

Chempedak isolates were found to be more susceptible to cadmium and copper at MIC range of 50-100 µg/ml. Interestingly, almost all isolates from Pantai Batu Hitam displayed high resistance to both chromium and nickel with MIC > 500 µg/ml and half of the isolates showed high resistance to both copper and cobalt with similar MIC. The resistance pattern associated with Teluk Chempedak and Pantai Batu Hitam were Cr > Ni > Co > Cd = Cu and Cr = Ni > Co > Cu > Cd respectively.

Table 3 depicts selected isolates from both location that exhibited both antibiotic and heavy metal resistance capabilities. Isolate TC 46 was resistant to 8 antibiotics and resistant to chromium, cadmium and cobalt with MIC range of 350-400 µg/ml while TC 48 was resistant to 7 antibiotics and resistant to chromium, cadmium and cobalt with MIC of 450 µg/ml. TC50, however, was resistant to 7 antibiotics and resistant to chromium and cadmium with MIC >500 µg/ml and MIC for cobalt of 450 µg/ml. Isolates BH 45 and BH 50 were resistant to 7 antibiotics and all were highly resistant to chromium, nickel, copper and cobalt with MIC > 450 µg/ml. Similar results was also obtained on isolate 6 that was resistant to 8 antibiotics. The genes encoding resistance to heavy metals can be located together with antibiotic resistance genes on either the same genetic structure or different genetic structures within the same bacterial strain Metal and antibiotic resistances are the most common features that bacteria gain due to the abuse of metals and/or antibiotic [12]. Recent studies have suggested that the presence of metal contamination in natural environments could play a role in the maintenance and proliferation of antibiotic resistance [13,14].

The 16S rRNA gene sequence analysis of isolate TC 50 revealed that it has the closest relationship to *Staphylococcus saprophyticus* ATC 15305 (100 %), while isolate BH 50 possess the closest relationship with 99 % similarity to *Brevundimonas vesicularis* IAM 12105. *S. saprophyticus* is a gram-positive coccus commonly found in urine specimen and a common cause of urinary tract infection. Reports on multiple antibiotic resistant *S. saprophyticus* were mostly related to clinical isolates from hospitals but none from the marine environment [15]. High number of tourists in Teluk Chempedak might implicate to high volume of waste water (excrete products, urine) discharged into the marine environment and which eventually led to its residing in the marine sediment. Additionally, there was no report on *S. saprophyticus* to possess both antibiotic and heavy metal resistant capabilities. Hence, this is the the first report of such incidence. Meanwhile, *B. vesicularis* can be isolated from the human end cervix, natural soil environments, bottled water and hospital environments [16]. This bacterium can cause arthritis, endocarditis, meningitis and primary bloodstream infection in immunocompromised as well as immunocompetent patient [17]. Similar to *S. saprophyticus*, reports on *B. vesicularis* were mostly on its multiple antibiotic resistance ability but none on the heavy metal resistant ability. Hence, this is the the first report of such incidence for both *S. saprophyticus* and *B. vesicularis*. Results obtained in this study indicated the possibility of marine environments of Teluk Chempedak and Pantai Batu Hitam were of poor microbiological quality which has led to bacterial isolates to gain resistance against several antibiotic agents and together with high numbers of heavy metal resistant bacteria could be the result of agrochemical and industrial pollution.

Table 1: Heavy metal tolerance in bacteria from marine sediment of Teluk Chempedak

Heavy Metal	MIC (µg/ml) with number of tolerant isolates						
	< 50	50-100	110-200	210-300	310-400	410-500	>500
Cd		45	5				
Co		11	29		5	5	
Cr				14	15	13	8
Cu		45	5				
Ni	10		17	16	2	3	2

Table 2: Heavy metal tolerance in bacteria from marine sediment of Pantai Batu Hitam

Heavy Metal	MIC (µg/ml) with number of tolerant isolates						
	< 50	50-100	110-200	210-300	310-400	410-500	>500
Cd		7	32				
Co		2	2	1	9	7	18
Cr		1					38
Cu		18		6			15
Ni		1					38

Table 3: Selected antibiotic and heavy metals resistance isolates from Teluk Chempedak (TC) and Pantai Batu Hitam (BH)

Isolates	Antibiotic	Heavy Metal
TC 46	Vancomycin, Ampicillin, Tetracycline, Chloramphenicol, Penicillin G, Streptomycin, Gentamycin, Rifampicin	Chromium, Cadmium, Cobalt
TC 48	Vancomycin, Ampicillin, Tetracycline, Chloramphenicol, Gentamycin, Rifampicin, Penicillin G	Chromium, Cadmium, Cobalt
TC 50	Vancomycin, Ampicillin, Tetracycline, Chloramphenicol, Gentamycin, Rifampicin, Penicillin G	Chromium, Cadmium, Cobalt
BH 6	Vancomycin, Ampicillin, Tetracycline, Chloramphenicol, Penicillin G, Streptomycin, Gentamycin, Rifampicin	Chromium, Copper, Cobalt, Nickel
BH 45	Vancomycin, Tetracycline, Chloramphenicol, Erythromycin, Streptomycin, Gentamycin, Rifampicin	Chromium, Copper, Cobalt, Nickel
BH 50	Vancomycin, Tetracycline, Chloramphenicol, Erythromycin, Streptomycin, Gentamycin, Rifampicin	Chromium, Copper, Cobalt, Nickel

4. CONCLUSION

In conclusion, the occurrence of antibiotic and heavy metal resistant bacteria in Pahang coastal water indicated the impact of human activities on the marine environment which constituting a risk to the public health and pose a threat to the marine ecosystem. In addition, marine sediment in both locations probably represents a reservoir of multiresistant genetic elements which bacteria can attain through horizontal gene transfer.

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REFERENCES

- [1] Yamina, B.T. 2012. Isolation and screening of heavy metal resistance bacteria from wastewater: A study of heavy metal co-resistance and antibiotic resistance. Water Science and Technology. A Journal of the International Association on Water Pollution Research, 66 (10), 2041-2048.
- [2] Hosono, T., Su, C.C., Siringan, F., Amano, A., Onodera, S.I. 2011. Effects of environmental regulation on heavy metal pollution decline in core sediments from Manila Bay. Marine Pollution Bulletin, 60 (5), 780-785.
- [3] Mondragón, V.A., Llamas-Pérez, D.F., González-Guzmán, G.E., Márquez-González, A.R., Padilla-Noriega, R., Durán-Avelar, M.J., Franco, B. 2011. Identification of Enterococcus faecalis bacteria resistant to heavy metals and antibiotics in surface water of Mololoa River in Tepic, Nayarit, Mexico. Environmental Monitoring and Assessment, 183 (1-4), 329-340.
- [4] Fu, F.W., Qi, W. 2011. Removal of heavy metal ion from wastewaters: A review. Journal of Environmental Management, 92 (3), 407-408.
- [5] Lopez-Maury, L., Garcia-Dominguez, M., Florencio, F.J., Reyes, J.C. 2002. A two-component signal transduction system involved in nickel sensing in the Cyanobacterium *Synechocystis* sp. PCC 6803. Molecular Microbiology, 43 (1), 247-256.

- [6] Silver, S. 1998. Genes for all metals a bacterial view of the periodic table. *J Ind Microbiol Biot*, 20 (1), 1–12.
- [7] Miranda, C.D., Castillo, G. 1998. Resistance to antibiotic and heavy metals of motile aeromonads from Chilean freshwater. *Science of the Total Environment*, 224 (1-3), 167–176.
- [8] Matyar, F. 2007. Distribution and antimicrobial multiresistance in Gram negative bacteria isolated from Turkish sea bass (*Dicentrarchus labrax* L., 1781) farm. *Annals of Microbiology*, 57 (1), 35–38.
- [9] Gul-Seker, M., Mater, Y. 2009. Assessment of metal and antibiotic resistance in marine bacteria isolated from Izmit Bay and Bosphorus entrance of Marmara and Black Sea, Turkey. *Fresenius Environmental Bulletin*, 18, 2192–2202.
- [10] Matyar, F. 2012. Antibiotic and heavy metal resistance in bacteria isolated from Mediterranean Sea coast. *Bulletin of Environmental Contamination and Toxicology*, 89 (3), 551–556.
- [11] Rahman, N.A., Chowdhury, A.J.K., Abidin, Z.A.Z. 2015. Antibiotic resistant bacteria from sediment of coastal water of Pahang, Malaysia. *Jurnal Teknologi*, 77 (24), 65-70.
- [12] Baker-Austin, C., Wright, M., Stephanauskas, R., McArthur, J.V. 2006. Co-selection of antibiotic and metal resistance. *Trends Microbiology*, 14, (4), 176–182.
- [13] Summers, A.O. 2002. Generally overlooked fundamentals of bacterial genetics and ecology. *Clinical Infectious Diseases*, 34 (3), 85–92.
- [14] Matyar, F., Kaya, A., Dincer, S. 2008. Antibacterial agents and heavy metal resistance in Gram-negative bacteria isolated from seawater, shrimp and sediment in Iskenderun Bay, Turkey. *Science of the Total Environment*, 407 (1), 279–285.
- [15] Sabzehali, F., Goudarzi, M., Goudarzi, H., Azimi, H. 2017. Distribution of Aminoglycoside Resistance Genes in Coagulase-Negative Staphylococci Isolated from Hospitalized Patients. *Archives of Pediatric Infectious Diseases*, 5 (3), e57297. [doi: 10.5812/pedinfest.57297](https://doi.org/10.5812/pedinfest.57297)
- [16] Han, X.Y., Andrade, R.A. 2005. *Brevundimonas diminuta* infections and Its Resistance to Fluoroquinolones. *Journal of Antimicrobial Chemotherapy*, 55 (6), 853-859.
- [17] Zhang, C.C., Hsu, H.J., Li, C.M. 2012. *Brevundimonas vesicularis* Bacteremia Resistant to Trimethoprim-Sulfamethoxazole and Ceftazidime in a Tertiary Hospital in Southern Taiwan. *Journal of Microbiology, Immunology and Infection*, 45 (6), 448-452

