value higher than 0.2 indicate the tested samples were highly exposed to tested antibiotics (Lee et al., 2010).

RESULTS

A total of 200 bacterial isolates of *Salmonella* spp. was successfully isolated and identified from 80 live Asian clams. The total plate count of bacteria from *C. fluminea* tissue sample was 7.85×10^7 colony farming unit (CFU)/ml. In the present study, total antibiotic sensitive case for *Salmonella* spp. isolated from *C. fluminea* sample was 50% or 1800 cases. This was followed by antibiotic resistance case 45% or 1620 cases and intermediary antibiotic sensitive case (5% or 180 cases) (Fig 1). Tetracycline, doxycycline, oxytetracycline and flumequine were found to effectively control all present bacterial isolates. Whereas, oleandomycin, sulphamethoxazole and lincomycin failed to control any present bacterial isolates. Intermediary sensitivity was reported only for nalidixic acid (10%), spiramycin (40%) and kanamycin (40%). Most (90%) of the present bacterial isolates were sensitive to oxolinic acid,

nalidixic acid, chloramphenicol and florfenicol, whereas more than 60% of the present bacterial isolates were sensitive to novobiocin, erythromycin, ampicillin and fosfomycin. Recorded MAR index was 0.36.

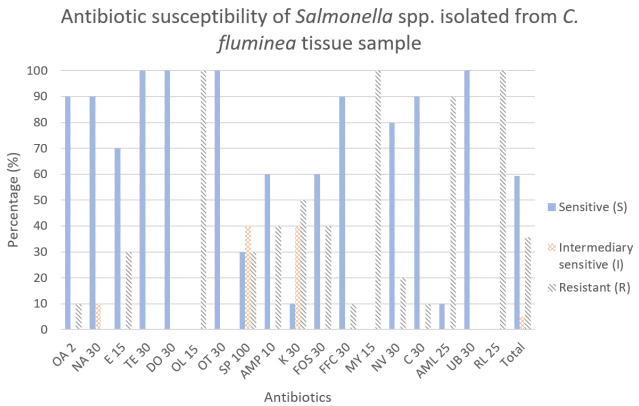

Fig 1. Antibiotic susceptibility of *Salmonella* spp. isolated from tissue sample of Asian clam *Corbicula fluminea*

R – resistant, I – intermediary sensitive, S – sensitive

DISCUSSION

The prevalence of *Salmonella* spp., human pathogen bacteria of fecal contamination origin, in seafood such as mussels (*Mytilusgallo provincialis*) (Mannas et al., 2014), oysters (Martinez-Urtaza et al., 2003), fish parts, raw crustacean (Heinitz et al., 2000), short-necked clam (*Orbicularia orbiculata*) (Hamdan et al., 2008) and Asian seabass (*Lates calcarifer*) (Lee et al., 2010) poses risk to consumers. This bacteria is reported as one of the main etiological agents of human gastroenteritis (Costa et al., 2012). According to Heinitz et al. (2000), *Salmonella* spp. incidence from fish or shellfish that led to outbreak was found in 8 of 160 outbreaks. However, far too little attention has been paid to the incidence of *Salmonella* in seafood from Malaysia. Only one study on the presence of Salmonella spp. from freshwater Asian seabass (*Lates calcarifer*) (Lee et al., 2010) could be found. In this study, a high population of *Salmonella* spp. was found in sampled *C. fluminea* tissue. This may be due to bacteria uptake from their filter feeding activity. The high bacteria count found in *C. fluminea* tissue sample indicated that raw or undercooked *C. fluminea* should be avoided as it will pose health risk to consumers (Hamdan et al., 2008). However, further study should be conducted to find out factors that lead to a high concentration of *Salmonella* spp. *C. fluminea* flesh.

In the present study, only tetracycline, doxycycline, oxytetracycline and flumequine were found to be able to control all isolated *Salmonella* spp. effectively. Thus, the findings suggested that these antibiotics should be used for the treatment of *Salmonella* spp. infection diseases in case that they are caused by consumption of *C. fluminea*. Tetracycline, doxycycline and oxytetracycline are under the same group of antibiotics. Hence, we may conclude that *Salmonella* spp. was susceptible to this group of antibiotics. Doxycycline is a tetracycline antibiotic. It is commonly used in bacterial infection in humans, whereas tetracycline and oxytetracycline are antibiotics commonly used in aquaculture. These antibiotics have been banned in shrimp farming due to misuses and overuses in Malaysia (Lee & Wendy, 2012). Flumequine is a common antibiotic widely used in aquaculture (Rogstad et al., 1993), cattle (Mevius et al., 1990) and poultry (Mirzaie et al., 2010). This antibiotic can degrade in fish pond by the exposure of fish pond sediment to sunlight (Lai & Lin, 2009). The effectiveness of flumequine in controlling various bacteria and the fact that it is easily degraded in the environment are the reasons for its frequent use in bacteria disease treatment.

High MAR index is indicating that *Salmonella* spp. accumulated in the sampled clams by their filtration activities (or filter feeding) was highly exposed to the tested antibiotics. Many studies have similar findings in the literature. For example, Lee et al. (2009) reported that bacteria from American bullfrog (*Rana catesebeina*) exhibited high MAR. Lee & Wendy (2011) shared similar findings which revealed that this bacteria isolated from wild Asian seabass performed high MAR index. MAR index presents a preliminary tool which uses bacteria as bioindicator in order to detect the level exposure of animals or environment to the antibiotic residues. However, further studies need to be carried out for example analysis of antibiotic residues in animal tissues by using atomic absorption spectroscopy (AAS) for confirmation before reaching a conclusion.

CONCLUSIONS

High accumulation of *Salmonella* spp. was found in the samples of *C. fluminea* flesh from Malaysia. Antimicrobial sensitivity tests showed that tetracycline, doxycycline, oxytetracycline and flumequine are the best antibiotics in controlling Salmonella infections in this area.

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SAŽETAK

PROCJENA ANTIMIKROBNE OTPORNOSTI *Salmonella* spp. IZOLIRANE IZ AZIJSKOG ŠKOLJKAŠA *Corbicula fluminea* IZ UZGOJA

U ovom istraživanju, *Salmonella* spp. je uspješno izolirana iz azijskog školjkaša *Corbicula fluminea* primjenom Ksiloza Lizin Deoksiolata (XLD) selektivnog agara. Ukupno je

izolirano 200 bakterijskih kolonija iz živih azijskih školjkaša i podvrgnuto ispitivanju osjetljivosti na antimikrobne tvari primjenom disk difuzije. U istraživanju je primijenjeno ukupno 18 antibiotika, i to: oksolinska kiselina, nalidiksična kiselina, eritromicin, tetraciklin, doksiciklin, oleandomicin, oksitetraciklin, spiramicin, ampicillin, kanamicin, fosfomicin, florfenikol, linkomicin, novobiocin, kloramfenikol, amoksicilin, flumekvin i sulfametoksazol. Rezultati ove studije ukazali su da je ukupni uzorak osjetljivih na antibiotike za *Salmonella* spp. izolirane iz uzorka *C. fluminea* bilo 50% ili 1800 slučajeva. Potom su uslijedili slučajevi rezistencije na antibiotike od 45% ili 1620 slučajeva i posredno osjetljivih na antibiotike (5% ili 180 slučajeva). Na temelju rezultata, tetraciklin, doksiciklin, oksitetraciklin i flumekvin pokazali su najveću inhibiciju izolirane *Salmonella* spp. Indeks višestruke rezistencije na antibiotike (MAR) bio je 0,36, što ukazuje da su uzorci školjkaša bili visoko izloženi testiranim antibioticima.

Ključne riječi: Azijski školjkaš, *Corbicula fluminea*, *Salmonella* spp., antibiogram

REFERENCES

Asai, Y., Kaneko, M., Ohtsuka, K., Morita, Y., Kaneko, S., Noda, H., Hara-Kudo, Y. (2008): *Salmonella* prevalence in seafood imported into Japan. Journal of Food Protection, 71, 7, 1460–1464.

Azzam, M. I., Ezzat, S. M., Othman, B. A., El-Dougdoug. (2017): Antibiotics Resistance Phenomenon and Virulence Ability in Bacteria from Water Environment. Water Science, 31, 109–121.

Chouhan, S. (2015): Enumeration and Identification of Standard Plate Count Bacteria in Raw Water Supplies. *IOSR* Journal of Environmental Science Toxicology and Food Technology Ver. II, 9, 2, 67–73.

Costa, R. A., Carvalho, F. C. T. D. C., Vieira, R. H. S. D. F. (2012): Antibiotic Resistance in *Salmonella*: A Risk for Tropical Aquaculture. RFID Technology, Security Vulnerabilities, and Countermeasures, 195–206.

Elhadi, N. (2014): Prevalence and Antimicrobial Resistance of *Salmonella* spp. in Raw Retail Frozen Imported Freshwater Fish to Eastern Provience of Saudi Arabia. Asian Pacific Journal of Tropical Biomedicine, 4, 3, 234 –238.

Hamdan, R. H., Musa, N., Musa, N., Lee, S., Sarman, A. (2008): Isolation and enumeration of coliform bacteria and *Salmonella* spp . from short-.necked clam *Orbicularia orbiculata* at East coast, Malaysia. Internet Journal of Food Safety, 10, Anon 2004, 58–64.

Heinitz, M. L., Ruble, R. D., Wagner, D. E., Tatini, S. R. (2000): Incidence of *Salmonella* in fish and seafood. Journal of Food Protection, 63, 5, 579–592.

Lai, H. T., Lin, J. J. (2009): Degradation of oxolinic acid and flumiquine in aquaculture pond waters and sediments. Chemosphere, 75, 4, 462-468.

Lee, S. W., Najiah, M., Wendy, W., Nadirah, M., Faizah, S. H. (2009): Occurrence of heavy metals and antibiotic resistance in bacteria from internal organs of American bullfrog (*Rana catesebeina*) raised in Malaysia. J Venom Anim Tox Trop Dis. 15, 2, 353-358.

Lee, S. W., Musa, N., Wee, W. (2010): Bacterial flora from a healthy freshwater Asian sea bass (*Lates calcarifer*) fingerling hatchery with emphasis on their antimicrobial and heavy metal resistance pattern. Veterinarski Arhiv, 80, 3, 411–420.

Lee, S. W., Wee, W., Che Manan, Z., Amin, M. R., Hajisamae, S. (2013): A study of *Edwardsiella tarda* colonizing live Asian clam, *Corbicula fluminea*, from Pasir Mas, Kelantan , Malaysia with the emphasis on its antibiogram, heavy metal tolerance and genetic diversity. Veterinarski Arhiv, 83, 3, 323–331.

Lee, S. W., Wendy, W. (2011): Antibiogram and heavy metal resistance pattern of *Salmonella* spp. isolated from wild Asian sea bass (*Lates calcarifer*) from Tok Bali, Kelantan, Malaysia. Jordan J Biol Sci. 147, 621, 1-4.

Lee, S. W., Wendy, W. (2012): Characterization of *Vibrio alginolyticus* isolated from white leg shrimp (*Litopenaeus vannamei*) with emphasis on its antibiogram and heavy metal resistance pattern. Veterinarski Arhiv, 82, 2, 221-227.

Mannas, H., Mimouni, R., Chaouqy, N., Hamadi, F., Martinez-Urtaza, J. (2014): Occurrence of *Vibrio* and *Salmonella* species in mussels (*Mytilus galloprovincialis*) collected along the Moroccan Atlantic coast. SpringerPlus, 3, 1, 1–11.

Martinez-Urtaza, J., Peiteado, J., Lozano-Le, N, A., Garcia-Martin, O. (2004): Detection of *Salmonella senftenberg* associated with high saline environments in mussel processing facilities. Journal of Food Protection, 67, 2, 256–263.

Martinez-Urtaza, J., Saco, M., Hernandez-Cordova, G., Lozano, A., Garcia-Martin, O., Espinosa, J. (2003): Identification of *Salmonella* serovars isolated from live molluscan shellfish and their significance in the marine environment. Journal of Food Protection, 66, 2, 226–232.

Mevius, D. J, Breukink, H. J., Miert, A. S. J. P. A. M. (1990): *In vitro* activity of flumequine in comparison with several other antimicrobial agents against five pathogens isolated in calves in the Netherlands. Vet Quarterly 12, 4, 212-220.

Mirzaie, S., Hassanzadeh, M., Ashrafi, I. (2010): Identification and characterization of *Salmonella* isolates from captured house sparrows. Turk J Vet Anim Sci, 34, 2, 181-186.

Muziasari, W., Managaki, S., Pärnänen, K., Karkman, A., Lyra, C., Tamminen, M. (2014): Sulfonamide and trimethoprim resistance genes persists in sediments at Baltic Sea aquaculture farms but are not detected in the surrounding environment. PLoS ONE, 9, 3, e92702.

Muziasari, W., Pärnänen, K., Johnson, T., Lyra, C., Karkman, A., Stedfeld , R., et al. (2016): Aquaculture changes the profile if antibiotic resistance and mobile genetic element associated genes in Baltic Sea sediments. FEMS Microbiology Ecology, 92, 4, fiw052.

Menninger, H. (2013). The Asian Clam, *Corbicula Fluminea*: A Brief Review of the Scientific Literature. In *Vital Signs*. Retrieved from http://vitalsignsme.org/sites/default/files/content/blog082511_asian_clam_brief_nyisri_2.pdf.

Raufu, I. A., Lawan, F. A., Bello, H. S., Musa, A. S., Ameh, J. A., Ambali, A. G. (2014): Occurrence and antimicrobial susceptibility profiles of *Salmonella* serovars from fish in Maiduguri, sub-Sahara, Nigeria. Egyptian Journal of Aquatic Research 40, 59–63.

Rogstad, A., Ellingsen, O. F., Syvertsen, C. (1993): Pharmacokinetics and bioavailability of flumequine and oxolinic acid after various routes of administration to Atlantic salmon in seawater. Aquaculture, 110, 3-4, 207-220.

Sicuro, B. (2015). Freshwater Bivalves Rearing: A Brief Overview. International Aquatic Research 7, 2, 93–100.

Tamminen , M., Karkman , A., Lõhmus , T., Muziasari, W., Takasu , H., Wada, S., et al. (2011). Tetracycline resistance genes persist at aquaculture farms in the absence of selection pressure. Environment Science Technology, 45, 2, 386-391.

Watts, J. E. M., Schreier, H. J., Lanska, L., Hale, M. S. (2017): The rising tide of antimicrobial resistance in aquaculture: sources, sinks and solutions. Marine Drug Journal, 15, 158.

Zhao, S., McDermott, P. F., Friedman, S., Qaiyumi, S., Abbott, J., Kiessling, C., White, D. G. et al. (2006): Characterization of antimicrobial-resistant *Salmonella* isolated from imported foods. Journal of Food Protection Food Prot, 69, 3, 500–507.